Microbiologist

The magazine of the Society for Applied Microbiology ■ March 2011 ■ Vol 12 No 1 ISSN 1479-2699

Microbiomes

INSIDE

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editorial

Lucy Harper muses on the marvels of microbiomes

I hope I don't need to convince readers of *Microbiologist* that microbes are fascinating. Despite leaving the bench some time ago I find I'm drawn to this conclusion more and more over time. There isn't one particular species I'm smitten with or a genus I consider genius. More, it's the way microbes work together and with their hosts to survive, grow and evolve, I find fascinating. And it's the influence they have on each other, as well as the effects they have on their host species' which will never cease to amaze.

So, with this in mind, this issue of *Microbiologist* has the theme 'microbiomes'. Wikipedia describes microbiomes as: "*the totality of microbes, their genetic elements (genomes), and environmental interactions in a defined environment. A defined environment could, for example, be the gut of a human being or a soil sample. Thus, microbiome usually includes microbiota and their complete genetic elements.*"

The National Institutes of Health in the USA is running a project to investigate the human microbiome. According to their website (http://commonfund.nih.gov/hmp/): "*The…* ***Human Microbiome Project (HMP)*** *aims to characterize the microbial communities found at several different sites on the human body, including nasal passages, oral cavities, skin, gastrointestinal tract, and urogenital tract, and to analyze the role of these microbes in human health and disease. HMP includes the following initiatives*:

- *Reference set of microbial genome sequences and preliminary characterization of the human microbiome*
- *Relationship between disease and changes in the human microbiome*
- *New technologies and tools for computational analysis*
- *Data and analysis coordinating center (DACC)*
- *Resource repository*
- *Ethical, legal, and social implications (ELSI)*"

In this issue of *Microbiologist*, we look at a number of different microbiomes of a variety of environments:

In our first feature article, as part of the HMP, Derrick E. Fouts and colleagues at the J. Craig Venter Institute look at the microbiome of the human vagina and how this relates to the condition bacterial vaginosis (page 22). For those who don't know, this microbial infection can cause preterm birth in some pregnant women. A better understanding of the microbiology of this often symptomless condition is needed to enable us to potentially prevent unnecessary preterm births.

Our second article moves the focus away from us, to the gut microbiome of *Drosophila melanogaster*. Ed Yong explains how the diet of these fruit flies has been shown to influence their sexual behaviour through the changes that occur to their gut microflora (page 26).

A concept which can cause controversy is the potential cross-over between science and art. I have met people who are quite cynical about this and who believe that there is no such cross-over — that artists hold no place in a scientists world and *vice versa*. However, I don't think anyone can argue that the images produced by Caleb Charland on page 32 are anything other than a great example of microbes creating aesthetic beauty.

As always, if you feel strongly about any of the topics or articles that appear in *Microbiologist*, please do let us know. I welcome emails from readers (lucy@sfam.org.uk) or you can contact me via Facebook or Twitter (sfamtweets).

contribute

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

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

Lucy Harper

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

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

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benefits

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

- 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 PDF downloadable 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.

■ **Retirement 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.

■ **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.** Alternatively, write to her at:

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

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Tweeting for SfAM

The competition for this issue of *Microbiologist* is based on Twitter. For those who don't know about Twitter, you can read about it in a previous issue of *Microbiologist* (September 2009, Vol. 10, No. 3, p14) but in a nutshell, it's a way to share information online about you or your organization with organizations and people you are interested in. Each message or 'tweet' is 140 written characters in length (including punctuation) and can be about anything you like. The SfAM Twitter feed tweets every day: we talk about microbiology-related news or link our tweet to news of upcoming SfAM events or news. Now we are looking for the most interesting, inspiring and attention-grabbing microbiology-related tweets from you.

To get you started, here's a tweet I read this morning from @NHSChoices:
"*Daily Mail reports that giving antibiotics to children may lead to IBS. Original research acknowledges link unproven http://at.nhs.uk/ehHc3u*"

So as you can see, tweets can be full of vital information and point you in the direction of more complete sources. They can also be fun, frivolous and just plain factual.
To find inspiration, why not sign up to Twitter and follow @sfamtweets?

Send your entries by email to the Editor (lucy@sfam.org.uk) by **11 April 2011** to be in with a chance of winning an Amazon voucher. But remember, any entries longer than 140 characters will be disqualified!

president's and ceo's column

Geoff Hanlon and **Philip Wheat**
highlight some exciting new initiatives

Once again at the beginning of a new year we would like to share some exciting new initiatives for 2011 which will further enhance the benefits of membership. In this first issue for 2011, the President and CEO felt it was appropriate to write a joint column to highlight these new initiatives

As we have previously described, membership of the Society is still gradually increasing. This is in contrast to many other learned societies who are seeing a gradual decline in membership. As part of the efforts to maintain and increase membership, it is essential that any membership organization keeps in touch with its members and indeed meets, or even exceeds their needs and expectations. One way this can be achieved is to regularly seek feedback from members. Last year we chose to seek feedback using a membership survey which specifically focussed upon Society grants and awards. We are delighted to share with you some of this feedback which has now been considered and below we detail several new initiatives which we intend to implement during the coming year.

After evaluating and discussing a number of the suggestions and comments from members' feedback, we have introduced a new grant, the **Scientific Meeting Grant**. This grant is intended for members (Student and Full) to help them attend scientific meetings. Whilst preference will be given to individuals who are presenting at the meeting it is not essential that the member is presenting. The meetings which will be eligible for this grant include all Society scientific meetings, including the annual *Environmental Microbiology* lecture. However, this grant is also available for use in attending non-S*f*AM scientific meetings — it is not exclusively for S*f*AM meetings.

When applying, each member will be asked to describe the meeting they wish to attend and each meeting will be assessed on its own relevance to the field of applied microbiology. The grant will be up to £300 (or equivalent in local currency) and can be used to assist with registration, travel and subsistence costs. So for instance, a member from the UK who wishes to attend the S*f*AM Summer Conference in 2011 can attend the meeting (registration cost £250

including three nights hotel accommodation) and still have £50 towards the cost of travel and subsistence. Applicants for this grant will be expected to pay the cost of the meeting they wish to attend and claim up to £300 back once the meeting has ended. Any repayments will only be made with appropriate receipts.

We must emphasize that all our grants are available to all the relevant member categories irrespective of their country of residence. Full details of the terms and conditions for all grants and awards can be found on our website (www.sfam.org.uk).

Once again, following feedback from the members' survey we have also increased the value of some of the long established grants we have on offer. The popular **President's Fund** has been increased in value from up to £1000 to £1200 per successful applicant. In addition, the value of the **Students into Work Grant** has been increased from up to £1600 (payable to the student) to £2000 with up to £500 for consumable costs. Previously, the maximum amount available was £2100 and this has now increased to a maximum of £2500. Although we have used pounds sterling to illustrate these grants, once again it is worth making the point that both these grants are available to members where other currencies operate e.g. US$ and €.

We have also decided that from 2011 we will no longer offer a jointly funded (with the Society for General Microbiology [SGM]) **Regional Meeting Grant**. Previous arrangements for this grant were that applicants had to be a member of either the S*f*AM or the SGM. Instead of this arrangement we have decided still to offer the grant but just to S*f*AM members. The grant will in future be wholly administered by the Society's office.

Further feedback from members indicated the need to improve and further develop the

Society's website. This had already been identified by the management of the Society and we can tell you that significant resources have been identified and late in 2010 a company was appointed to help to transform the website so that members will have a far better online experience. Changes will include the facility to apply for all grants online, there'll be a blog, a member's area/directory and many other facilities are planned. We expect most if not all of these online improvements to be completed by the end of 2011.

Another new initiative for 2011 which we are proposing, is to be more active in the area of outreach and public engagement of applied microbiology in schools. We have agreed to support at least two projects where microbiology is introduced to children at a variety of ages, from as young as 9 to10 years old to older children of 13 to14 years. Reports of these events will appear in future issues of the *Microbiologist*. In addition we hope to make resources used in these activities, available to other members who are involved with the promotion of microbiology to school children.

Regular readers of the *Microbiologist* will be aware of one of the regular feature articles — StatNote. This series of articles by Drs Hilton and Armstrong from Aston University, Birmingham, cover statistics which is of particular use to biologists and has been made into a book published by the Society's publishing partner Wiley-Blackwell. We will be promoting the book at the various exhibitions we are attending during the year. We also hope to have some copies available at discounted rates to members and prospective new members. In the longer term we are in discussions with Wiley-Blackwell about branding relevant microbiology text books with the Society logo. We envisage that these books will be offered to members at a discounted rate (above the already discounted rate provided to members by Wiley-Blackwell). We see this as yet another opportunity to even further enhance the benefits of membership.

Once again in 2011 some of the Society's Staff and Trustees will be attending a number of scientific meetings outside the UK. So far those at which we have confirmed attendance include:

- American Society of Microbiology, New Orleans, USA. 21-25 May
- International Food Technology, New Orleans, USA. 11-15 June
- International Association of Food Protection, Milwaukee, USA. 31 July- 3 August

If you are attending any of the above meetings please call by the stand — we would be glad to meet you. In addition, whilst we are discussing forthcoming meetings, we are writing this column late in 2010 and already we have a number of delegates registered for the Society's Summer Conference (Dublin 4-7 July). If you have not done so already, we strongly urge you to register for this meeting, as places are limited and we expect to be full well before the published deadline dates.

Finally, in this joint column we would like to share some good news concerning a Staff member of the Society. During 2008, Dr Lucy Harper (Communications Manager and amongst many other things the Editor of the *Microbiologist*), started studying part-time for a Master of Business Administration (MBA) at Aston University Business School, Birmingham. We are delighted to inform you that Lucy has successfully completed her studies and been awarded a MBA. In addition, because she achieved an average mark of above 70% she has also been awarded a distinction. Her final module was her project where she achieved an outstanding mark of 75%. We are sure all of you would like to congratulate Lucy (lucy@sfam.org.uk) on her success which was very well deserved after all her hard work and effort. Very well done Lucy, many congratulations!

Professor Geoff Hanlon
President of the Society

Philip Wheat
Chief Executive Officer

SfAM Communications Award
Call for nominations!

The SfAM **Communications Award** aims to recognize individuals who have communicated their work/applied microbiology to the general public. The overall aim of this award is to raise the profile of applied microbiology and SfAM. The award will be for £1000 and nominations must be from Full Ordinary or Student Members with a deadline in April each year. Nominations should be in writing, providing detailed information about all relevant media/communications work of the nominee. Nominations should be made by members of SfAM but nominees do not have to be members of the Society. Nominees could include:

- Professional communicators: broadcasters, authors or science writers/journalists
- Scientists who are recognized science communicators
- Scientists who are not yet recognized science communicators but have significant experience of working with the media
- Teachers/lecturers
- Artists

The nature of the communication can be local, national or international factual or fictional works including: fiction books, factual books, popular science books, newspaper / magazine articles, film, television (series or documentary), lectures or lecture series, classroom demonstrations, works of art / exhibitions or any other format a nominee considers appropriate.

The award will be presented every year at the Summer Conference dinner and the winner will be asked to give an after dinner speech as a condition of receiving the award. Members who make a nomination are responsible for contacting the nominee to ensure they are available on the date of the Summer Conference dinner. **The closing date for applications is 15 April 2011.**

To make a nomination, please download and complete a pdf application form from the website: www.sfam.org.uk/grants.php and send five copies of the application together with the nominated newspaper article, magazine, DVD or other medium to the SfAM office.

membership matters

Call for nominations to Committee

There will be up to two vacancies on the SfAM Committee in **July 2011.** Nominations are invited from all Full Members of the Society for these vacancies. Nominations must be made in writing and received by the Society Office by **6 May 2011**.

Should nominations exceed vacancies, election will be by a system of postal voting arranged by Committee.

Call for nominations for W H Pierce Prize

Do you know a young microbiologist (under 40 years of age) who has made a substantial contribution to microbiology? If so, why not nominate them for this prestigious and substantial award which is now worth £3,000. The award was instituted in 1984 by Oxoid to commemorate the life and works of the late W H (Bill) Pierce, former chief bacteriologist at Oxoid Ltd and a long time member of the Society. The prize is presented annually at the Summer Conference. Full Members wishing to make a nomination for the 2011 prize should write in confidence to the Honorary General Secretary, Dr Mark Fielder, at the Society Office in Bedford, including a full CV of the nominee and a letter of support. Please note that application is through nomination by Full Members of SfAM only and that there are no official forms for this award. **Closing date for nominations is Thursday 21 April 2011.**

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.

Bahrain

S. Al-Thawadi

Canada

E. Beckett Sward

Estonia

T. Kramarenko; M. Kuningas; K. Lilles

Ireland

K. Twomey

Kosovo

A. Kurti

Nepal

S. R. Kandel

New Zealand

S. Simpson

Nigeria

B. O. Amechi; S. O. Kareem; M. A. Oguntayo; O. A. Olumboyede

Pakistan

F. Hasan

Poland

J. Kozdroj

Sweden

A. Christiansson

UAE

B. Sarbudeen

UK

A. R. Abdulrahman; S. S. Ahmed; H. Al-Khanaq; T. C. Ardis; P. Ashton; S. Baugh; R. G. Bello; J. W. Betts; R. S. Bhangal; L. E. Bingle; J. Blaxland; E. N. Boadu; J. A. Butler; E. Cetinkaya; R. A. Culak; S. Dehlawi; L. Eland; J. Evans; J. M. Evans; R. G. Fernandes; J. Fothergill; F. Fraser; A. Green; V. Hall; S. E. Hiscott; R. W. Jackson; D. M. Jamrozy; E. J. Jawad; M. Jayaweera Bandara; A. Jones; C. A. Jones; M. Joshi; H. Kadhim; M. Karamanlioglu; A. J. Kermode; H. Kerridge; J. Key; E. King; L. Knapp; K. Lai; S. H. Lau; W. J. Law; K. D. Longmaid; M. N. Mohd Zairi; S. C. Moody; L. Ogilvie; M. Ojeil; T. C. Okezie; K. M. Pawlowsky; T. Prince; J. Ramsay; F. Rao; S. Rogerson; J. Russell; F. Sargent; K. Sim; N. M. Thorpe; E. Wachnicka; U. Zafar

USA

R. Crowley; G. Cuadra; D. Gibbs; R. Helton; K. McDonald

Losses

We were saddened to learn of the death of the following member of the Society:

Mrs A J Baxter (NCIMB Ltd) — full member since 1993 — died December 2010

journal**Watch**

News about the Society's journals

Journal of Applied Microbiology

Read the top cited articles published in *Journal of Applied Microbiology* in 2009 and 2010:

Fe(III) oxide reduction and carbon tetrachloride dechlorination by a newly isolated *Klebsiella pneumoniae* strain L17. Li, X.M., Zhou, S.G., Li, F.B., Wu C.Y., Zhuang, L., Xu, W. and Liu, L. **Vol. 106**, No. 1

The role of biofilms and protozoa in *Legionella* pathogenesis: implications for drinking water. Lau, H.Y. and Ashbolt, N.J. **Vol. 107**, No. 2

Role of the alternative sigma factor σ^B on *Staphylococcus aureus* resistance to stresses of relevance to food preservation. Cebrián, G., Sagarzazu, N., Aertsen, A., Pagán, R., Condón, S. and Mañas, P. **Vol. 107**, No. 1

The effect of climate change on the occurrence and prevalence of livestock diseases in Great Britain: a review. Gale, P., Drew, T., Phipps, L., David, G. and Wooldridge, M. **Vol. 106**, No. 5

Elevation of *Francisella philomiragia* subsp *noatunensis*, Mikalsen *et al*., (2007) to *Francisella noatunensis* comb. nov [syn. *Francisella piscicida* Ottem *et al*., (2008) syn. nov.] and characterization of *Francisella noatunensis* subsp. *orientalis* subsp nov., two important fish pathogens. Ottem, K., Nylund, A., Karlsbakk, E., Friis-Møller, A. and Kamaishi, T. **Vol. 106**, No. 4

Letters in Applied Microbiology

Read the top cited articles published in *Letters in Applied Microbiology* in 2009 and 2010:

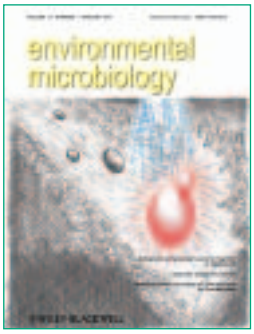
Fabrication of silver nanoparticles by *Phoma glomerata* and its combined effect against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Birla, S., Tiwari, V., Gade, A., Ingle, A., Yadav, A. and Rai, M. **Vol. 48**, No. 2

Inactivation of *Vibrio parahaemolyticus* in pure culture, whole live and half shell oysters (*Crassostrea virginica*) by X-ray. Mahmoud, B. S. M. and Burrage, D. D. **Vol. 48**, No. 5

The benefits of silver in hygiene, personal care and healthcare. Edwards-Jones V. **Vol. 49**, No. 2

Occurrence of *Cryptosporidium* and *Giardia* genotypes and subtypes in raw and treated water in Portugal. Lobo, M., Xiao, L., Antunes, F. and Matos, O. **Vol. 48**, No. 6

Auxin production by plant associated bacteria: impact on endogenous IAA content and growth of *Triticum aestivum*. Ali, B., Sabri, A., Ljung, K. and Hasnain, S. **Vol. 48**, No. 5



Environmental Microbiology

Read the top cited articles published in *Environmental Microbiology* in 2009 and 2010:

Wrinkles in the rare biosphere: pyrosequencing errors can lead to artificial inflation of diversity estimates. Kunin, V., Engelbrektson, A., Ochman, H. and Hugenholtz, P. **Vol. 12**, No. 1

Bacteria rather than *Archaea* dominate microbial ammonia oxidation in an agricultural soil. Jia, Z.J. and Conrad R. **Vol. 11**, No. 7

Dynamics and functional relevance of ammonia-oxidizing archaea in two agricultural soils. Schauss, K., Focks, A., Leininger, S., Kotzerke, A., Heuer, H., Thiele-Bruhn, S., Sharma, S., Wilke, B.-M., Matthies, M., Smalla, K., Munch, J. C., Amelung, W., Kaupenjohann, M., Schloter, M. and Schleper, C. **Vol. 11**, No. 2

Clostridium difficile PCR ribotype 078 toxinotype V found in diarrhoeal pigs identical to isolates from affected humans. Debast, S. B., Van Leengoed, L. A. M. G., Goorhuis, A., Harmanus, C., Kuijper, E. J. and Bergwerff, A. A. **Vol. 11**, No. 2

Comparative day/night metatranscriptomic analysis of microbial communities in the North Pacific subtropical gyre. Poretsky, R. S., Hewson, I., Sun, S., Allen, A. E., Zehr, J. P. and Moran, M. A. **Vol. 11**, No. 6

Environmental Microbiology Reports

Read the top cited articles published in *Environmental Microbiology Reports* in 2009 and 2010:

Honeybee colony collapse due to *Nosema ceranae* in professional apiaries. Higes, M., Martín-Hernández, R., Garrido-Bailón, E., González-Porto, A. V., García-Palencia, P., Meana, A., Del Nozal, M. J., Mayo, R. and Bernal, J. L. **Vol. 1**, No. 2

Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. Paerl H.W. and Huisman J. **Vol. 1**, No. 1

South American native bumblebees (Hymenoptera: Apidae) infected by *Nosema ceranae* (Microsporidia), an emerging pathogen of honeybees (Apis mellifera). Plischuk, S., Martín-Hernández, R., Prieto, L., Lucía, M., Botías, C., Meana, A., Abrahamovich, A. H., Lange, C. and Higes, M. **Vol. 1**, No. 2

The global methane cycle: recent advances in understanding the microbial processes involved. Conrad, R. **Vol. 1**, No. 5

Environmental, genomic and taxonomic perspectives on methanotrophic *Verrucomicrobia*. Op den Camp, H. J. M., Islam, T., Stott, M. B., Harhangi, H. R., Hynes, A., Schouten, S., Jetten, M. S. M., Birkeland, N.-K., Pol, A. and Dunfield, P. F. **Vol. 1**, No. 5

Lucy Collister
Wiley-Blackwell

bio **focus**

Mark Downs reports on the latest developments from the Society of Biology

SOCIETY OF Biology

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

Accreditation of biology degrees

The Society of Biology acts as a single voice for over 80,000 biologists in the UK on key generic issues of policy. Science policy and science funding are of course critical but as both a professional body and a charity, education will always be high on our agenda. The recently published* Browne review recommends sweeping changes to the way in which higher education is funded and obviously presents significant challenges to all concerned.

Understandably the media focus has been almost exclusively on the student loan element of the proposals and the increased variable cap on tuition fees for higher education institutions. However, Lord Browne's report also seeks to address the gaps between the skills required by employers and those which university graduates are able to demonstrate. University education must, surely, above all be about development of intellectual rigour and the analytical skills so valuable for life whatever the chosen career of the individual. Nonetheless, if students are to pay the significantly enhanced

fees, their interest in employability skills will inevitably grow.

The Browne review highlights something which professional institutions have known for a long time — and have been acting on: that graduates need specific skills for individual professions in addition to their basic educational grounding and, once in employment, need to continually develop their professional skills. The Society of Biology, in common with many other professional organizations, offers a chartered route to recognizing professional skills, alongside a continuous professional development programme to ensure standards are maintained. But there is also an important role to be played in helping students to identify courses which have the strongest likelihood of providing them with the skills and education they require for a particular career path. It is for that reason that the Society of Biology has been working for the last year to develop an accreditation programme for undergraduate biology degrees.

As has frequently been made clear in the run up to the comprehensive spending review announcements, science in its totality contributes enormously to our economic and social prosperity. The life sciences are a particularly successful story for the United Kingdom. In many areas we are second in the World only to the United States and often first. Over the last 10 years, university life science research groups have spun out over 200 companies, worth in excess of £720 million. Our success at postgraduate and post doctoral level is clear to see but there remains a gap for graduates who often lack the skills suitable for research careers. Working with the Office for Life Sciences, and with support from the Biotechnology and Biological Sciences Research Council (BBSRC), and a dedicated team of skilled volunteers, the Society of Biology has, after wide consultation, developed a new framework for the accreditation of biology degrees for students who hope to embark upon a research career.

These degrees would typically be at least four years in length, including a major project with hands on experience in either industry or within a university research group. Intellectual rigour and experimental design will be high on the agenda, along with a capability to demonstrate strength in mathematics. Biology is far from a soft option amongst the sciences and a future career in the life sciences inevitably involves the application of numeric skills. It is an issue which has been ignored for far too long.

Of course, biology is a huge field and the Society cannot hope to accredit the entire breadth of degrees in one step. With this in mind, we are starting with a pilot programme in *in vivo* sciences and biochemistry. Host institutions have already expressed interest and we hope that the first students will be recruited in 2011. The challenge is to ensure that the system is not over bureaucratic, does not place undue cost burdens on universities, and meets the needs and expectations of employers. Above all though, an accredited degree needs to meet the expectation and aspiration of students.

To try and meet these challenges and requirements, the Society has opted for an outcomes based approach. We will not be dictating to universities the way in which they should teach a subject, or the particular topics they should cover. Rather, we will set out clearly the outcomes we (and employers) expect from the degree programme, based on consultation. We believe this programme, alongside our Chartered Biologist and Continuing Professional Development approaches can contribute, along with many other professional bodies, to help meet some of the aspirations outlined in Lord Browne's report.

However, university degrees are financed, and whatever the debt students incur, one thing is certain: with a more market based approach the customer (or can we still say student?) will become increasingly demanding in return for the investment they make. The Society of Biology is keen to make sure we can enable students to make more informed choices and to be more certain of the outcomes they can expect from their university education.

*correct at time of going to press December 2010

further information

■ For more information please visit:
http://www.societyofbiology.org/education/hei/accreditation

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

13 April 2011

Spring Meeting

5th broadening microbiology horizons in biomedical science meeting

Latest developments in respiratory tract infections (RTIs)

- Including the Procter and Gamble Applied Healthcare Microbiology Award lecture

The Stratford Q Hotel, Stratford upon Avon, UK

IBMS CPD ACCREDITATION 6 POINTS

Programme

These preliminary programme times and titles were correct at the time of going to press. For the latest programme please visit: www.sfam.org.uk/spring_meetings.php

09.15-10.15	**Coffee, trade exhibition and registration**
10.15-10.20	**Chairman's Welcome**
10.20-11.00	**Procter and Gamble Applied Healthcare Microbiology Award Lecture: *Salmonella* — always one step ahead?** John Threlfall, Health Protection Agency, Colindale, London
11.05-11.35	**Respiratory Bordetella Infections** Norman Fry, Health Protection Agency, Colindale, London
11.35-12.05	***Legionella* spp** Tim Harrison, HPA, Colindale, London
12.05-12.35	**RTIs in animals** Robin Nicholas, VLA, Weybridge
12.35-14.00	**Lunch and trade exhibition**
14.00-14.30	**RTIs and the paediatric patient** Patricia Fenton, Sheffield Children's Hospital, Sheffield
14.30-15.00	**RTIs and the intensive care patient** John Simpson, Newcastle University
15.00-15.30	**What a CF Doctor thinks he wants from a microbiologist** Frank Edenborough, Northern General Hospital, Sheffield
15.30-16.00	**RTIs and the immunocompromised patient** Derek Macallan, St George's University of London, London
16.00	**Close, tea and coffee**

To register online for the Spring Meeting please visit www.sfam.org.uk/spring_meetings.php or contact Sally Cryer ■ Email: sally@sfam.org.uk. Telephone: +44 (0)1234 761752

SPRING MEETING 2011 BOOKING FORM and INVOICE

SfAM SPRING MEETING WEDNESDAY 13 APRIL 2011

Only ONE person per form please. CLOSING DATE FOR REGISTRATIONS: Wednesday 6 April 2011
EARLY BIRD DISCOUNT of £30.00 is applied to all bookings made before **Wednesday 16 March 2011**

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

FEES	Before 16/03/2011	Between 17/03/2011 and 6/04/2011
Full Member	£50 ☐	£80 ☐
Student Member	£30 ☐	£60 ☐
Honorary Member	£30 ☐	£60 ☐
Associate Member	£30 ☐	£60 ☐
Retired Member	£30 ☐	£60 ☐
Student Non–Member	£60 ☐	£90 ☐
IBMS Member	£75 ☐	£105 ☐
Non–Member	£100 ☐	£130 ☐

***Non–Members please note:** You can add 1 year's membership to your event booking using this form, then register at the member rate and spend the same amount of money or less!

*ADD MEMBERSHIP TO YOUR BOOKING

Add Student membership (£25.00): ☐ **Add Full membership (£50.00):** ☐

YOUR DETAILS

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

Address:_____

_____ **Postcode:**_____

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

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

_____

YOUR NAME BADGE

Please enter the information below in **BLOCK CAPITALS** as you would like it to appear on your name badge

First Name:_____ **Family name:**_____

Organization/Affiliation:_____

YOUR PAYMENT

● For all participants: **The Society DOES NOT INVOICE for conference fees. Please treat your completed booking form as an invoice. Cheques must be in £ STERLING ONLY and made payable to 'The Society for Applied Microbiology'. Foreign cheques/ drafts MUST be negotiable for the full amount due.** We accept payment **ONLY** by the following credit and debit cards: VISA, Mastercard, Eurocard, Delta, Electron, JCB, Maestro and Solo.

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

TOTAL Amount enclosed/ to be charged: £_____

Card number: ☐☐☐☐ ☐☐☐☐ ☐☐☐☐ ☐☐☐☐ Solo Cards only: ☐☐☐

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

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Signature:_____ Date:_____

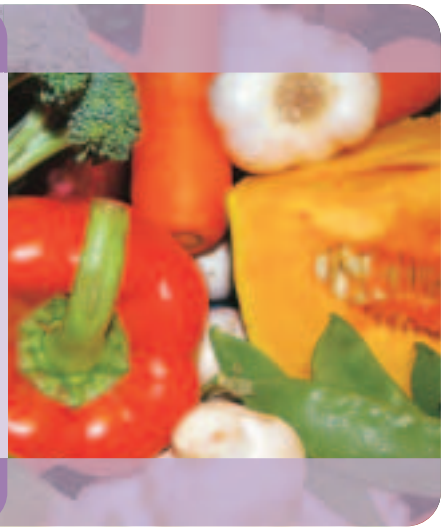
Please return the completed form by fax (post if you are enclosing a cheque) to: **The Society for Applied Microbiology, Bedford Heights, Brickhill Drive, Bedford MK41 7PH, UK. Tel: 01234 761752 Fax: 01234 328330. Email: meetings@sfam.org.uk**

4 - 7 July 2011

Summer Conference

Food microbiology

- Including the Lewis B Perry Memorial Lecture, followed by drinks, buffet and tour of the Guinness Storehouse
- Conference dinner with Irish entertainment and tutored whisky tasting session at the Jameson Distillery



Clontarf Castle, Dublin, Ireland

CALL FOR ABSTRACTS!

- We are now accepting abstracts for posters and the Student session at the 2011 Summer Conference at Clontarf Castle in Dublin. These can be on any topic in applied microbiology.
- There are prizes of **£150**, **£100** and **£50** available to winners of first, second and third prize for posters. For the best student oral presentation there is a prize of **£300!**

For more information or to submit your abstract visit: www.sfam.org.uk/summer_conference.php

STUDENTSHIP GRANTS

- Don't forget that we offer studentship grants to enable student members to attend Society meetings. The grant covers registration, accommodation, meals (where appropriate) and modest travel expenses.
- To be considered for a studentship grant please complete the application form at **www.sfam.org.uk/grants.php**

For more information about *Sf*AM grants visit: www.sfam.org.uk/grants.php

To register online for the Summer Conference please visit www.sfam.org.uk/summer_conference.php or contact Sally Cryer ■ Email: sally@sfam.org.uk. Telephone: +44 (0)1234 761752

SUMMER CONFERENCE 2011 BOOKING FORM and INVOICE

SfAM SUMMER CONFERENCE 4 — 7 July 2011

CLOSING DATE FOR REGISTRATIONS: Monday 20 June 2011. EARLY BIRD DISCOUNT of £50.00 is applied to all bookings made before 6 June 2011

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

FEES BEFORE 6 JUNE 2011	Full Member	Student, Honorary, Associate & Retired Member	Student Non–Member	Non–Member
Full Conference Rate: (inc accommodation)	£250.00 ☐	£200.00 ☐	£400.00 ☐	£600.00 ☐
Conference Rate: (no accommodation)	£100.00 ☐	£50.00 ☐	£100.00 ☐	£200.00 ☐
Conference Day Rate:	£50.00 ☐	£25.00 ☐	£50.00 ☐	£100.00 ☐

FEES BETWEEN 7 JUNE and 20 JUNE 2011	Full Member	Student, Honorary, Associate & Retired Member	Student Non–Member	Non–Member
Full Conference Rate: (inc accommodation)	£300.00 ☐	£250.00 ☐	£450.00 ☐	£650.00 ☐
Conference Rate: (no accommodation)	£150.00 ☐	£100.00 ☐	£150.00 ☐	£250.00 ☐
Conference Day Rate:	£100.00 ☐	£75.00 ☐	£100.00 ☐	£150.00 ☐

Conference Day Rate delegates please tick the day you wish to attend: Mon 4th ☐ Tue 5th ☐ Wed 6th ☐ Thur 7th ☐

RISK ASSESSMENT WORKSHOP: please tick this box if you would like to attend the workshop taking place on Monday 4 July 11.00 – 17.00 ☐

LEWIS B PERRY MEMORIAL LECTURE: please tick this box if you would like to attend the lecture and social event at the Guinness Storehouse on Monday 4 July ☐

QUIZ NIGHT with wine and buffet: please tick this box if you would like to attend at Clontarf Castle on Tuesday 5 July ☐

CONFERENCE DINNER: please tick this box if you would like to attend the dinner at the Jameson Distillery on Wednesday 6 July (extra fee applies) **£50.00** ☐

***Non–Members please note: You can add 1 year's membership to your event booking using this form, then register at the member rate and spend the same amount of money or less!**

*ADD MEMBERSHIP TO YOUR BOOKING

Add Student membership (£25.00): ☐ **Add Full membership (£50.00):** ☐

YOUR DETAILS

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

Address:_____

Postcode:_____ **Tel No:**_____ **Email:**_____

Special dietary or other requirements:_____

YOUR NAME BADGE

Please enter the information below in **BLOCK CAPITALS** as you would like it to appear on your name badge

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

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

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Signature:_____ Date:_____

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Summer Conference 2011 Programme

Monday 4 July 2011

11.00-17.00 **Risk assessment workshop in the Great Hall, Clontarf Castle**

17.30 **Coaches leave Clontarf Castle to travel to the Guinness Storehouse**

18.00-19.00 **Tour of the Guinness Storehouse**

19.00-20.00 **Lewis B Perry Memorial Lecture in the Arrol Suite, Guinness Storehouse.**
Alan Reilly, Chief Executive, Food Safety Authority of Ireland

20.00-21.00 **Drinks reception and buffet in the Guinness Storehouse**

Tuesday 5 July 2011

Session 1: Pathogen updates, The Great Hall, Clontarf Castle

09.00-09.35 **Verotoxigenic *Escherichia coli***
Chris Low, Scottish Agricultural College, UK

09.35-10.10 ***Campylobacter***
Simon Park, University of Surrey, UK

10.10-10.45 **Foodborne viruses**
Marion Koopmans, National Institute for Public Health and the Environment (RIVM), The Netherlands

10.45-11.15 **Tea and coffee**

11.15-11.50 ***Salmonella***
John Threlfall, HPA, London, UK

11.50-12.25 ***Clostridium botulinum* and foodborne botulism**
Mike Peck, Institute of Food Research (IFR), Norwich, UK

12.25-13.25 **Buffet lunch in the Great Hall foyer and reception**

13.25-14.00 ***Cronobacter* spp.**
Seamus Fanning, University College Dublin, Ireland

14.00-14.35 ***Bacillus***
Niall Logan, Glasgow Caledonian University, UK

Session 2: Epidemiology of foodborne disease

14.35-15.10 **Current challenges to microbial food safety — estimating the global burden of foodborne diseases**
Danilo Lo-Fo-Wong, World Health Organization (WHO), Geneva, Switzerland

15.10-15.45 **Food safety in the European Union: ECDC's role in tracking the burden of disease and trends**
Andrea Ammon, European Centre for Disease Prevention and Control (ECDC), Sweden

15.45-16.00 **Tea and coffee in the Great Hall foyer and reception**

16.00-16.35 **Climate Change and the challenge of new pathogens**
Marion Wooldridge, Veterinary Laboratories Agency, Weybridge, UK

16.35-17.10 **Food safety — the retailer's perspective**
Alec Kyriakides, Sainsburys, UK

17.10-18.10 **Student session**

17.15-19.30 **Trade show with wine, The Great Hall foyer, Clontarf Castle**

19.30-20.30 **Buffet and wine in Indigo Lounge, Clontarf Castle**

20.30 onwards **Quiz night in Indigo Lounge, Clontarf Castle**

Wednesday 6 July 2011

09.00 onwards **Posters**

Session 2: Epidemiology of foodborne disease (continued) The Great Hall, Clontarf Castle

These preliminary programme times and titles were correct at the time of going to press. For the latest programme please visit: ww.sfam.org.uk/summer_conference.php

09.00-09.35 **The threat of antibiotic resistance in the food chain for human health**
Hilde Kruse, WHO, Europe

Session 3: Microbiological risk assessment

09.35-10.10 **Recent global risk assessments and impact on Codex standard setting**
Sarah Cahill, Food and Agricultural Organization (FAO), Rome, Italy

10.10-10.45 **Recent developments in *Campylobacter* risk assessment**
Maarten Nauta, Technical University of Denmark

10.45-11.15 **Tea and coffee in reception**

11.00-12.00 **Attended poster viewing**

12.00-13.00 **Lunch**

13.00-13.35 ***Salmonella* risk assessment in Finland**
Pirkko Tuominen, Finnish Food Safety Authority, Finland

13.35-14.10 ***Listeria* risk in butter**
Phil Voysey, Campden, UK

14.10-15.10 **Student presentations**

15.10-15.30 **Tea and coffee**

SfAM Award Lectures

Chair: President of the Society

15.30-15.35 **Introduction to New Lecturer Research Grant**

15.35-16.10 **SfAM New Lecturers Research Grant Lecture**
Speaker to be confirmed

16.10 -16.15 **Introduction to the WH Pierce Prize**
SfAM President

16.15-16.45 **W H Pierce Prize**

16.45-17.15 **Annual General Meeting**

18.00 **Coaches leave Clontarf Castle to travel to the Jameson Distillery**

18.30 **Delegates taken on a tour of the Jameson Distillery**

19.30 **Tutored whisky tasting session**

19.45-22.00 **Dinner in the Jameson Distillery with Irish entertainment — music and dancing**

22.30 **First coach arrives to take delegates back to Clontarf Castle**

23.00 **Last coach arrives to take delegates back to Clontarf Castle**

Thursday 7 July 2011

Novel technologies to control safety and stability

09.00-09.35 **Novel technologies — overview**
Bala Balasubramaniam, Ohio State University, USA

09.35-10.10 **Pulsed electric fields**
Stefan Toepfl, University of Applied Science, Osnabruck, Germany

10.10-10.45 **Tea and coffee in The Great Hall foyer and reception**

10.45-11.20 **Modified Atmosphere Packaging (MAP) and active packing**
Frank Devlieghere, Ghent University Belgium

11.20-11.55 **High-pressure processing /pressure assisted thermal sterilization (HPP/PATS)**
Alejandro M. Amezquita, Unilever, UK

12.00-13.00 **Lunch and close**

A metagenomics approach to understanding the relationship between the vaginal microbiome, bacterial vaginosis and preterm birth

Derrick Fouts and Karen Nelson at the J. Craig Venter Institute (JCVI) in Rockville, Maryland, have had a long history of working together. Initially they produced a number of papers on genomes of bacterial species that are related to human and environmental health. More recently they have been active in the world of metagenomics having published studies on humans and various animal species. Fouts also works in the field of viral ecology and has developed approaches to the study of different viruses. Most recently they have received a National Institutes of Health (NIH) award along with colleagues Bryan White and Brenda Wilson at the University of Illinois (UIUC), and Doug Creedon at the Mayo Clinic, to study the relationship between bacterial vaginosis (BV) and preterm birth. Specifically, this collaborative research project, led by Bryan White, will fill an important knowledge gap: the relationship between the vaginal microbiome and two significant problems impacting women's health, BV and preterm birth, and their possible linkage to each other.

BV, previously called nonspecific vaginitis or *Gardnerella*-associated vaginitis, is a condition that is estimated to affect 10 to 15% of women of reproductive age. The condition is caused by an imbalance of the naturally occurring vaginal bacterial flora and is associated with genital tract infections, resulting in an increased risk of ectopic pregnancy, pelvic inflammatory disease, chronic pelvic pain, and tubal factor infertility. BV is diagnosed based on positive results of three of four clinical criteria commonly known as "Amsel's criteria": presence of a homogeneous discharge; vaginal pH greater than 4.5; potassium hydroxide test for volatile amines, and the microscopic presence of clue cells, which are vaginal epithelial cells having a stippled appearance due to being covered with bacteria (Amsel *et al.*, 1983). This condition is thought to stem from a shift in the vaginal microbial community from mainly *Lactobacillus* species to a more diverse polymicrobial community although the same microbial species can be found in the vaginal tract of asymptomatic women as well (Josey & Schwebke, 2008). This paradox illustrates a clear gap between the clinical assessment of this condition and our understanding of the pathophysiology of the female genital tract.

This gap in our knowledge is widened when we consider the impact of BV on pregnancy. For pregnant women, BV increases the risk of late miscarriage, preterm labour, and preterm premature rupture of membranes, resulting in preterm delivery (Denney & Culhane, 2009). In addition, the risk of developing chorioamnionitis and endometritis is increased with BV. In the United States alone, preterm delivery is associated with an annual cost to society of $26.2 billion and is the single most significant factor contributing to neonatal morbidity and mortality (Behrman & Butler, 2007). The World Health Organization reported that about 13 million premature births occur globally each year directly causing nearly 28% of all neonatal deaths (1 million each year) (Beck *et al.*, 2009) and overall the world's preterm birth rate has continued to rise. For example, the rate of singleton preterm births has increased by 14% since 1990, and now accounts for 11.1% of deliveries worldwide. In 2006, preterm births constituted 12.8% of live births in the United States, an increase of 20% since 1990.

A recent study demonstrated a role for BV in preterm birth by showing that BV more than doubles the risk of preterm delivery in asymptomatic patients and of preterm labour in patients with symptoms, as well as significantly increasing the risk of late miscarriages and maternal infection (Leitich & Kiss, 2007). Microbial infection may contribute to approximately 25% of preterm births, with bacterial colonization rates as high as 79% for birth at 23 weeks of gestation, declining to 11% at 31 to 34 weeks. Moreover, there are specific associations between various vaginal microbes linked to BV (*Mycoplasma hominis*, *Ureaplasma urealyticum*, *Gardnerella vaginalis* and *Atopobium vaginae*, alone or together) and preterm birth (Donders *et al.*, 2000; Menard *et al.*, 2010). Abnormal vaginal flora has also been associated with shortening of the cervix in pregnancy, a known risk factor for preterm delivery (Donders *et al.*, 2010). It is puzzling that although treatment for BV with metronidazole was shown to clear symptoms and signs of BV (Spiegel *et al.*, 1980), metronidazole treatment was unable to decrease rates of preterm delivery (Andrews *et al.*, 2003). The question remains, then, "*What is the relationship between the vaginal tract microbiome and preterm birth*?"

Culture-based identification of vaginal microbes has provided an incomplete and biased understanding of the vaginal microbiome (Weir, 2004). This approach resulted in a common belief that the vaginal microbiome included only a few microbial species, primarily the "*beneficial*" bacteria from the genus *Lactobacillus*. It was thought that an overgrowth of bacterial species such as *G. vaginalis* in addition to a reduction in the lactobacilli population resulted in BV (Spiegel *et al.*, 1980; Josey & Schwebke, 2008). The advances in modern molecular culture-independent techniques and next-generation sequencing approaches allow us to characterize microbial communities in detail which was previously not possible. These techniques have been applied to survey the human vaginal microbial community (Forney *et al.*, 2010; Ling *et*

al., 2010) and amniotic fluid during preterm labour (DiGiulio *et al.*, 2008). The diversity of species present has exceeded that of classic culture-based methods by several orders of magnitude (Ling *et al.*, 2010). Not only is there incredible diversity of microorganisms present in individual women, but also the vaginal populations in different healthy women are highly varied. A recent study by co-principle investigator (PI) Wilson supports this observation (Kim *et al.*, 2009).

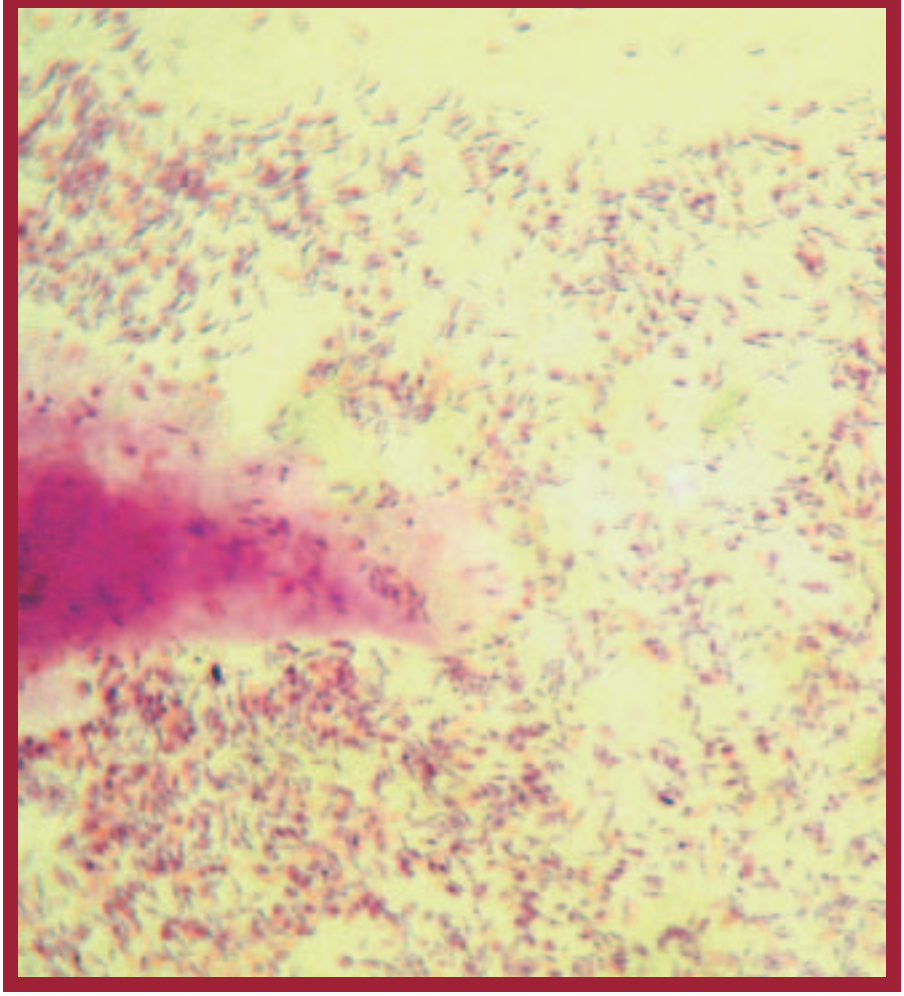
While we still know very little about how the vaginal microbiome is impacted by age, menstrual cycle, genetic background or other factors in the daily lives of women, demonstration projects and consortium sequencing through the NIH funded Human Microbiome Project (HMP; http://nihroadmap.nih.gov/hmp/fundedresearch.asp) will provide important microbiome and metagenome baseline data. In addition, the PIs (White and Wilson) of the NIH award at the UIUC are supplying vaginal isolates to the JCVI for reference genome sequencing under the HMP U54 mechanism, and this has resulted in the release and publication of the complete genome of *G. vaginalis* 409-05 (Yeoman *et al.*, 2010). Reference genome sequencing is in progress at the JCVI for at least nine other vaginal isolates from genera other than *Gardnerella*. As such additional biological, technological, and reference genome resources are now available, enabling the application of translational biomedical approaches based on genomics, metagenomics and computational biology to improve women's reproductive health.

All previous culture-independent studies of the vaginal microbiome focused only on the prokaryotic community by sequencing the 16S rRNA gene (rDNA), neglecting the other potential key players: bacteriophage, viruses and fungi (Fredricks *et al.*, 2005; Zhou *et al.*, 2007). In addition, none of these investigations looked at the metabolic potential (functional gene content) of the vaginal microbiome, which we will investigate in our study, as has been done in other environmental systems (Brulc *et al.*, 2009). We will use high throughput genomic technologies (454 and Illumina) to survey the fungal, bacterial, phage and viral microbiomes as they relate to BV and preterm birth. Microbial and gene predictions (annotation and construction of genomes based on microbial reference genomes from the HMP) will be used to test the hypothesis that a defined set of microbial taxa or gene(s) can be predictors (quantitative trait loci or QTLs) of increased risk and incidence of preterm birth. These microbial genetic markers will lead to new and rapid diagnostics that will ultimately provide medical professionals with predictors of risk for these clinical outcomes that have profound impacts on women's health.

Specifically, we will use deep, tag-based 454 sequencing of the V1-V3 hypervariable regions of the bacterial 16S rDNA and the internal transcribed spacer (ITS) region of fungal rDNA, and metagenomic sequencing of viral/bacteriophage genomes to assess the structure of the vaginal microbiome and associations of specific taxonomic groups with BV and preterm birth. In addition, we will use a combination of 454 and Illumina metagenomic sequencing, whole genome assemblies from metagenome sequencing and comparative analyses to determine the metabolic potential of non-BV versus BV-associated vaginal microbiomes and of vaginal microbiomes associated with preterm birth. This will allow us to define the identifiable microbiome taxa or gene(s) that are linked to an increased risk and incidence of BV and preterm birth.

Our overall approach will provide a tremendous amount of new information about the factors that have the greatest impacts on vaginal microbiome dynamics and provide critical data on risk factors and how changes in the vaginal ecosystem accompany or predict disease development and progression. Ultimately, we envision these studies leading to personalized (genomic) medical diagnostics that would be capable of predicting the risk for these clinical outcomes, which impact women's health.

Gram-stained vaginal smear from a case of BV showing small mobiluncus



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Derrick E. Fouts

Bryan A. White

Karen E. Nelson

The Gut microbiome of *Drosophila melanogaster*

In the second of our series of articles on microbiomes, **Ed Yong** reports on a paper describing the effect of gut bacteria composition on the sexual preference of fruit flies. Ed Yong is an award-winning science writer who writes the blog **Not Exactly Rocket Science**. This article first appeared on his blog on 1 November 2010

Imagine taking a course of antibiotics and suddenly finding that your sexual preferences have changed. Individuals who you once found attractive no longer have that special allure. That may sound far-fetched, but some fruit flies at Tel Aviv University have just gone through that very experience. They're part of some fascinating experiments by Gil Sharon, who has shown that the bacteria inside the flies' guts can actually shape their sexual choices.

The guts of all kinds of animals, from flies to humans, are laden with bacteria and other microscopic passengers. This 'microbiome' acts as a hidden organ. It includes trillions of genes that outnumber those of their hosts by hundreds of times. They affect our health, influencing the risk of obesity and chronic diseases. They affect our digestion, by breaking down chemicals in our food that we wouldn't normally be able to process. And, at least in flies, they can alter sexual preferences, perhaps even contributing to the rise of new species.

Sharon was inspired by experiments by Diane Dodd, who raised two strains of fruit flies on different diets, and found that after 25 generations, their menus had affected their sex lives. Those reared on a menu of starch preferred to mate with other 'starch flies', while those reared on maltose had a bias towards 'maltose flies'. These results were odd. Dodd had set up an artificial evolutionary pressure for diet but somehow, the flies' mating habits had changed as well.

To work out why, Sharon repeated Dodd's experiment with the fly *Drosophila melanogaster*, and raised two strains on

either molasses or starch. After just two generations, he found the same effect that Dodd did: the flies were more attracted to individuals reared on the same diets. Something in their food was changing their behaviour.

Bacteria in our guts change according to the food that we eat, so Sharon suspected that something similar was happening in the flies. This idea was dramatically confirmed when he gave the insects a dose of antibiotics. Immediately, their sexual bias disappeared and they were just as likely to mate with flies from either group.

As further evidence, Sharon isolated bacteria from the food that the flies had eaten and added them to vials of sterile food. When the antibiotic-treated flies ate this food, laced with a drizzle of bacteria, they regained their sexual preferences *after a single generation*. Those that ate food containing 'starch bacteria' preferred to mate with starch flies, and those that ate food containing 'molasses bacteria' preferred to mate with molasses flies.

Many bacteria are probably responsible for this effect but Sharon singled out *Lactobacillus plantarum* for special attention. It's particularly common in starch-based food and while every molasses fly harbours around 2,600 of these bugs, starch flies contain around 23,000 apiece. After a dose of antibiotics, flies that are infected with *this bacterium alone* take a fancy to starch flies over molasses flies.

It's possible that the bacteria influence the levels of sex pheromones that affect the fly's attractiveness, either by producing those chemicals themselves or stimulating the fly to do the same. That's not too far-fetched: bacteria can alter the smells given off by many animals, and smell certainly affects sexual behaviour. Indeed, Sharon found five different chemicals that are present at different amounts on the outer shells of starch and molasses flies. Antibiotics brought the levels of these chemicals down to similar levels. For a fruit fly, these seem to be the smells that make the differences between striking lucky and getting rejected.

These experiments could have important implications for the origin of new species. Imagine that two wild populations of the same species mate with each other less and less often, because they start to harbour different bacteria (just as the starch and molasses flies did). The stronger these preferences, the greater the odds that these populations will diverge into separate species.

Diet, of course, is just one thing that affects the membership of the bacterial club in our bodies, so it's unlikely that a species would split in two just because two groups started eating different foods. However, Sharon speculates that diverse diets could intensify the effects of other barriers that restrict the flow of genes, such as physical obstacles.

In any case, the study suggests that you can't understand an animal's evolution simply by considering the evolutionary pressures that act on its genome. You also have to consider the genes of the bacteria and other passengers that live inside it, which also create variations in its behaviour and affect its chances of survival. Sharon calls this the hologenome — the combined genes of a host and all the microbes it contains.

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Ed Yong

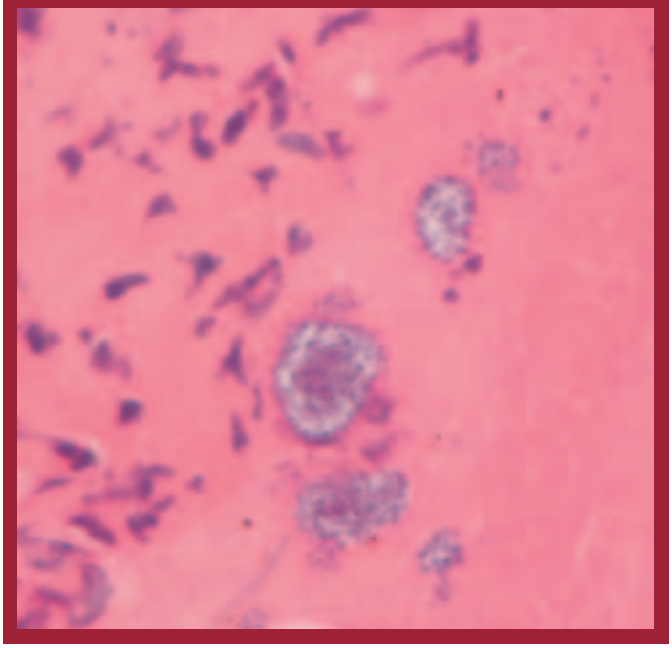
Microbiology of bird nest boxes

Ten day-old great tit (Parus major) sits in a typical wooden nest box

Humans have long had a special relationship with birds. One example of this, building artificial nesting sites, has taken place for centuries. Initially, many of these early nest boxes provided an easy way to obtain food. For example, the majority of Norman castles had nest chambers to attract breeding pigeons, and artificial nests sites constructed to attract wildfowl and facilitate egg collection were used extensively by the Lapps in the Middle Ages. Times have changed however, and the nature of our relationship with wild birds has shifted. These days, nest boxes are usually put up for the conservation of rare, declining, or ecologically-important species, as well as to provide ecological interest in sites such as gardens and schools. Indeed, the increasing availability of inexpensive nest box cameras has brought the private world of the bird nest into our living rooms.

Numerous species will breed successfully in the relatively safe and convenient nesting site that a nest box provides. Common European nest box-occupying species include tits (including that perennial favourite, the blue tit), nuthatches, treecreepers and flycatchers, while typical American residents include tree swallows and eastern bluebirds. Many of these birds use nest boxes in preference to natural sites, as their solid construction means that they offer better protection from weather and predators and breeding success is usually higher. The propensity of birds to use nest boxes, the ability for boxes to be used year after year, and the fact that it is seemingly advantageous for birds to nest in them, has resulted in nest boxes being used to study aspects of avian reproduction and breeding behaviour. Indeed, some of the most prominent post-war ornithologists, such as David Lack, Lars von Haartman and Huijbert Kluijver studied birds in nest boxes to provide many of the principles of avian reproduction that still underpin what we know today. However, until very recently, only the target nest box occupants (the birds) were considered worthy of research. Other, rather less visible nest box occupants, including nest box microbes, had been largely ignored, despite the likelihood that they could interact with,

Staphylococcus hyicus

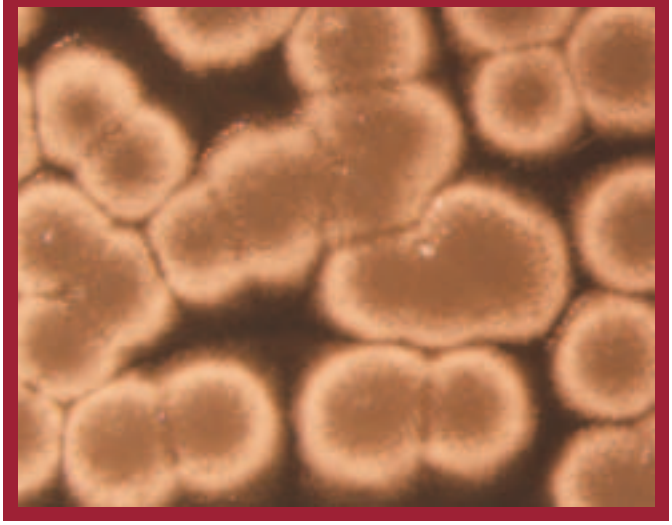


and influence the success of, the feathered occupants.

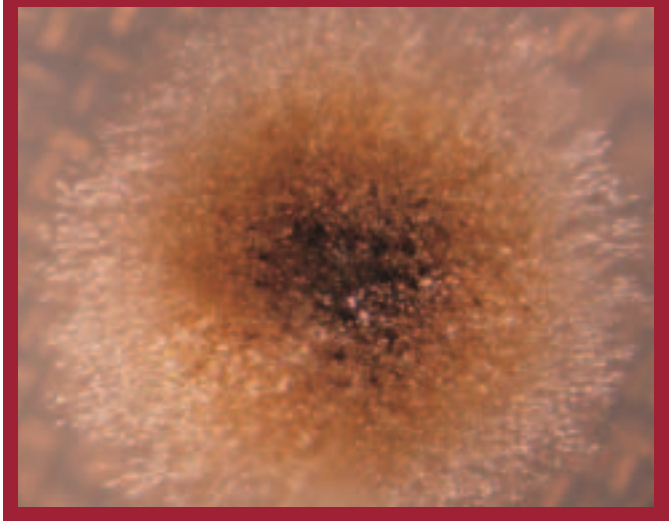
Over the past decade the importance of bird-microbe interactions has started to gain recognition by ornithologists and microbiologists alike. We now know that birds have species-specific cloacal bacterial communities (Maul *et al.*, 2005) (the cloaca, from the Latin word for sewer, is the single opening for the intestine, genital and urinary tracts in birds), distinctive feather bacteria that influence plumage colouring (Shawkey *et al.*, 2007) and gut bacteria that differ according to diet (Blanco *et al.*, 2006). Together, these studies indicate that, as might be expected, bird-microbe relationships are complex. However, comparatively few studies have examined the relationship between microbes and wild birds during one of their most important times of the year — the breeding season. In fact, not only do we know little about the relationships between microbes and birds (both parents and chicks) during the breeding season, we also know very little about what bacteria and fungi actually occur within nests. This is, therefore, an example of where seemingly '*basic*' information about otherwise well-characterized natural systems is missing.

To try to fill at least part of this knowledge gap, we have been involved in a recent series of projects focusing on bacteria and fungi in the nest boxes of small songbirds (blue tits, great tits, nuthatches and pied flycatchers), breeding at the Nagshead RSPB reserve in the Forest of Dean (Gloucestershire, UK). These projects, carried out over the last four years and still ongoing, involve swabbing nesting material and the nest box itself with sterile swabs to sample the microbial communities present. Laboratory analysis is used to reveal which microbial species inhabit bird nesting environments, together with their abundance. Results are related to biological parameters, such as the bird species, the number of chicks and relative fledging success (the proportion of chicks to survive until they are fully feathered and can fly), as well as environmental parameters, such as distance to the nearest water source.

***Pseudomonas veronii* colonies taken at 10x magnification**



***Cladosporium cladosporioides* 24hr colony taken at 10x magnification**



We use a multifaceted approach to identify microbial isolates. Fungal isolates are typically identified using macroscopic and microscopic characteristics. Identification of bacteria is more involved, usually combining biochemical characteristics (such as ability to hydrolyse gelatin and UV fluorescence) with Fatty Acid Methyl Ester (FAME) analysis. Some cryptic bacterial isolates are identified using DNA sequencing, following DNA extraction, amplification of the 16S rRNA gene, electrophoresis, and purification.

The results that we have obtained thus far are striking for two reasons. Firstly, we have been surprised how high the microbial loads of nesting material and nest boxes are: for example, a typical 30 second swab of great tit nesting material, extracted from nest boxes immediately after the young fledged, yields around 2.5 billion CFUs. The microbial load of blue tit nesting material is even higher at about 4.4 billion CFUs. A 30 second swab of the nest box itself typically yields 15 million CFUs before the breeding season, a number that increases to an average 120 million CFUs in occupied boxes (and 60 million in unoccupied boxes) in June.

The second striking thing is the sheer diversity of bacteria and fungi that are supported within nest boxes. In total, we have cultured 35 bacterial species (in 10 different genera) and 13 fungal species (in nine different genera) from nest material swabs and nest box swabs over the last four years. The bacterial community is typically dominated by *Pseudomonas* species (particularly *Ps. putida*, *Ps. fluorescens* and *Ps. agarici*), with *Bacillus* and *Staphylococcus* also being abundant. The fungal community is typically dominated by *Cladosporium*, with *Eupenicillium* being abundant in the winter (but strikingly absent in the breeding season) and *Epicoccum* species being abundant in the breeding season (but only rare during the winter). Bacterial colonies typically outnumber fungal colonies three-to-one during the breeding season, but fungi outnumber bacteria about two-to-one during the winter. There are other patterns in our data, many of which are not straightforward to interpret. For example, the abundance of actinomycetes is significantly higher immediately after the breeding season than immediately before the breeding season when the same nest boxes are sampled, while the abundance of *Mucor*, *Chrysosporium*, and *Aspergillus* fungi typically decreases. Overall, our findings suggest that there are important seasonal changes in the microbes associated with birds, which could be due to the obvious environmental differences between seasons (primarily ambient temperature), or by more subtle differences caused by the birds themselves. Boxes in the winter will either be unoccupied or only used as cold-night roosts, whereas most of those in the summer are used for breeding.

Our work has recorded four genera, and numerous individual species, occurring in bird nests for the first time. In fact, almost half of the species we have recorded have not previously been documented in this environment. Previously unrecorded bacteria include *Paenibacillus* and *Roseomonas* (new genera) and *Pseudomonas fluorescens*, *Ps. putida*, *Ps. agarici*, *Bacillus circulans* and *Staphylococcus lentus* (new species that occur in high numbers in our studies). Previously unrecorded fungi include *Epicoccum* and *Eupenicillium* (new genera, which are abundant in our studies) and *Microsporum gallinae* (new species).

We have been particularly interested to find numerous keratinolytic microbial species in the nesting material and nest box environments. These species have, as their name suggests, the ability to decompose keratin. Feathers are composed mainly of keratin therefore the presence of keratinolytic microbes is potentially important. These microbes are often referred to as feather degrading bacteria, although since fungi can also be keratinolytic, feather degrading microbes is perhaps a more accurate term. The main feather degrading microbes that we have found include *Pseudomonas stutzeri* and *Bacillus subtilis* (bacteria) and *Chrysosporium tropicum* and *Microsporum gallinae* (fungi). High loads of these types of microbes might cause feather degradation in parent birds, particularly those that incubate eggs and brood young, and reduce the ability of developing chicks to grow high-quality feathers. Although it is not clear whether the birds transfer these microbes to the nest material or vice versa, it is worth noting that feather degrading fungi occur in most of the empty/unoccupied nest boxes that we have tested.

We have also been surprised by the number of potentially-pathogenic species that we have isolated from nest boxes. The

most prevalent pathogenic bacteria are typically *Staphylococcus hyicus* and *Enterobacter cloacae* with the former causing conjunctivitis in birds, and the latter being a common recorded gut pathogen of certain ducks (Aguirre *et al.*, 1992; Silvanose *et al.*, 2001). Common pathogenic fungi include *Microsporum gallinae*, which can cause ringworm in poultry (Droual *et al.*, 1991), *Candida albicans*, which can cause candidiasis, and *Aspergillus flavus*, an important cause of avian aspergillosis (Wobester, 1981).

Some species have only (or mainly) been found on the wood of the nest box rather than in the nesting material. These include the bacteria *Eupenicillium javanicum* and *Yersinia* spp., and the fungus *Epicoccum purpurascens*. These are all associated primarily with wood and suggest that the nest box itself is an important source of microbes, some of which could potentially be pathogenic. This is further suggested by the fact that a substantial microbial load is present even before the breeding season begins. Indeed, work done over the winter suggests that the microbial load is high all year-round, particularly for fungi, with high numbers of *Cladosporium* and *Eupenicillium* being found during November/December. The distance to the nearest water source (a key factor determining the internal humidity of the nest box), does not appear to affect overall microbial load. However, one species, the fungus *Eupenicillium javanicum*, occurs in substantially-higher numbers (more than an order of magnitude) in boxes within 200m of a permanent water source. Within the breeding season, nest mass does not appear to influence overall nest microbial load, although *Staphylococcus lentus*, which is often isolated from sheep, is typically only found in nests containing sheep's wool. Further research on how the nesting environment shapes its microbial community is needed.

Interestingly, the overall microbial load of nest boxes before nesting starts does not appear to influence whether that box is used by a bird or not, nor the species of bird that occupies it. In other words, it does not appear that nest box microbial load influences the choice of nest site (an important aspect of ecology) in our study populations, although further work is still needed in this area. The overall microbial load does not appear to be influenced by the number of young and it does not appear to affect the number of young to successfully fledge. There might, however, be more subtle influences of microbes on the condition of the young (or their parents) and this could be worth investigating. For example, a '*highly-infected*' box might not affect how many chicks fledge, but it might influence how robust those chicks are as adults, their chances of surviving the winter, their attractiveness as mates, or their fertility. Determining the link between presence of various microbes and their effect on birds is clearly the next important step in this research.

The microscopic nest box occupants discussed in this article were hidden to those who first constructed nesting sites in Norman castles and during the Middle Ages. Now, however, with increasing access to high-powered microscopes, fatty acid profiling, DNA extraction and sequencing, and a whole host of biochemical analyses, this hidden world is starting to give up its secrets. Avian biologists are therefore starting to consider what Burtt (1999) termed the ornithlogical "*frontier of the small*": the results that we have so far are intriguing, and are clearly just the start of a fascinating orni-microbiological journey.

Nest box swab

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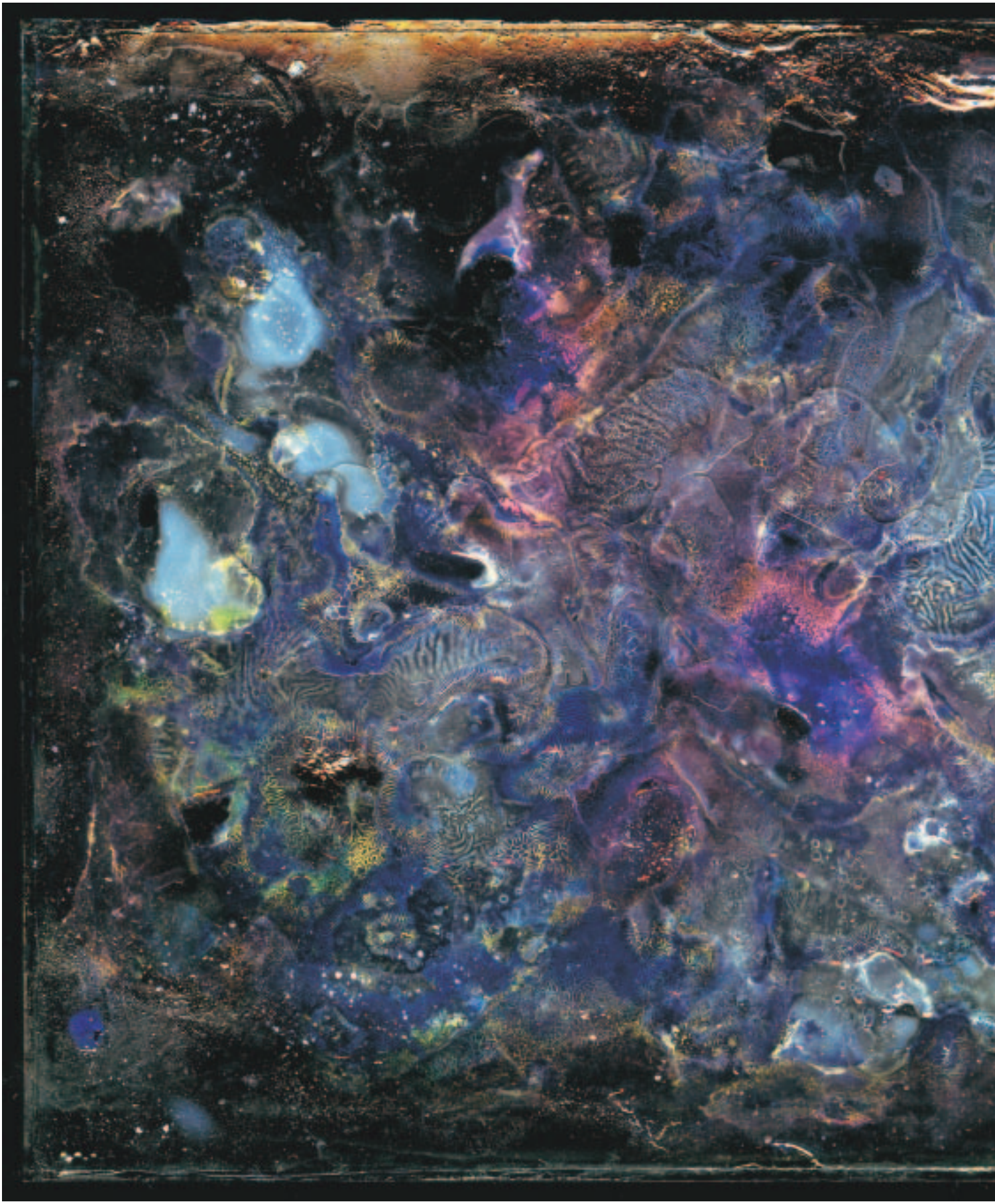


Violet Surface ■ image © Caleb Charland 2009

Bacterial artists

Beauty can appear in strange places, as contemporary photographer **Caleb Charland** knows well. But how did he discover the artistic power of microbes? **Lucy Goodchild** finds out

Sometimes you can find beauty in the most unexpected things, in the most unexpected ways. Everybody has seen a beautiful photograph, for example, but the real surprise comes when a photograph reveals a new, unfamiliar beauty in something you thought you knew. Like bacteria.

There is something familiar yet very alien about the images on contemporary photographer Caleb Charland's website. Perhaps it is this surprising beauty in objects we take for granted, like a pen, a fan or a drill, or the movement of time in the still images. "*I've had some interesting reactions*," says Caleb. "*People really respond to the everyday objects photographed in new ways — everyone has had those experiences. It excites people*."

Caleb grew up in rural Maine, USA, and helping his father work on their house had a big impact on his art, as he explains: "*I used to help my Dad a lot, he would take vacations to work on our house. When I was seven or eight we doubled the size of our house in one summer — it was amazing to watch my Dad do that. Helping him, getting in the middle of it, being outside in the sun in July really gave me the freedom to use materials and express myself. Those experiences have since allowed me to be experimental and scientific about creating images*."

Even a brief look at his photographs shows that Caleb is passionate about science. But he wasn't always interested in the field. "*I didn't even take physics in high school! I came to it later. I finished my undergrad degree in 2004 and moved home to Maine. I wasn't ready to go to grad school, so I enrolled on a two year program to become an x-ray technician. We studied things like biology and algebra; it was interesting because I hadn't really used the more logical parts of my brain since high school. Being exposed to science again really got my brain going*."

After that, Caleb started looking at children's books of science experiments. "*I think they're fun, fascinating fodder for creativity,"* he says. *"I always ask the question 'Is that possible?' — that's the whole point for me, to use photography to look at the world. A lot of the work is about creating forms out of light. For me it's the whole point of photography, the recording of light in time. Only with photography can you express that in one visual plane*."

Although it is clearly inspired by science, Caleb says his work is more illustrative than scientific. "*I guess I've been lucky and found this grey area between art and science, where both are still fun and hopefully not too pretentious*."

One of his projects, 'BioGraphs', definitely sits neatly between art and science. "*I made that work in my first semester of grad school, in Fall 2008, when I had a Trustees Fellowship at the School of the Art Institute of Chicago. I had been making black and white photographs for a while, based on*

Violet Surface detail ■ image © Caleb Charland 2009

simple physics experiments, but going to grad school you want to start over. I wanted to make something new. On the drive from Maine to Chicago, I was thinking about new projects. I had done the black and white work in physics, and I thought next time it should be biology."

Caleb remembers his time studying biology, "*like when you can swab your mouth and coat the petri dish and watch the grossness grow*". And that's exactly what he did. After Googling around to work out how to make an agar plate, Caleb went shopping for film, agar and the secret ingredient — raw chicken. He then coated the photographic film in agar and smeared it with the raw chicken, before placing the whole lot on a plastic plate inside a black bag for a couple of weeks. Which begs the question did he get sick?

"*I did not actually*," he says proudly. "*In my job I have to be pretty safe*

with chemicals, so I'd always wear rubber gloves. And I used to work in mental healthcare, where you have to be clean. The plates did smell pretty funky though."

The physical process, says Caleb, was "*kind of by accident*". "*I use science experiments for the basis of my photographs, but I don't follow them step by step, as that produces non-interesting images. Researching how things work and how people approach experiments definitely informs my image making process. In this case, the bacteria were making their own drawings. I kind of just set it up and they did the work.*"

Fascinated by the notion that the bacteria were moving the silver on the photographic film, Caleb began to control the process, sometimes letting several batches of bacteria grow on one plate, one after the other. "*I just assumed they ate all the food that was available on the film and died, but maybe the silver killed them. We'd need to know all the facts to be scientific about it. I noticed that around ten days to two weeks was the end point, there would be no more growth or change in the image patterns after that.*"

After the bacteria had stopped growing, Caleb took high resolution scans of the plates, positive, negative and reflective. "*There was a topography to the bacteria on the films, which you sort of lose in the 2D scans. With the reflective scans, you get more of a sense of the topography, you can see highlights. I was giving myself as many options as I could visually. Whatever it takes for me to get an image, I guess.*"

The images are not instantly recognizable, instead hinting at different natural phenomena, such as sand in a delta or images of the universe. "*I'm still amazed that it worked and that it translated into such a fascinating visual field," he says. "When they're actually shown in a gallery, the photos are pretty big — 30 by 40 inches. The thing about images at that scale is you can really get down to the grain of the film and see the detail.*"

Is there more bacterial art on the horizon? "*It was fun, but after a while I felt like it ran its course (no pun intended). I'm now starting to step in front of the camera to embrace and explore the landscape a bit. It's definitely more performative. I made an image using my arm as a compass in the dark, holding a cigarette lighter. I made arcs from my hip straight over my head, leaving arcs of flame on the film, like lines of longitude on a globe. And when we had a big blizzard here last winter, I got a spotlight and pushed back the night. I'm trying to keep it interesting and fun. I don't know where the next idea's going to come from. You know what they say, it's one percent inspiration, ninety-nine percent perspiration.*"

A Picture Of Grey Eaten By Bacteria
■ image © Caleb Charland 2009

Caleb focuses on expressing a sense of wonder, which he sees as lying somewhere between knowledge and uncertainty. A scientist strives to achieve certainty, to gain knowledge, while recognizing the uncertainties they encounter. For Caleb, though, the art is in the wonder. "*It comes from being a viewer of artwork. If you're looking at a painting or photograph, every image proposes a question. As a viewer you're caught in this in-between space that you didn't create — you're stuck in that spot of wonder. With really good work you never leave that space, you're always uncertain about it. It should be interesting and make you think about the world differently forever.*"

The sense of wonder is what drives the images, as well as what they provide the viewer. "*That goes back to me questioning what would this look like — I wonder. I don't know where you go, but you leave the world for a second, you try to figure out what it is. You're focused on the beauty, not on work or anything else.*"

Lucy Goodchild

Figure 1. *Black stem rust on wheat, showing asexual (uredial) infections on stems (Photo by Yue Jin, USDA-ARS)*

Figure 2. *Black stem rust on Berberis vulgaris, showing the aecidial structures arising from sexual fusion (Photo by the author)*

historical**Perspectives**

Black stem rust

"…two blighted ears of wheat, which few persons would have thought it worthwhile to carry with them round the world…"

Charles Darwin, exploring La Plata in Argentina in 1833, encountered fields of wheat destroyed by "*Polvillo*", saying "*This blight is a prodigious evil to the country*..." He collected samples and returned them to J. S. Henslow at Cambridge, who was delighted, identifying them as afflicted by a common scourge of European wheat, black stem rust (Figure 1) and praising Darwin's acumen with the comment that forms the subtitle of this article (Henslow, 1844).

In the last 50 years or so, black stem rust has become a minor pathogen, until the variant known colloquially as Ug99 appeared. With luck, our response to Ug99 will represent a problem caught, understood and managed before it becomes again a prodigious evil. Without luck and investment, the pathogen could dramatically reduce wheat yields in many parts of the world, bringing price rises, food shortage and terrible hardship for poorer people. But the fungal diseases known as rusts have been a part of farmers' lives since the cultivation of cereals began, just as they are part of the natural forces shaping vegetation all over the world. Other strains of the same fungus will be found in most gardens, on a range of wild grasses. So what is special about black stem rust?

Rusts represent a very highly specialized group of basidiomycete fungi — distant relatives of mushrooms. They are obligate parasites, drawing nourishment from haustoria (a portion of a parasitic fungus, or root of a parasitic plant that draws nutrients from host cells) expanded inside the cytoplasm of host plant cells, ramifying within host tissues, and reproducing by production of tough spores. These spores are desiccation and UV resistant, with dimensions of the order of 10μm. The difficulty of evading plant immune defences leads to extreme specialization, with single strains generally only able to infect a small range of genotypes of a single plant host species. The ecological problem of survival when no host tissue is available has been overcome both by the evolution of very tough and long-lived spores, and by the most extraordinary adaptation of the group, which is at once marvellous and a nightmare to teach: the ability to infect two quite unrelated hosts, with a succession of up to five different spore types. Often one host is a short-lived annual, on which multiplication is rapid, repeated and via asexual spores; the other host is often a long-lived perennial, within which the sexual cycle is completed. In the case of black stem rust of grasses, the alternate hosts are species of the genus Berberis including *Berberis. vulgaris*, Barberry (Figure 2).

The general class of fungi to which black stem rust belongs can be identified in writings from classical Rome and Greece, and their importance is indicated by the cult of a goddess, Rubigo, believed to control a vaguely described blight of the wheat. St Augustine charmingly uses the existence of this cult to undermine pagan religion, arguing that it is quite illogical to worship both this goddess and a goddess of happiness, since if you were happy, why worry about your crops, which are only a tool towards happiness (St Augustine, 426 [1998]: Book 4, ch 21)?

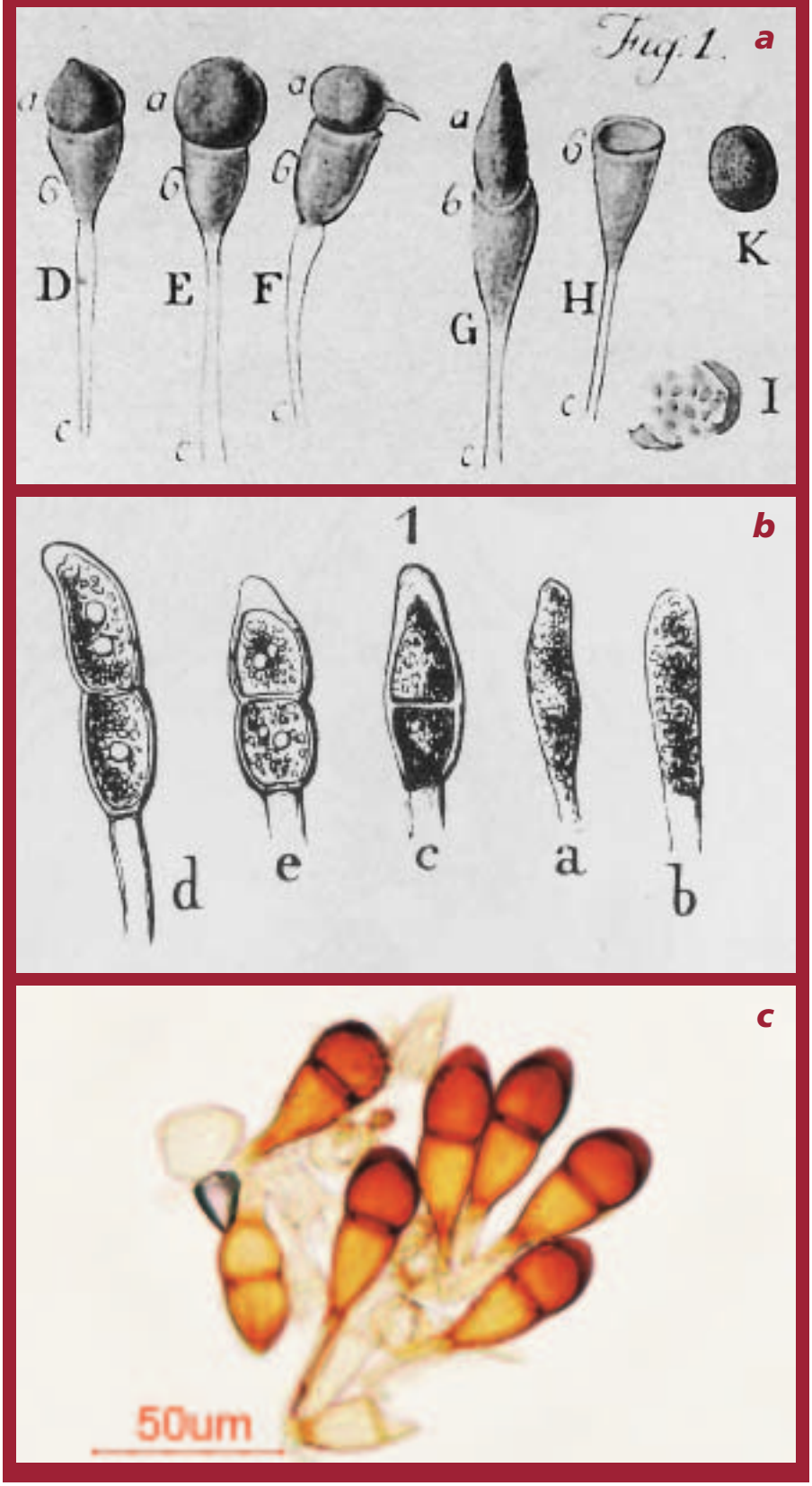
Rusts seem to have certainly coexisted with wheat cultivation for the whole history of agriculture. There must have been folk wisdom and observations within farming communities, or features of the farming system, which made obvious damage by rust sporadic or minimal; as with almost all features of pre-modern farming, we have no way of finding out the details of this knowledge. Certainly poor fertility, mixed cropping, and crops consisting of the unconsciously selected survivors of previous seasons would all combine to reduce the identifiable impact of rust.

By the early 18th century, rust had become an identifiable subject of study. A very severe attack on Tuscan wheat in 1766 seems to have prompted both Felice Fontana, Director of the Physical Museum in Florence, and Giovanni Targioni Tozzetti, Professor of Botany in Florence, to publish their observations (Fontana, 1767 [1932]; Targioni Tozzetti, 1767 [1952]). Targioni also reviewed previous opinion, giving a glimpse into the wide network of discussion, observation, obsession and crankiness out of which understanding arose. By this period, the necessity of original observation and experiment was well established; but the problem that faced natural philosophers was to know of which observations to take notice. There was a large body of empirical evidence that (severe) rust came following weather with hot days and heavy dews, and this was what farmers told their aristocratic enquirers. Without any paradigm of microbial life — and working with a strict

division of living things into animals, plants and people — most observers concluded that the rust was a disorder of plant growth. The growing sophistication of microscopes made it possible to examine in more detail the rusted portions of plants, and both observers argued that the visible rust, which we know to be masses of spores, was composed of microscopic plants rooted into the wheat, and linking them explicitly to parasitic plants like mistletoes and dodders (Figure 3). Not a bad description as it happens, though the roots (mycelium) were quite invisible. However, this analogy led Fontana to search in crushed spores for the "*true seeds*" of the rusts, rather than realizing that he had the seeds. Furthermore, in a climate which took spontaneous generation of life more or less for granted, there was less necessity to ask the question which to us is obvious, "*where did the 'seeds' come from*?" Neither the framework nor the tools of experimental design were present in the discourse of the time to make much more advance and as late as 1837 John Lindley wrote of black stem rust spores "*…if it is really anything more than the diseased tissue*" (Peterson, 2001).

At the same time, farmers continued to make useful empirical observations, and both botany and medicine advanced. In particular, in certain areas, some farmers became convinced of a link between early attack by stem rust and the European native prickly hedging plant, *B. vulgaris*. There are 18th and even 17th century ordinances from several countries attempting to ban its use or planting. Experiments showing infection from *Berberis* to wheat were actually undertaken in England in 1805 (Peterson, 2001) and in Sweden in 1818 (Pritchard, 1911). It would be fascinating to determine why these experiments were forgotten in the academic world. Was it for reasons of social class, language barriers or mental preparedness, or was it that propagation of an idea is analogous to an epidemic and most epidemic chains die out in the early stages by pure chance? Why farmers' own intuitions were ignored and *Berberis* continued to be planted for so long is more curious; a partial answer may lie in the multiple uses of the bush in a mixed farming system, for the edible berries and as stock-proof hedging. A moderate rust attack leaves

Figure 3. *(a) Fontana's 1767 interpretation of teliospores, as "acorns in a cup". Note the crushed distal cell, with the contents interpreted as "seeds". (b) de Bary's 1854 interpretation as developing 2-celled spores. (c) Teliospores photographed with a modern microscope (Creative Commons Attribution: Taken by Cesar Calderon, Organization: USDA APHIS PPQ)*



more harvestable wheat than will a herd of cows.

By the mid 19th century microscopes, stains, fixatives and knives, social contacts and university structures, scientific journals, and accumulated success had transformed what could be undertaken. The energy and accomplishment of outstanding individuals is particularly noteworthy. Anton de Bary published his major synthesis of previous studies of rust fungi (de Bary, 1853 [1969]) with his own observations in 1854, the year after he graduated in medicine at the age of 23. This book was powerfully argued and extremely influential; in particular, it threw weight behind the increasing disbelief in spontaneous generation. It presented clear evidence that rust spores were indeed spores, which germinated in water to produce mycelium, and which could be infectious agents. By this time, the intellectual understructure was probably ready for such a synthesis.

However, it is intriguing that de Bary both mentioned that country-folk believed there was a connection between *Berberis* and rust of wheat and argued strongly against such a connection (de Bary, 1853 [1969]: p72, footnote 1). Twelve years later, however, de Bary (by now famous and surrounded by research students), published experimental proof, accompanied by microscopic details, that the germinated products of the black spores (teliospores) (Figure 3) could infect Barberry and produced a succession of structures culminating in spores which were unable to infect Barberry.

The impact of this discovery, thanks perhaps to de Bary's prestige, was swift. In 1866 M. J. Berkeley summarized de Bary's conclusions in the widely read British journal *Gardeners' Chronicle and Agricultural Gazette* (Berkeley, 1866), stating that the issue had been "*much debated*". Removal of Barberry hedges seems to have followed quite quickly: legislation to remove them followed in Denmark in 1903 and by 1923 the eminent US pathologist E. C. Stakman was arguing for elimination of Barberry in the wheat-growing areas of the US on the basis that it had hugely reduced damage from stem rust in England (Stakman, 1923). Damage from the disease in Europe has continued to decrease: I have never seen it on wheat in the wild. In the US, a federally funded programme continued through the 1920s, with spends of around $400K/yr (farmworkers' wages were around $800/year). As part of the new deal, nearly 3000 people were employed in the mid 1930s to search for and destroy remaining bushes, with a budget in the millions of dollars (Campbell & Long, 2001).

The result of this removal of the alternate host was to greatly reduce both the potential over-wintering sites for the rust, and turn the population of rust in much of the world from a sexual one to a clonal one (Kolmer *et al.*, 1991). In turn, this facilitated breeding efforts and led to success in producing wheat varieties with "*stacked*" (multiple) resistance genes — each detecting some aspect or side effect of the invasion process and triggering a defensive reaction of the plant. With an asexual pathogen population, the theory is that there is no evolutionary route to combine the necessary changes to enable infection. Varieties based on gene combinations from the international centre for improvement of maize and wheat in Mexico (CIMMYT [Centro Internacional de Mejoramiento de Maíz y Trigo]) are grown all over the warmer parts of the world, and for the past 40 years or so, black stem rust has been a sporadic shadow (Singh *et al.*, 2006). But in Uganda in 1999, the pathogen again destroyed crops. The strain responsible was Ug99, which had by some means combined genetic changes allowing it to attack all currently grown high-yielding wheat varieties. The epidemic is unfolding; if breeding and multiplication of resistant varieties is too slow, the results could be as lethal as a contagious disease of humans.

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Michael Shaw

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

multiple regression

StatNote 24

In previous StatNotes (Hilton & Armstrong, 2008a, 2008b, 2009a, 2009b), the application of correlation and regression methods to the analysis of two variables (X,Y) was described. These methods can be used to determine whether there is a linear relationship between the two variables, whether the relationship is positive or negative, to test the degree of significance of the linear relationship, and to obtain an equation relating Y to X. This StatNote extends the methods of linear correlation and regression to situations where there are two or more X variables, i.e., 'multiple linear regression'.

As in previous StatNotes, the variables under study are referred to as Y the 'dependent', 'outcome', or 'response' variable and X the 'independent', 'predictor', or 'explanatory' variable. Multiple linear regression determines the linear relationship between one dependent variable (Y) and 'multiple' independent variables (X_1, X_2, X_3 etc.). Multiple regression analysis has many uses. First, it enables a linear equation involving the X variables to be constructed that predicts Y, e.g., it may be useful to predict bacterial biomass under a set of conditions specified by a series of X variables, such as pH, temperature, and amount of nutrient medium. Second, given several possible X variables that could potentially be related to Y, an investigator may wish to select a subset of the X variables that gives the best linear prediction equation. Third, an investigator may wish to determine which of a group of X variables are actually related to Y and to rank them in order of importance. For example, an investigator may wish to determine which culture variables, e.g., pH, temperature, nutrient availability, etc. are most closely related to the growth of a bacterial culture and in which order of importance. Multiple regression is most useful, however, in deciding whether there are any significant variables influencing Y and therefore, should be thought of as an

Figure 1. *Multiple regression with two independent (X) variables influencing the dependent variable (Y). With two X variables, the position of any point (A) is described in three-dimensional space by three co-ordinates (x_1, x_2, y)*

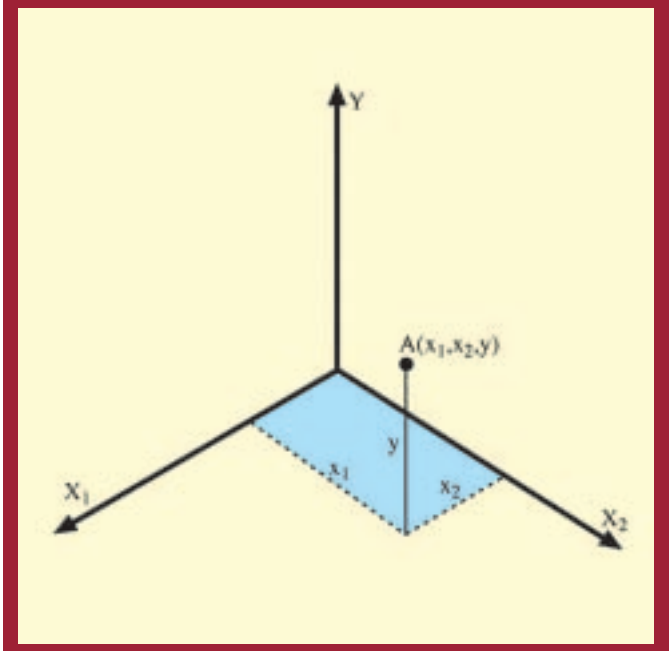
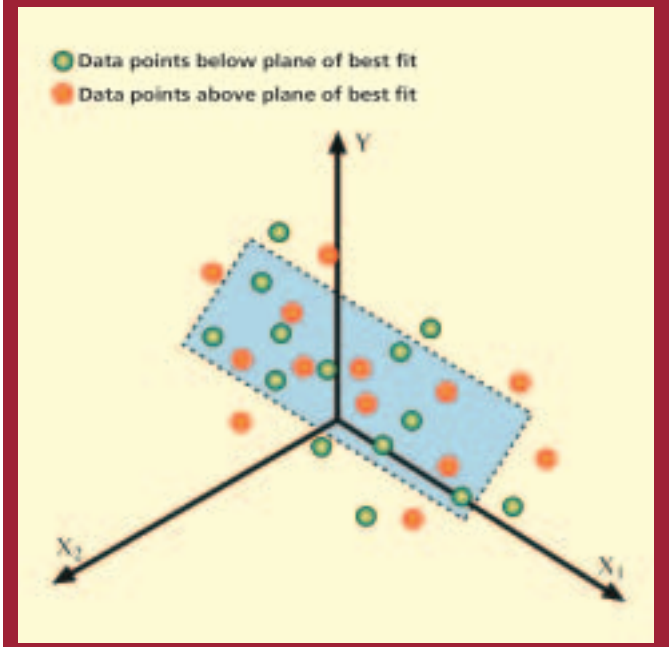


Figure 2. *Multiple regression with two independent variables influencing Y. The data are fitted by the 'plane of best fit'. The data points are scattered about the plane with some data points (y) above the plane and some below the plane in three-dimensional space. The degree of scatter of the points above and below the plane of best fit indicates the failure of the plane to fit the points*



exploratory method, the results of which can then be tested on a new set of data and preferably, by a more rigorous experimental approach.

Scenario

Lichens, a symbiotic association between a filamentous fungus and an alga, are often dominant in particularly stressful environments such as the surfaces of rock and tree bark. Under these conditions, lichens experience extremes of temperature, moisture supply, and low availability of nutrients. As a consequence, lichens sequester a high proportion of their carbon production for stress resistance rather than for growth. Hence, as a group, lichens are slow growing organisms with the radial growth rate of many species being less than 2mm $year^{-1}$ while some grow at less than 0.5mm $year^{-1}$ (Hale, 1967).

Slow radial growth rates and the difficulty of growing lichens for long periods under controlled laboratory conditions have made it difficult to study the influence of environmental factors on growth. In the absence of such studies, investigation of the seasonal variations in growth in the field has been one method of examining the affects of environmental factors. Significant correlations between growth and climatic variables suggest hypotheses about the causal factors limiting growth which can then be tested by more controlled physiological experiments. In the present study, the radial growth rate (RGR) of thalli of the crustose lichen *Rhizocarpon geographicum* was measured in successive three-month periods over a total period of 51 months in North Wales, UK. The radial growth of 20 thalli of *R. geographicum* (Armstrong & Smith, 1987) was measured at between eight and ten randomly chosen locations around each thallus at three-month intervals from April 1993 to June 1997 using the method described by Armstrong (1973). Essentially, the advance of the hypothallus is measured using a micrometer scale in relation to fixed markers on the substratum. Radial growth in each period was averaged for each thallus and then over the 20 thalli to examine the pattern of seasonal growth. Data for eight climatic variables were obtained from the Welsh Plant Breeding Station, Plas Gogerddan, near Aberystwyth and included records of: (1) total rainfall over each three-month period, (2) the total number of rain days, (3) maximum (*Tmax*) and minimum (*Tmin*) temperature recorded on each day and averaged for each three-month period, (4) the total number of both air and ground frosts, (5) the total number of sunshine hours, and (6) average daily wind speed.

Hence, the data comprise a single dependent (Y) variable, *viz.*, radial growth of the lichen in each three-month period and eight possible defining (X) variables and are presented in Table 1.

How is the analysis carried out?

Theory

When there are only two variables (X and Y), the distribution of data points in space can be represented by a two-dimensional (2D) surface but with three variables (Y, X_1 and X_2) 3D geometry is required. The theory of multiple regression will be described with reference to two independent variables, but the same principles apply to any number of X variables. Figure 1 illustrates the relationship between Y and two X variables (X_1, X_2) such that any point

Table 1. Radial growth rate (RGR) (Y) of the lichen *Rhizocarpon geographicum* in 17 successive three-month periods in North Wales, UK in relation to eight measured climatic variables (X)

	Independent (X) variables							
RGR Y	***Tmax*** (°C)	Air frosts	Rain days	Rainfall (mm)	***Tmin*** (°C)	Ground frosts	Sunshine hours	Wind speed (ms^{-1})
0.04	7.6	8	59	207.7	6.2	16	609.0	8.6
0.58	20.1	0	38	306.0	11.6	0	237.3	8.1
0.15	10.6	3	47	317.7	5.5	33	181.9	10.7
0.18	11.6	17	51	194.5	1.9	49	171.6	11.8
0.07	8.3	8	24	97.8	6.1	33	619.0	8.1
0.37	19.6	0	41	287.4	11.4	1	287.4	8.7
0.33	19.4	3	68	457.7	6.0	25	186.9	9.3
0.17	6.8	43	44	175.8	0.26	57	276.8	8.7
0.10	13.9	2	48	295.6	6.9	19	488.5	10
0.29	17.9	0	63	328.0	12.0	0	318.4	10
0.16	10.6	17	52	318.8	4.2	41	200.6	8.7
0.35	19.8	34	49	233.4	0.87	52	217.4	8.4
0.18	14.5	2	48	197.8	7.0	16	521.2	8.5
0.14	18.0	0	42	231.1	11.3	3	495.5	7.5
0.22	10.9	13	57	463.9	5.0	26	223.0	8.6
0.20	8.9	8	72	349.8	4.0	17	199.2	12.4
0.34	17.9	2	31	140.3	7.2	20	220.3	6.7

Tmax = maximum temperature, *Tmin* = minimum temperature

(A) in the 3D space is defined by three co-ordinates (x_1, x_1, y). The relationship between Y and a single X variable is described by the 'line of best fit' as determined by the method of least squares. By contrast, with two X variables the data are fitted by a surface or plane (the 'plane of best fit') (Figure 2) which is described by the equation:

$$Y_R = a + b_1X_1 + b_2X_2$$

'a' is a constant, 'b_1' measures the change in Y when X_1 increases by one unit, X_2 remaining constant while 'b2' measures the change in Y when X_2 increases by one unit, X_1 remaining constant. 'b_1' is called the 'partial regression coefficient' of Y on X_1 and 'b_2' the 'partial regression coefficient' of Y on X_2. Note that small case letters such as 'b' will be used to indicate a sample regression coefficient which is estimated from the data and upper case Greek letters such as 'β' the population or 'true' value of the regression coefficient. As in the case of a single X variable, the 'y' values are considered to be normally distributed about the regression plane (Figure 2) and the coefficients of the regression equation are chosen to minimize $\Sigma(Y - Y_L)^2$, where Y_L represents the points on the regression plane and 'Y' the actual points. Deviations of the points from the regression plane are a consequence of random error and the existence of variables that influence Y but which have not been included in the study.

Table 2. Analysis of variance (ANOVA) of the multiple regression data in Table 1

Source of variation	***DF***	***SS***	***MS***	***F***	***P***
Regression	8	0.2463	0.030798	5.69	<0.05
Error	8	421.8348	0.005416		

DF = Degrees of freedom, *SS* = Sums of squares, *MS* = Mean square, *F* = Variance ratio, *P* = Probability

Goodness of fit test of the points to the regression plane

As in the case of a single X variable, the sums of squares (SS) of the deviations of the Y values from their mean (Y^*) can be partitioned into two components, *viz.*, the SS of the fitted points on the plane (Y_L) from their mean and the SS of the deviations of the data points (Y) from the fitted values. The goodness of fit test of the regression plane to the data points can then be carried out using an 'F' test. The 'F' test determines whether any of the X variables included in the regression are related to Y, i.e., of the hypothesis that $b_1 = b_2 = 0$. Alternatively, a 't' test of the significance of each of the regression coefficients (b_1, b_2) can be made since $(b_1 - \beta_1)/s_b$ converts 'b_1' so that it is a member of the 't' distribution with

Table 3. Estimation of the regression coefficients of the multiple regression data in Table 1.

Variable	***b***	***SE of b*** (s_b)	***'t'***	***P***
Mean Tmax	0.51	0.21	2.43	<0.05
Air frosts	0.45	0.29	1.53	ns
Rain days	-0.38	0.30	-1.26	ns
Total rainfall	0.08	0.26	0.31	ns
Mean Tmin	-0.44	0.56	-0.78	ns
Ground frosts	-1.02	0.55	-1.86	ns
Sunshine hours	-0.57	0.22	-2.56	<0.05
Wind speed	0.006	0.22	0.03	ns

b = Regression coefficient, *SE* = Standard error, *t* = 't' test, *P* = Probability, (*Tmax* = Maximum temperature, *Tmin* = Minimum temperature, *ns* = Not significant

N – k degrees of freedom (DF) where N is the number of data points, 'k' the number of *X* variables, and 's_b' the standard error of 'b_1'. Even if the regression coefficients are statistically significant, it is not uncommon for the fraction of the variation in the *Y* values attributable to or 'explained' by the regression to be considerably less than 50%, i.e., much of the variance in *Y* may be explained by variables that have not actually been included in the study. A multiple regression analysis in which less than 50% of the overall variance in *Y* is explained by the chosen *X* variables will have limited value (Norman & Streiner, 1992).

Multiple correlation coefficient ('R')

The multiple correlation coefficient ('R') is defined as the simple correlation between *Y* and its linear regression on all of the *X* variables included in the study. Hence, 'R^2' is the fraction of the SS of deviations from the mean of the *Y* values attributable to the regression as a whole while $1 - R^2$ is the proportion of the SS not associated with the regression. As suggested above, a multiple regression should account for at least half the variance of the data, i.e., 'R' should be at least 0.7 ($R^2 = 0.49$). If nearly all of the variance is associated with a single *X* variable, a multiple regression analysis adds little to that of a simple linear regression.

Regression coefficients

Multiple regression analysis is used extensively to disentangle and measure the effects of different *X* variables on a single *Y* variable. Nevertheless, there are several important limitations of this procedure especially in observational studies. In any study, there will be *X* variables related to *Y* which have not been included. These may be variables thought to be unimportant, too difficult to measure, or are unknown to the investigator. Hence, the regression coefficient of a variable, e.g., 'b_1' is not an unbiased estimate of 'β_1' but of 'β_1' in combination with possible effects not measured. It is therefore advisable, at least initially, to include in the study all *X* variables that are likely to affect *Y* or to study a population in which variables not of direct interest can be controlled. Introducing more variables into an analysis, however, adds to the data collection effort, may contribute only noise to the prediction, and may reduce the sensitivity ('power') of the analysis. Hence, introducing new variables costs 'power' unless each variable individually can explain significant amounts of the variance. Deciding which variables to include in a study is usually a compromise between trying to achieve good predictive power while excluding irrelevant variables.

Interpretation

The multiple regression analysis of the lichen growth data in relation to the eight climatic variables was carried out using STATISTICA software (Statsoft Inc., 2300 East 14th St, Tulsa, Ok, 74104, USA). The value of 'R^2' for the present data set was 0.85, i.e., the regression accounts for over half of the variance and indicates that it is worthwhile to proceed with the analysis. The ANOVA of the multiple regression is shown in Table 2. The value of 'F' ($F = 5.69$) is significant at the 5% level of probability ($P < 0.05$) and therefore, at least some of the regression coefficients are not zero. Estimates of the various regression coefficients are shown in Table 3. This analysis suggests that the regression coefficients for mean maximum temperature ($t = 2.43$, $P < 0.05$) and total sunshine hours ($t = -2.56$, $P < 0.05$) are the only climatic variables out of the eight measured to have had a statistically significant effect on growth. Hence, lichen growth may be positively related to maximum temperature but negatively related to total sunshine hours. The likely explanation is that although warm temperatures may promote growth processes in *R. geographicum*, prolonged periods of hot dry weather actually inhibit growth presumably because of the drying out of the thalli.

Conclusion

Multiple linear regression determines the linear relationship between one dependent variable (*Y*) and multiple independent variables (X_1, X_2, X_3 etc.) and has many potential uses. An investigator should always have a clear hypothesis in mind before carrying out such a procedure and knowledge of the limitations of the analysis. In addition, multiple regression is probably best used in an exploratory context, identifying variables that might profitably be examined in more detailed studies. Where there are many variables potentially influencing *Y*, they are likely to be intercorrelated and to account for relatively small amounts of the variance. Any analysis in which 'R^2' is less than 50% should be suspect as probably not indicating the presence of any significant variables. A further problem relates to sample size. It is often stated that the number of samples taken must be at least 5 to 10 times the number of variables included in the study (Norman & Streiner, 1992). This advice should be taken only as a rough guide but it does indicate that the variables included should be selected with great care as inclusion of an obviously unimportant variable may have a significant impact on the sample size required.

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Careers

A career in microbial genomics — home and away

I have worked in the UK for 10 years now, but I grew up in Clinton, Connecticut, in the Northeastern USA. My interest in biology started in childhood, having perhaps been influenced by my parents. My father was a registered Respiratory Therapist and Pulmonary Function Technologist. My mother was a registered nurse. They met at Yale University Hospital in the US. Growing up, my family's dinner conversations tended to include quite a bit of biological science and as a result I could see a direct correlation between my lessons in school and their application. For one of my middle school science fairs, my father and I, in our basement workshop, made a spirometer for measuring the vital capacity of the lungs. The mother of one of my best friends was a microbiologist, who would occasionally bring Petri dishes to our school lessons to demonstrate to us what grew under our fingernails.

When it came time to go to university, I was determined to find the best place for me. I believed I wanted to be a veterinarian and had some experience at a local veterinary clinic. In the US veterinary training is done at the postgraduate level, so I was seeking a strong undergraduate biology or pre-veterinary programme.

To complicate matters, I had been playing guitar since I was 10-years-old and was determined to also study music at university. Although my application for university was competitive enough for the Ivy League, I ended up finding my best fit at the College of William and Mary in Williamsburg, Virginia, USA. There I was able to double major in biology and music at a highly competitive and highly ranked university.

The College of William and Mary is the only US university with a Royal Charter, issued by King William and Queen Mary in 1693. I pushed myself to graduate a full year early from my double major undergraduate programme so that I could be part of the 1993 tercentenary celebrations, including a Charter Day speech from Prince Charles.

During my time as an undergraduate, my desire to be a veterinarian waned and I developed a passion for genetics. I decided to do a PhD. It seemed to me that my pursuing some form of postgraduate study had been a given; my parents certainly believed I could and should do it. It was recommended that I should first do a Masters degree, as human genetics PhDs were believed to be highly competitive and a Masters would give my application an advantage. As it turned out, I was accepted for a genetics PhD without the Masters, but decided to continue my studies at William and Mary.

My Masters in Biology was a two year programme, during which I gained extensive teaching experience in exchange for the university paying my tuition fees and a small stipend. This was hard work, but has served me as a valuable experience. For up to nine hours per week, I taught practical laboratory sessions to undergraduates, as well as marking weekly quizzes, and assisting with examination marking. I thought I would hate teaching, but I discovered that I enjoyed it very much and found teaching introductory biology to non-scientists to be the most rewarding. Being a 'liberal arts' university, even students studying art and business had to take a laboratory science. Getting them interested in the science happening in their practical sessions was great.

Also during my Masters studies, I conducted a research project. This is where I entered microbiology. One of my taught classes was microbiology with Professor Carl Vermeulen. In his introductory lecture, he presented a research discovery that had been made by one of his students that summer. I immediately signed up to the project and embarked on my microbiology career by working on *Escherichia coli* lipopolysaccharide. It was a phenomenal experience to present this work at the American Society for Microbiology General Meeting, the size and scope of which was wonderfully overwhelming (Wilson *et al.*,1994).

When it came time to pursue my PhD I sought a programme strong in microbiology and molecular genetics and found one by that name at Emory University in Atlanta, Georgia, USA. This was a minimum five year programme, which included a research project, teaching experience, and classes, some of which my Masters excused me from. The university paid my tuition fees and a stipend. In the first year, students did 10 week laboratory rotations with supervisors who would potentially supply a suitable PhD project. My first rotation was with Professor Charlie Moran, an expert in *Bacillus subtilis* sporulation. My third rotation was with Professor Steve Morse at the US Centers for Disease Control and Prevention (CDC) on *Neisseria gonorrhoeae* iron use. My second rotation was with the man who ultimately became my PhD supervisor, Professor Bill Shafer. In my 10 week rotation, I worked on the gonococcal *mtr* antimicrobials efflux pump system and contributed to a publication (Hagman *et al.*, 1997). In attempting to find MtrR transcription regulator binding sites in the incomplete *N. gonorrhoeae* strain FA1090 genome sequence, I discovered the then unannotated neisserial *dcw* cluster and investigated *Neisseria*-specific genes therein (Snyder *et al.*, 2001). Although my PhD involved a lot of hard work, my time in Bill Shafer's laboratory was a phenomenal experience. I consider myself lucky to have had that opportunity and am grateful to have my PhD supervisor's continuing support in my career.

In 2001, I was offered a postdoctoral position at the Sir William Dunn School of Pathology at the University of Oxford. There I continued my comparative genomics work on *N. gonorrhoeae* and *Neisseria meningitidis* (Snyder *et al.*, 2005a). I also assisted with the annotation of the *N. gonorrhoeae* strain FA1090 genome sequence and designed microarray probes to it and all of the available *N. meningitidis* genome sequences, as well as other available gene sequences. This was used in several genomic and transcriptomic studies (Snyder *et al.*, 2004; Snyder *et al.*, 2005b). Additionally, I participated in the analysis of the *N. meningitidis* strain FAM18 genome sequence with the Wellcome Trust Sanger Institute (Bentley *et al.*, 2007).

During my time in Oxford I continued teaching through college tutorials and departmental seminars. I was a College Lecturer at various times for St Hilda's College, Keble College, and Somerville College. I consider myself lucky to have been given these teaching opportunities. Many Oxford-based researchers do not have college associations. When applying for these positions, I was thankful that my CV included my previous teaching experience from the US.

Eventually, grant funding for my time in Oxford ended. Fortunately, an opportunity presented itself at the University of Birmingham working on Professor Mark

Pallen's *x*BASE bacterial genomic database (http://www.xbase.ac. uk/). Although this took me out of the laboratory, it did allow me to do comparative genomic analyses on the computer full-time. I was also fortunate to be involved in several next-generation bacterial genome sequencing projects (Loman *et al.*, 2009). As in my previous jobs, I also found opportunities to teach at the University of Birmingham. I helped coordinate the running and grade reporting for an undergraduate module as well as lecturing and teaching bioinformatics practical sessions. I very much enjoyed my time in Birmingham. I learned a great deal and met some wonderful scientists.

After less than two years at the University of Birmingham, Kingston University advertised for a Senior Lecturer in Biotechnology. I knew I did not want the uncertainty of being a postdoc my whole life. I knew that I enjoyed teaching and wanted to be at an institution that would value my teaching skills. I also knew that I did not want to give up my research. Kingston offered me the right opportunity. I now teach postgraduate and final year undergraduate students. I conduct my own research, led by my own vision and implemented by me, my research students, and my project students. Having started at the College of William and Mary, I have experience in designing research that could be conducted by undergraduate and postgraduate students in their research projects. At the University of Oxford, I gained experience with UK student projects, including how to get publication-quality results. Having been at the University of Birmingham, I have experience in bioinformatics-based genomic research, which has proven to be useful on those days when I cannot get into the laboratory, but can further my research on the computer in my office (Snyder *et al.*, 2010). My time at Emory University provided strong ties to the *Neisseria* research community and now that I am an independent researcher, I have been able to establish collaborations.

My experiences and their influence on my CV have meant that I can have a job that I love. I get to teach and inspire students. There are long hours associated and some frustrations, but there are also rewards and sometimes thanks from unexpected sources. I get to pursue my research. I haven't yet been successful in obtaining grant funding, but Kingston University has been very supportive of me and I have benefited from internal funding sources. I am Course Director for the MSc in Biotechnology, which means a lot of co-ordination and problem solving, especially because this programme is also taught by staff from St George's University of London and Royal Holloway, University of London. Juggling teaching and project placements across three institutions and industrial placements is challenging, but the students appreciate the experience, which makes it worthwhile. It also means that I have more connection with researchers at the other two institutions than I think I would otherwise. In all, a balanced career path with both teaching and research has served me well.

Based on my experiences, I think the number one recommendation that I would make to students who want to do a PhD would be to select the right supervisor and laboratory. The people I shared my PhD experience with were a great benefit to my education, over and above what I learned from Professor Shafer. The relationship a PhD student has with their supervisor is not just for the time of the PhD. That person can be a mentor and support years after the viva is done. Yes, there will be stresses during the project. There might even be shouting and arguments. The thesis will be drafted and redrafted and then redrafted again. But, this is part of the experience and should be taken as a sign that the supervisor cares for their student and for the project. Don't be dismayed; we have all been there.

I would also say that it is important to grab opportunities that come. I spent some time in the CDC. I made a leap across the pond and decided to stay. There are grant programmes available to do short industrial placements or spend time in other research laboratories. These enrich your understanding of how research is done, in addition to enhancing your CV. Don't be afraid to try different institutions, different countries, different research techniques, and even different organisms. I started with *E. coli* and I have worked on a variety of other bacteria, from MRSA to *Laribacter hongkongensis* (Woo *et al.*, 2009), but *N. gonorrhoeae* captivated my attention during my PhD and continues to hold it even 10 years later.

Finally, I would advise that you teach. It can be difficult in a research post to find opportunities to teach, but grab them when they are available. Even if you end up in a post that is entirely research, you will still need to do oral presentations at research conferences. Teaching can provide an element of confidence in public speaking that cannot be obtained through occasional research talks. Assessing other people's writing can help improve your own. Talking to students about your field and your research can bring out new insights and new ideas. A CV that balances plenty of research papers with teaching experience is a valuable thing. Mine was able to secure me a job I truly enjoy.

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Lori Snyder
Kingston University

In the loop pecs

News from the S*f*AM Postgraduate and Early Career Scientist Committee

PECS Committee changes

Back row, left to right: Emmanuel Adukwu, Simon Gould and Phillip Humphryes. Front row, left to right: Samantha Price and Amara Anyogu

With the recent changes to the PECS Committee we thought we would take the opportunity to introduce ourselves and the roles we play within the Committee

Dr Simon Gould — Chairman

As the Chairman of PECS my role involves; organizing the PECS Committee's quarterly meetings, ensuring action points are followed up and liaising with the S*f*AM Honorary General Secretary, Dr Mark Fielder, who feeds back PECS ideas to the S*f*AM Main Committee. I'm currently based at Kingston University where I am a Lecturer in Medical Microbiology.

Amara Anyogu — Secretary

As Secretary of PECS I am responsible for writing and disseminating minutes of Committee meetings and work with other members of the Committee to organize student events at the Summer Conference. After completing my undergraduate degree in Microbiology at London Metropolitan University, I stayed on to carry out research towards a PhD studying survival mechanisms of enteric pathogens in traditional African fermented foods. I was originally involved with PECS as a member of the steering group and joined the Committee when a position became available. Our biggest goal is to get as many student members of S*f*AM involved in PECS as possible and we're always happy to hear from you so contact us to get involved!

Phillip Humphryes — Communications Officer

As Communications Officer I am responsible for organizing (and sometimes writing) this PECS column in *Microbiologist*, contributing to the monthly S*f*AM e-bulletins, and acting as the point of contact for members of the group via the PECS email service; allowing me to act as a liaison between the steering group and the Committee. I am currently in the final year of my PhD at Royal Holloway University; based full time at the Veterinary Laboratories Agency. My project involves using proteomics to develop an *in vitro* alternative to current *in vivo Leptospira* vaccine batch potency tests. Prior to starting this project I gained an MSc from the University of Sussex in Genetic Manipulation and Molecular Cell Biology and a BSc from the University of Surrey in Molecular Biology.

PECS NEWS

The PECS Committee met in October and appointed a new events officer, Jo Griffin. PECS would like to welcome Jo to the Committee and to wish her luck in her new position. If you would like to become more involved with PECS or wish to be featured in our articles please contact Phillip at **pecs@sfam.org.uk**.

If you know of a S*f*AM student or early career scientist who has been awarded a PhD/prize/award then get in touch, email Phillip Humphryes at: **P.C.Humphryes@rhul.ac.uk**

Phillip Humphryes
PECS Communications Officer

Emmanuel Adukwu — Events Officer

I am part of a three-person events team tasked with organizing events for the PECS group. My role includes: planning events for the PECS group at the Summer Conference such as the 'icebreaker'; the student session and any other social activities during the conference. I am currently a PhD student in my second year at the University of Northampton looking at the use of antimicrobial agents against community acquired strains of bacteria. Prior to undertaking this programme I worked in clinical research following completion of an MRes in Microbiology at Manchester Metropolitan University, where I studied the effect of essential oils and essential oil blends against a range of Gram-positive and Gram-negative bacteria.

Samantha Price — Events Officer

I'm part of the events team alongside Emmanuel and Jo. As Emmanuel mentioned we're responsible for organizing the Summer Conference PECS events and ensuring you have a good time! We've already got some exciting ideas planned for 2011. I did my undergraduate degree in Biomedical Science gaining a first class honours degree at De Montfort University in Leicester. I am now in the second year of my PhD at De Montfort University in Microbiology, with the Pharmacy research team, developing a disinfectant using novel catalytic technology developed by the university. Prior to this I worked as a student Biomedical Scientist in the NHS pathology labs and completed my professional portfolio.

Jo Griffin — Events Officer

I am a new member of the events team and am looking forward to organizing some of the student activities at the Summer Conference in Dublin. I am currently in the first year of my PhD at De Montfort University looking at healthcare laundries and their contribution to reducing *Clostridium difficile* spores, in partnership with the NHS. Previously I studied for an MSc in Advanced Taxonomy at The Natural History Museum and Imperial College London. I am looking forward to meeting all the Student Members at the upcoming Summer Conference and revisiting the great Jameson Distillery!

Students into Work Grant reports

am I eligible — can I apply?

Yes — if you are FULL Member who can offer an undergraduate microbiology student the chance to obtain work experience. If you would like to read about the experiences of students who have benefited from this grant, you can do so below.

For further information visit: **www.sfam.org.uk/grants.php**

Developing and refining homologous recombination-mediated allelic exchange in *Klebsiella* species

In my fourth year of Medical School at the University of Leicester, I chose to undertake an intercalated BSc degree in Microbiology and Microbial Genetics with Dr Kumar Rajakumar. With his help and support, I started a project in which I screened for and analyzed novel genomic sequences in *Klebsiella* strains isolated from human blood stream and urinary tract infections. The project was highly challenging and proved to be intellectually stimulating and rewarding. I subsequently became more interested in unravelling the molecular basis of pathogenicity in organisms and applied for an intercalated PhD which would start October 2008. In the period prior to commencing my PhD studies, I was very fortunate to obtain a Students into Work Grant from the Society for Applied Microbiology. I anticipated that the 10 week project would allow me to further expand and develop my laboratory skills and expertise and immerse myself within the theoretical and practical aspects of molecular biology, microbiology and bacterial genetics, all of which would be of great benefit at the start of my PhD studies.

Bacterial genomes consist of two parts: the core and flexible genome. The core genome is highly conserved between almost all strains of the same species, whilst the remainder, the flexible genome, consists of strain-specific genes. The flexible genome often harbours segments of horizontally transferred mobile genetic elements, such as transposons, phages, plasmids and integrative genomic islands, which are large multi-gene elements that confer a variety of functions. Pathogenicity islands are a subset of genomic islands with virulence associated genes that may encode determinants such as toxins, adhesins and type III bacterial 'syringes'. Previous work by many research groups has shown that *Enterobacteriaceae* often possess novel genes within their flexible genome. During my BSc project, we also confirmed this to be true for *Klebsiella* by identifying a number of novel genomic islands, one harbouring a putative type I fimbrial operon. *Klebsiella pneumoniae* is a clinically important pathogen that causes community acquired pneumonia, blood stream infections and urinary tract infections.

To functionally characterize genes and/or a genomic region, it is often

necessary to delete the gene or region from the genome and compare the deletion mutant to the wild-type strain using a defined set of phenotype assays. The main aim of this project was to design a protocol for generating *Klebsiella* mutants, which would allow further characterization of the novel genomic islands identified in earlier work. We decided to focus our efforts on the mutagenesis of *fimK*, a non-essential *Klebsiella* type I fimbrial operon-specific gene, which had previously been deleted from a *Klebsiella* strain.

Primers were designed for splice overlap extension-PCR and were used to construct a mutant allele that was comprised of *fimK* and immediate flanking DNA with approximately 400bp of the gene itself deleted and replaced by a selectable gentamicin cassette (*fimK::Gen*). The mutant allele was cloned into the multiple cloning site of plasmid pDS132, a lambda pir-based suicide vector. The plasmid can then be transferred from *E. coli* S17.1λ *pir*, a conjugative *E. coli* donor strain, to a variety of bacteria because it harbours the broad host range *mobRP4* origin of transfer. pDS132 and its derivatives are unable to replicate in most *Klebsiella* strains because these organisms do not possess the essential phage-encoded *pir* protein that is required for pDS132 replication. Hence, the plasmid 'suicides' and is lost unless, as in the rare instance, it has integrated into the chromosome via homologous recombination. The pDS132 vector backbone also possesses a chloramphenicol resistance cassette and the *Bacillus subtilis sacB* cassette, which confers sucrose sensitivity to *Klebsiella* strains.

K. pneumoniae strain KR116 and *E. coli* S17.1λ pir\pJKO-1b (pDS132 with the allele *fimK::Gen*) were conjugated on filter paper and transconjugants were selected for on M9 minimal media supplemented with gentamicin. Whilst neither the donor nor recipient strain would be able to grow on this medium *K. pneumoniae* clones, harbouring a chromosomally integrated pJKO-1b suicide vector (transconjugants), would be able to replicate normally on this medium. Colonies were subsequently screened for resistance to gentamicin and chloramphenicol, the expected phenotype of the transconjugant, using antibiotic-supplemented LB agar plates. The merodiploid nature of clones exhibiting the correct resistance phenotype was confirmed by PCR assays to detect the two antibiotic resistance cassettes, and a third PCR assay that used a genome- and vector-specific primer to verify genomic integration of pJKO-1b. High numbers of clones exhibiting positive results in both the phenotype and PCR screen assays demonstrated that we were able to efficiently transfer and integrate suicide plasmids into *K. pneumoniae* strain KR116. However, due to time constraints I was unable to perform the final step of the process that would have led to the replacement of the wild-type *fimK* allele with the mutated *fimK::Gen* allele. In theory this final step would have been achieved by selecting for clones that had undergone a second homologous recombination event, through the use of the counter-selectable properties of the pDS132 vector backbone-borne *sacB* cassette, exerted during growth on LB medium supplemented with 6% sucrose.

After completing the S*f*AM Students into Work Grant placement I continued establishing and optimizing this *Klebsiella* mutagenesis protocol. Following extensive troubleshooting, I have been able to develop a highly efficient mutagenesis protocol, which I have successfully applied in my PhD project to mutate and/or delete three different genetic loci in five different *Klebsiella* strain backgrounds.

Apart from the practical experience that I gained from the Students into Work scheme, it has also introduced me to the reality of independent laboratory work where self-motivation and persistence are essential, particularly so when the desired results are not obtained. I also significantly enhanced my planning, multitasking and experimental design skills. Ultimately, I hope to conclude my medical studies and specialize in clinical microbiology where I will be able to continue to pursue my research interests within academic medicine. I am very grateful to both Dr Rajakumar and his laboratory as well as the Society for Applied Microbiology for providing me with the opportunity to take up this placement, something that I would certainly recommend to anyone who wants to experience research first-hand.

Jon van Aartsen

Engineering *Bacillus subtilis* 168 to detect the presence of the lantibiotic subtilin

For 10 weeks I was part of the 2009 Newcastle University International Genetically Engineered Machines (iGEM) team. IGEM is a synthetic biology competition for undergraduate students. The aim is to design and implement novel genetic systems which produce interesting or useful behaviours. Teams are made up of students from a wide range of disciplines; our team of six included students of genetics, molecular biology, computing and bioinformatics. Our project involved engineering the Gram-positive *Bacillus subtilis* 168 to detect the presence of the lantibiotic subtilin in its environment, and respond by producing Green Fluorescent Protein (GFP). The work was proof of principle for a more ambitious project which we did not have time to complete in the time available.

The larger project aimed to turn *B. subtilis* into a biosensor capable of detecting pathogens in its environment and indicating their presence by producing varying levels of different coloured fluorescent proteins (FPs). Whilst bacteria naturally sense multiple input signals using a network of signal transduction systems, our problem required us to be able to design signalling circuits that would be capable of sensing a range of distinct quorum peptides.

In order to design systems that would be useful for the detection of a number of pathogens we modelled the required network computationally using a well known computing system called an Artificial Neural Network (ANN). ANNs are normally used to classify a range of input signals and produce a discrete output in response to the combined input signal.

Instead of implementing our ANN in software we wanted it to be implemented in the bacteria. We decided to use two-component quorum-sensing systems as the sensing layer, transcription factors as an intermediate layer and FPs as the output. We implemented a single input — a hidden layer component of this larger system. The circuit was designed on the

computer and synthesized commercially by GenScript Corp. We received our design in DNA form, cloned in the pUC57 vector in *E. coli*.

Once synthesized we needed to insert the DNA encoding the two component system, and the *gfp* reporter it regulated, into the chromosome of *B. subtilis* 168. Fortunately, there are well established techniques for exploiting the natural ability of *Bacillus* to take up and integrate DNA through natural competence coupled with homologous recombination. We subcloned the synthesized DNA insert into a vector designed to facilitate this integration at the *amyE* locus of *B. subtilis*. Transformants were obtained by screening for erythromycin resistance, encoded by the integration vector. I was successful in isolating transformants with the correct chromosomal insertions and then moved on to characterizing the response of the two-component system to subtilin. We cultured the subtilin producing strain, *B. subtilis* ACTC1633 overnight and isolated the culture supernatant that contained large amounts of the lantibiotic subtilin. We then characterized the response of our system to varying levels of subtilin using fluorescence microscopy and flow cytometry. The entire project was recorded on a web page at: http://2008.igem.org/Team:Newcastle_University.

At the beginning of November the entire team, plus instructors and some of our advisors, went to the Massachusetts Institute of Technology to present our work. We arrived late and tired on Friday night and joined the Edinburgh and Imperial College teams for dinner. We had to be at the Stata Center in time for breakfast in the morning. There were 84 teams from 21 different countries, so the presentations had to be organized into multiple parallel sessions. Our team presented in the afternoon. In the evening there was a poster session, where we had to answer questions from the judges and other teams about the work. The day wound up with a reception in the bar for those of 21 (the drinking age in the US), and an excellent ice cream bar for those with a sweeter tooth.

On Sunday the top six teams presented their work again, and the awards were then announced. The six UK teams achieved three Gold medals (Newcastle, Edinburgh and Imperial), one Silver (Bristol) and a Bronze (Cambridge). We spent the afternoon sightseeing in Boston, before heading back to the airport for the return leg of a whirlwind trip.

My vacation project was a unique experience. Exposure to a range of other disciplines, the opportunity to work with a tightly-focussed team, and being able to meet and talk with like-minded students from all over the world made it a great learning experience. The project was interesting, educational, and has given me a much greater understanding of the practicalities of doing independent research. I would like to thank the other iGEM team members, my supervisors Professor Anil Wipat, Dr Jennifer Hallinan and Dr Matt Pocock and, of course, S*f*AM for making it all possible.

Ria Chalder

Assessing the effect of tarnishing on the antimicrobial properties of copper

Having just completed my honours degree in biomedical science I was given the opportunity to work on a project in a microbiology research laboratory for 10 weeks at Kingston University based on the antimicrobial properties of copper. It is thought the National Health Service spends around a billion pounds a year on hospital acquired infections with the predominant method of transmission being via touch. Common surfaces which contribute to this form of transmission include door handles, push plates and bed rails. A number of studies have shown the antimicrobial properties of copper against stainless steel on many pathogenic bacteria such as meticillin resistant *Staphylococcus aureus* (MRSA), *Clostridium difficile*, *Mycobacterium tuberculosis* and a number of other nosocomial pathogens (Noyce *et al.*, 2006 a, b; Wheeldon *et al.*, 2008). These studies have focussed on clean, untarnished copper, but have not taken into account the affect of tarnishing on the antimicrobial properties of copper. The aim of the current study was to investigate how the antimicrobial properties of copper might change with tarnishing. This effect was assessed against important nosocomial pathogens including MRSA and *Pseudomonas aeruginosa* which can cause complications such as wound infections as well as other infections.

Overnight cultures of clinical isolates of either MRSA or *Ps. aeruginosa* were grown in nutrient broth. Cultures were then harvested in phosphate buffered saline (PBS) to give a cell density of approximately 10^9 CFU. Clean copper and stainless steel coupons were inoculated with aliquots of the resultant culture, these were then incubated at room temperature for 210 minutes with readings taken every 15 minutes. Following incubation viable counts were

determined. This was repeated over a total of four days.

The general trend in results had shown that survival time increases as tarnish levels increase. In some cases the survival time had more than doubled from untarnished copper on day one to tarnished copper on day two. Further increase had also been shown during day three and four showing that the antimicrobial properties of copper had been impaired. Contrastingly, stainless steel showed no significant difference in the number of viable cells per coupon throughout the experiment.

All the results showed that copper works best when not tarnished. Previous work by Airey and Verran (2007), showed problems with cleaning copper where once the copper was cleaned and reused, bacteria had remained present. This shows different cleaning agents would need to be tested in order to determine which product works best in removing tarnish and preventing a decrease in the antimicrobial properties of the copper.

The published literature on the antimicrobial properties of copper is growing and it is clear that copper affects a wide range of clinical pathogens (Noyce *et al.*, 2006 a, b; Wheeldon *et al.*, 2008). However, there is still considerable scope to further investigate copper before its uses can be fully appreciated and harnessed for use in the clinical setting. The conclusion of this study was whilst polished copper was able to reduce and completely kill the organisms tested, this antimicrobial activity was reduced when the copper was tarnished. This indicates appropriate cleaning measures will be needed, not only to retain the antimicrobial properties of copper but also prevent damage to this soft metal which may lead to soil and bacterial build up. In contrast, stainless steel has shown no significant difference in cell numbers and therefore confers no advantage in terms of reducing the transmission of potentially pathogenic bacteria.

As a result of having been given the opportunity to carry out this project, I have been able to gain experience of working in a research laboratory and I have gained many useful microbiological skills. I have also been able to experience the day to day tasks involved with the running of a laboratory. I now feel more prepared to be able to work towards my chosen career path. I would like to thank Dr Simon Gould for giving me the opportunity to be involved with this project and for developing my keen interest of working in microbiology. I would also like to thank Dr Mark Fielder and Dr Alison Kelly for their advice and assistance and of course S*f*AM for funding this project.

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Dipa Ghedia

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Bioremediation of PAHs: Why still a problem?

Bioremediation is the process of breaking down xenobiotics of a contaminated area by biological processes. In microbiological terms, we usually take this to mean by the action of microorganisms (bacteria or fungi) rather than other specific biological processes. Bioremediation is seen as, and often used as, a treatment for contaminated sites to remove the unwanted xenobiotic.

Polycyclic aromatic hydrocarbons (PAHs) are priority pollutants chemically consisting of two or more fused benzene and/or pentacyclic rings. One of the largest uses is (or was, in some countries) in creosote and in coal tars. Creosote is a persistent chemical mixture composed of approximately 85% PAHs, 10% phenolic compounds and 5% N-, S- and O-heterocyclics. It is used as a wood preservative for timber in applications such as building materials, fencing posts and telephone poles.

In the environment, creosote and

coal tar contamination occurs at the sites of usage but this is minimal compared to the sites of production of these formulations. Even if the compounds can run-off industrial sites to the surroundings or eventually leach into the ground soil. The lower molecular weight, lower numbered ring PAHs volatilize naturally and are relatively non-toxic, but the higher molecular weight, higher ringed (particularly those of five rings or more) PAHs are recalcitrant and very toxic. Thus, PAHs pose an environmental problem where used.

Isolation of the contaminated land is the best control against the spread of pollution, but if the land is to be reused then this approach is not possible. Removal of PAHs *ex situ* is the most effective way to control and stop the potential spread of pollution and clean the soil for reuse, but such practices are prohibitively expensive and are only used in extreme cases of environmental control or where the economic benefits are substantial enough to merit it. Treatment *in situ* is preferred for a number of economic and environmental reasons. Industrial/mechanical methods such as biosparging, bioventing and landfarming have all been proven to remove PAHs in soils, but for clean-up solutions for contaminated soils where these are not appropriate (be it economic, geographic, or time-related) the use of microorganisms provides an alternative.

The biodegradation of PAHs and bioremediation of PAH-contaminated soil is well documented. Isolated sole-degraders and communities involved in PAH have been researched to such a level that, in conjunction with the engineering solutions, a lay-person would find it difficult to understand why we have PAH contamination at all. However, this is the case and PAHs, as well as many other well researched xenobiotics, are still present in undesirable concentrations at undesirable locations. One of the largest problems is interpreting data into information and putting into action the conclusions reached — to remove the contamination.

Microbiological research has moved on to improve the efficiency and efficacy of PAH treatments to overcome the above problems. In fact, many of the engineering solutions rely on the stimulation of indigenous microbial communities present in order to degrade the xenobiotic. Investigations of the abundance of individuals or groups of individuals at the molecular level is now possible, and beyond this we can look at those organisms actively expressing specific genes and the abundance of relevant proteins produced.

This then leads to the question posed to many scientists involved in applied research: how do we use this information? The new techniques allow us to look at the whole soil microbial community, of which 90 to 99% is unculturable. The chances of obtaining a culturable high-performing sole degrader that can be worked on are minimal and thus we are left with altering the unculturable fraction. This can only be monitored using the molecular techniques (although the end result of actual loss of PAH is much easier) to assess the effect of treatments.

Furthermore, degradation is usually by a consortium of organisms and not single organisms acting independently. Approaches to stimulate whole communities are ongoing by many groups, and experiments are being carried out to assess the changes that occur during the industrially more 'traditional' techniques — after all, the effects of bioventing on xenobiotic concentrations are well-known but not the effects on the whole microbial community. Of course, the soil itself is important, as all are different (even in close geographic regions) and so what may be microbiologically advantageous for one soil may not be for another. It is still not understood how changing the majority of the microbial structure of a soil will affect it over time.

A brief search of recent research on the bioremediation of PAHs shows a focusing on microbial communities and, with the advent of available pyrosequencing and microarray technologies, the ability to monitor and then influence organisms involved in the degradation process is evermore increasing. These will most likely form a stepping stone to further manipulation on the genetic level and assessment of introducing changes, a myriad that cannot yet be currently undertaken.

Ultimately, economics will decide the future directions of the research, and this is not just limited to PAHs, although as xenobiotics they provide a suitable group of model compounds for study. Problems will remain with these and other compounds, but across the world priorities are different, and whilst pollution is undesirable, it doesn't have to mean it is always harmful and doesn't mean it will always be dealt with for a variety of reasons.

further reading

■ CL:AIRE. Contaminated Land: Application In Real Environments. 2 Queen Anne's Gate Building, Dartmouth Street, London SW1H 9BD. www.claire.co.uk. Information on bioremediation.

Russ Grant

Developing an *in vitro* biofilm model to assess the antimicrobial efficacy of topical wound treatments

It is generally accepted that all acute, traumatic, surgical and chronic wounds become contaminated with microbes. In some cases, microbial proliferation within the wound environment can result in a failure of the wound to move through the phases of healing in a timely manner. Wound infection causes increased morbidity and associated healthcare costs; surgical wound infection alone has been estimated to double both the length of hospitalization and cost of care (Ploughman *et al.*, 2001).

Systemic antibiotics are seen as essential in treating heavily infected wounds. However, for general control of the wound bio-burden and treatment of localized infection, topical antimicrobial agents are more widely advocated. It is thought that systemic antibiotics have poor localized tissue perfusion and the widespread use of broad-spectrum antibiotics is implicated in the rise of antibiotic resistance in clinically relevant

microbes. Therefore, new antimicrobial topical wound products tend to incorporate broad-spectrum antiseptic agents rather than antibiotics.

Many different *in vitro* microbiological models have been developed over the years to assess the antimicrobial efficacy of topical wound treatments. This has largely relied on two methodologies; zone of inhibition and log reduction/challenge testing, where a target species is incubated with the test dressing and the numbers of survivors quantified after a defined period of exposure. More recently, we have developed a simple *in vitro* static diffusion method that enables accurate determination of the kill rate (*K*) of a given treatment. *K* is the key comparator for different formulations, target species and physicochemical conditions (Thorn *et al.*, 2005).

Most existing methodologies use planktonic microbial cultures to assess antimicrobial efficacy. A growing body of evidence, however, now supports the presence of microbial biofilms in wounds. In fact, it has been hypothesized that progression of a wound towards infection is actually a model of a progressing biofilm (Percival *et al.*, 2004).

Research has shown that biofilms are more resistant to antimicrobial therapy than planktonic cultures due to numerous mechanisms (Lindsay and von Holy, 2006). Therefore, those *in vitro* antimicrobial susceptibility tests performed using planktonic inocula are unlikely to accurately predict the efficacy of a given treatment *in vivo* where a biofilm is present. To address this issue, a novel *in vitro* flat bed perfusion biofilm model was developed that would enable controlled, defined growth of reproducible biofilms, facilitate application of topical antimicrobial treatments and assessment of their efficacy.

The model biofilm system involves perfusing a complex or defined medium through a cellulose matrix in which the target organism is grown. This throughflow of fluid donates nutrients to the biofilm and removes waste products along with any newly shed daughter cells. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were grown separately within this system for 48 hours and found to form a mature quasi-steady state biofilm, whereby the proportion of daughter cells shed from the total biofilm matrix population remains constant, indicative of a constant growth rate. As a further development, a bioluminescent reporter strain of *Ps. aeruginosa* was integrated into this model, and used to monitor the metabolic activity of the biofilm *in situ*, without disturbance, using a low light photon camera. The top surface of the cellulose matrix is left exposed within the model growth chamber and this allows topical antimicrobial therapies to be applied to mature biofilms. If multiple biofilm systems are set up and treated simultaneously, the antimicrobial effects can then be monitored by measuring the viable counts and bioluminescence levels of both the actual biofilm population *in situ*, and the microbial population shed from the biofilm.

The antimicrobial kill kinetic data produced by the model accurately illustrates and differentiates both any bactericidal effects on the biofilm population itself and any bacteriostatic effects, measured by the reduction of growth rate of the system over given time points. Moreover, use of a bioluminescent reporter strain enables any antimicrobial effects on biofilm metabolism to be monitored in real time.

Numerous novel and existing topical treatments have been assessed using this model and found to exhibit very different antimicrobial profiles. This data can then be used to compare the strengths and weaknesses of either commercially available dressings or prototypes within research and development, where it is essential to be able to measure the effects of small step changes in formulation. Research into biofilms has been ongoing over the last 20 years, producing important results that can be applied to assist infection control within the clinical environment. The importance of biofilms within wounds is only now being fully appreciated and it is essential to have appropriate models to facilitate research in this field.

The award of the S*f*AM President's Fund enabled me to attend the World Union of Wound Healing Societies in Toronto, Canada, in June 2008 and this article is based in part on a poster presented at this conference. Financial support for this work was provided by Insense Ltd., Bedford, UK.

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Robin Michael Statham Thorn

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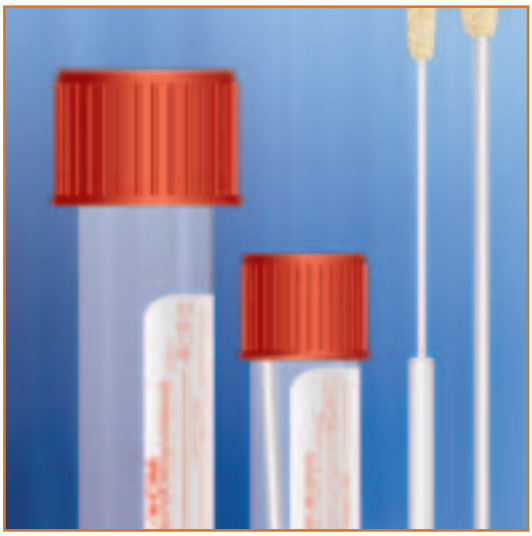

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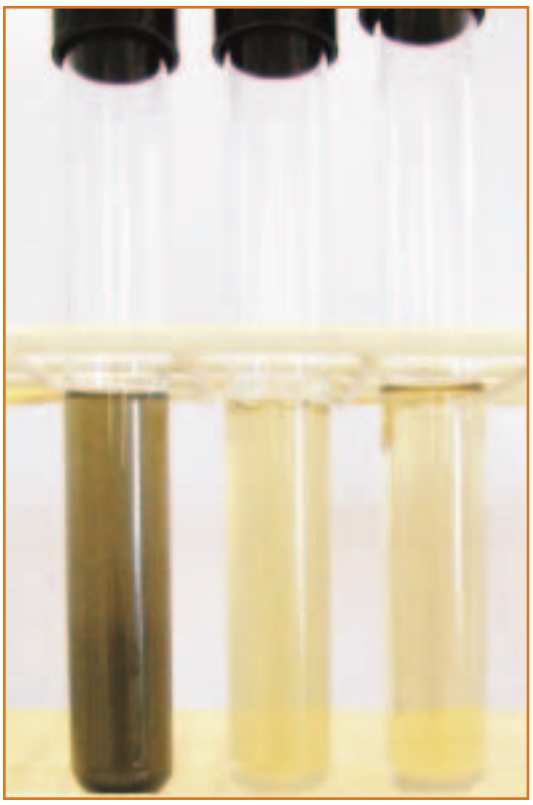

Lab M adds their new Modified Giolitti and Cantoni Broth (ISO) to culture media range

Microbiological culture media specialists Lab M have introduced their new Modified Giolitti and Cantoni Broth (ISO) for the detection and enumeration of coagulase-positive staphylococci. This adds to Lab M's expanding range of dehydrated culture media formulated to meet the specific ISO requirements of the food industry.

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Modified Giolitti and Cantoni Broth is optimised for samples where staphylococci may be stressed and/or present only in low numbers. Their growth is promoted by the inclusion of sodium pyruvate, glycine and high concentration mannitol. Potassium tellurite, which inhibits other Gram-positive organisms, and lithium chloride, inhibitory to Gram-negative bacilli, provide the necessary selectivity. Tellurite also serves as an indicator for coagulase-positive staphylococci , its reduction by these organisms resulting in a blackening of the broth or the presence of a black precipitate.

The introduction of this Modified Giolitti and Cantoni Broth (ISO) enhances Lab M's range of media for the isolation of coagulase-positive staphylococci in accordance with ISO 6888-3:2003.

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