

# Microbiologist

The magazine of the Society for Applied Microbiology ■ June 2006 ■ Vol 7 No 2

ISSN 1479-2699

## Microbiologist

Vol 7 No.2

June 2006

ISSN 1479-2699

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**Art and Design & layout:**  
Pollard Creativity

**Production and printing:**  
Pollard Creativity.  
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sfam@pollardcreativity.co.uk  
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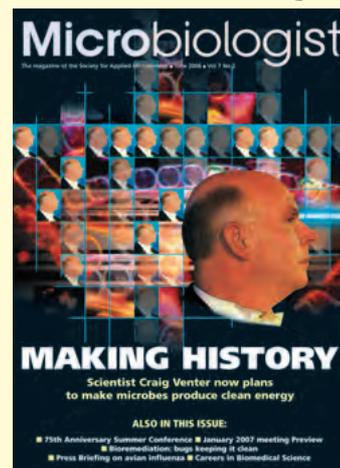
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## WRITE FOR US!

The editor is always looking for enthusiastic writers who wish to contribute articles to *Microbiologist* on their chosen microbiological subject.

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

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**Alun Anderson** reviews the controversial career of Scientist Craig Venter who discusses his latest plan to make microbes produce clean energy

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### Microbiologist copy

**Dates:** contributors please note that the final copy dates in 2006/2007 will be:

**Vol 7 No.3 Sept 2006**  
Friday 7 July 2006

**Vol 7 No.4 Dec 2006**  
Friday 15 Sept 2006

**Vol 8 No.1 March 2007**  
Friday 15 December 2006

**Vol 8 No.2 June 2007**  
Friday 9 March 2007

### How to submit material

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

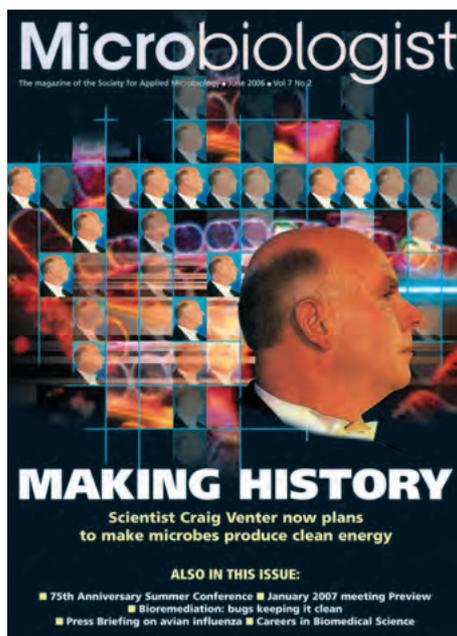
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**Website:** the society website is a timely source of up-to-date information on all Society matters and maintains a comprehensive archive of articles and reports on a variety of microbiological topics.

[www.sfam.org.uk](http://www.sfam.org.uk)

**I**N THE PREVIOUS ISSUE OF *Microbiologist* (March 2006, Vol 7; No 1) I asked a rather provocative question: When is a scientist not a scientist?" Answer: "When he / she is a business person".

I was delighted by the response I received. Many of you think that the two disciplines can never be separated, that they are far from mutually exclusive. It's true to say that a successful scientist and a successful business person will both have some very similar characteristics and transferable skills — time and project management, accuracy, numeracy, attention to detail, patience, and flexibility to name but a few. In this sense the two go hand in hand. What do you think? To join the debate contact me, the Editor at [lucy@sfam.org.uk](mailto:lucy@sfam.org.uk) or at the postal address opposite. A couple of examples of different perspectives on this thorny topic are described in the 'Mailbox' section (page 7).



A scientist who is also a business person is the subject of our feature article (page 28). Craig Venter, the man behind the commercial arm of human genome sequencing. Considered by some a maverick scientist — a 'bad boy' of science — he has now established both for-profit and non-profit companies all with a common goal — the exploitation of microorganisms for the commercial production of energy. You may remember that we ran an article back in March 2003 ([www.sfam.org.uk/pdf/features/playgod.pdf](http://www.sfam.org.uk/pdf/features/playgod.pdf)) in which we described the work of this controversial figure and his



role in the Human Genome Project, through his involvement in the company 'Celera Genomics.' This article was written just as he was about to embark upon new and apparently environmentally friendly research, so 'perhaps he's not such a bad boy after all.' We caught up with Venter at the latest stage of his endeavours, with an interview by Alun Anderson, former Editor-in-Chief of *New Scientist* magazine. Venter gives us not only his opinion on the relationship between science and business, but also describes the principles of the science involved, and its potential impact upon the environment.

On the subject of the environment, our second feature article is an overview of Bioremediation (see page 20) the dictionary definition of which is: 'The use of plants or microorganisms to clean up pollution or to solve other environmental problems.' This phenomenon has been recognised and researched scientifically since the 1940s but also has state of the art applications, contributing to an area which is high (though some might say not high enough) on the political agenda — the environment.

Bioremediation is also the subject of a session at our summer conference — Living Together: Polymicrobial Communities. The closing date for registrations is now looming so get your registration to us before 9 June 2006 to prevent paying the late booking fee! Spaces are limited and the conference is filling up fast, so don't delay. See page 26 for a booking form or you can register online at [www.sfam.org.uk/sumconf.php](http://www.sfam.org.uk/sumconf.php) where you can fill in an online registration form, or download a pdf application form to complete offline.

I look forward to seeing you all in Edinburgh!

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# MEDIAwatch



## Science Media Centre Press Briefing on avian influenza.

In my role as Communications Officer for SfAM I was lucky enough to be invited to a Press Briefing run by the Science Media Centre (SMC) on Avian Influenza. This was about one month before the dead swan found in Cellardyke Scotland, tested positive for H5N1. The briefing aimed to prepare journalists with good information about what would happen if a case of H5N1 were found in the UK.

The topics covered included:

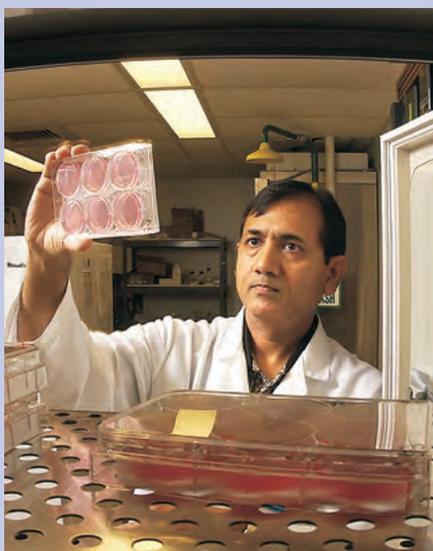
- Would it be safe to eat chicken and eggs?
- What options would be available to the poultry industry? For example vaccination or bringing free range birds inside.
- How would this affect the UK poultry industry?
- What measures should the person in the street take to avoid avian flu?
- Who is most at risk of catching avian flu?
- What would it mean for wild birds in the UK?

After a delayed rail journey, I was a few minutes late, but this didn't seem to

## MICROBIOLOGY IN THE NEWS

If you spot a story in the media which you think should feature in this column, then send it to the Editor at: [lucy@sfam.org.uk](mailto:lucy@sfam.org.uk).

To keep up to date, don't forget to look at our 'News' section on the SfAM website: [www.sfam.org.uk/news.php](http://www.sfam.org.uk/news.php)



have any effect on the warmth of the welcome I received from the Staff at the SMC. I crept into the back of the briefing room where Dr Bob McCracken, ex-President of the British Veterinary Association, Dr Judith Hilton, Head of Microbiological Safety, Food Standards Agency and Dr John McCauley, Institute of Animal Health were all explaining their position on avian influenza. They each gave us a five minute talk on their role and stance on the subject of food safety aspects of H5N1, and then opened the floor to questions. The charismatic director of the SMC, Fiona Fox was chairing the briefing, and she kept a tally of who was next to ask a question, referring to each journalist by name. At this point I found it hard not to feel a little starstruck: for example, "Ok, we'll have questions from Tom (Fielden — BBC



Journalists from the UK Media attending a press briefing

Today programme), Fergus (Walsh — BBC 10 o'clock News) and then Roger (Highfield — *Telegraph*)." The scientists answered the questions precisely and with great clarity and all dealt well with sometimes quite leading questions. For a group of people who are often considered to be sometimes ineffective communicators, I thought the scientists put across their points to great effect. Whether this was pure co-incidence, canny choices on whom to put in the spotlight, or a reflection of the effect the SMC is having on scientists attitudes to talking to the press, I don't know. But if more scientists knew what good they could do by talking to the press, science reporting can only get better. For more information about the work of the SMC, please call Claire Bithell, Senior Press Officer on 020 7670 2980 or visit [www.sciencemediacentre.com](http://www.sciencemediacentre.com).

The briefing was over quickly — to allow the journalists to return to their desks, with more informal discussion afterwards between the scientists and those journalists who could spare the time. For me this was definitely an hour well spent.

## SfAM POLICY ON THE MEDIA

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



## Hand in hand with Business

**FROM:** Russ Grant

**SUBJECT:** Science and Commerce

I read with great interest your editorial and the article on commercialisation of research in *Microbiologist*. I work as a post doctoral researcher but I am lucky enough to carry out a good deal of business and scientific consultancy. Your question, ‘When is a scientist not a scientist?’, and answer ‘When he/she is a business person.’, brings up several points that have been recently addressed by the BBC regarding innovation (Peter Day — ‘Universities Challenged’ and ‘Real World Innovation’ on the [www.bbc.co.uk](http://www.bbc.co.uk) website). However, it does rely on the fact that University innovation may be a good thing, with the best evidence actually showing the opposite.

As a person who works in both fields, my personal opinion is that science and business go hand in hand. Similarities far out weigh differences. In the lab I plan the experiments and order what is needed, taking care of the cost and time it will take – generally the same as any project manager. Keeping track of the grants involves very basic bookkeeping. I attend meetings and conferences to learn and share my work — in affect, marketing. I think of the best way to achieve the aim of the work — strategy. As a scientist I find myself doing many of the things I do in business. What is different however comes in the research exploitation, where potential commercialisation requires knowledge of fields outside those generally encountered in lab science.

The CEO of Intercell AG also recently wrote a report on commercialisation where expanding the knowledge of students is increasingly being seen as the way forward in commercialisation, to increase awareness of situations.

Additionally it is pointed out that there is little (but not no) opportunity for people to take sabbaticals from their tenure positions to pursue commercialisation activities — they have to choose between one or the other scientist or businessman (unlike the USA).

Referring to your editorial and from real-life experiences of science commercialisation from Universities, I can understand why some scientists may want to do their research for the love of it, but I cannot understand why, in this age of limited and competitive funding, they turn down the opportunity to obtain more funding to continue their work.

Contrary to this however in recent developments there is now the spectre of losing control if commercialisation occurs — often seen in start ups where the founder ends up as a scientific officer when more business experienced personnel are brought in and where shares are lost to raise further equity.

As for being essential, it may become this way, but at the same time over commercialisation will be damaging with unsustainable losses leading in turn to fewer funded exploitations. Furthermore, it has been found that Universities are not really the ‘hot- bed’ of innovation they have been made out to be, even in the US where such revenue generation is seen as huge. Without getting political it appears the recent investments are more public appeasing than anything else.

That University innovation commercialisation should be so low is no surprise if the evidence is examined — the biggest innovation comes from challenging University educated researchers with real world problems, in the real world. More on this appears in Peter Day’s articles from where interested parties can continue their own fact gathering.

Would you like to contribute to the debate? If these letters make you want to put pen to paper or fingers to keyboard, then please feel free to contact the Editor, Lucy Harper at: [lucy@sfam.org.uk](mailto:lucy@sfam.org.uk) or at the postal address at the front of the magazine.

We’ve had quite a response to the somewhat contentious issue of science and commerce discussed in the last issue of *Microbiologist*. Here are two examples:

## Quid pro quo?

**FROM:** Clive Blatchford

**SUBJECT:** Science and Commerce

I read your editorial with interest, because I believe we have a problem with scientists in business. I do not personally believe the two are incompatible, though I appreciate the basis of the question. If you look at the page facing your editorial, you will find a list of no doubt excellent scientists, of whom not one would appear to be in a conventional business role, this despite the fact that *SfAM* is very definitely and usefully orientated towards application. There may be many reasons for this bias, but it is self-perpetuating; my own contribution to *SfAM* being for many years limited to looking through *Microbiologist*. It is difficult to justify using my company’s time and money to attend meetings which always look interesting, but where there is little immediate value from attending. It is here that the difference lies; businesses invest money, which implies getting something tangible in return for the investment. It may be that we should just accept that applied for us goes as far as academic laboratories which work with industry, or food testing laboratories, but somehow when one thinks of the number of microbiologists in business industry, this seem a pity.

## Erratum

In the last issue of *Microbiologist* (March 2006), an article was published entitled: *The H. Pylori Saga ends with the Nobel Prize*. This article first appeared in the *ESCMID News* and was reproduced with permission from the Editor. An acknowledgement was mistakenly absent from our reproduction and the Editor of *Microbiologist* apologises for this error.



**Dr Margaret Patterson** looks at enthusing the young to take up science as a career

**E**XAMINATION SEASON IS upon us, making this one of the most stressful times of the year for young and old alike. Many young people are hoping for good grades at GCSE and A-level so that they can pursue their chosen career or university course. I wonder how many are planning on becoming 'a scientist'? Probably not that many, and this is not surprising due to the poor public perception of such a career. However, applied microbiology, in particular among the core sciences, cannot be accused of being irrelevant as it impacts daily on our health, our food and our environment. So what has gone wrong?

I was interested to read that the President of the Royal Society, Lord Rees of Ludlow, used the neglected and decaying bust of Sir Isaac Newton as symbolic of the way the UK regards science and its scientists. Lord Rees used his speech in National Science Week in March to highlight the falling popularity of science in schools and universities and the knock-on effect this will have on the UK's standing as a major player in the world of science and innovation.

It seems that Lord Rees is not the only one who feels that a strong science base is important if the UK economy is to grow and we are to compete effectively at the international level. Gordon Brown mentioned the words 'science' and 'scientific' at least 18 times in his March budget. He also announced plans for creating an extra 3,000 posts for specialist science teachers. Currently, the science curriculum in schools is not always taught by those with a degree in that particular subject. I was surprised to read in a recent report<sup>1</sup> that around 10% of A-level biology, chemistry and physics was taught by those who either held no qualifications in that subject above post-16 level, or whose highest qualification in the subject was itself at A-level.

This announcement on extra specialist science teachers is to be welcomed but I am not sure where they will suddenly be found, given the closure of a significant number of university science departments

and falling numbers of students studying core scientific disciplines. This point is echoed by the Biosciences Federation in its November 2005 report 'Enthusing the Next Generation'<sup>2</sup>. The report states: "Although the biosciences have not experienced the same course closures as the physical sciences, this masks undesirable variations in the strength of interest and engagement across different areas of the discipline. Subjects such as sports science, psychology and forensic science are increasingly popular in universities whilst core subjects such as biochemistry, pharmacology and microbiology are finding it harder to recruit students."



Those of us in the university sector will know that the unit of resource for teaching science subjects does not usually cover the costs of courses. As a result, many students now graduate with a BSc in a biological science but with little hands-on experience, as practical classes are too expensive to run. Even in schools, science teachers are shying away from practical experiments (which I remember as the most exciting part of the lesson) because of lack of resources, time and worries over health and safety. Perhaps it is not too surprising, therefore, that many young people are not overly enthusiastic about having a career in science, especially if it is perceived as being boring, difficult and irrelevant to everyday life.

In addition, there is the concern over the jobs that will be available to science graduates, especially those interested in R & D, what their salary will be and the career progression they can expect. The recent news that university lecturers are taking strike action over pay is a case in point. New lecturers, most of whom will have spent three years as a postgraduate studying for a PhD and possibly a few years more as a postdoctoral fellow working on a fixed term contract, can expect to earn only around £25,000 per year.

The private sector also needs to better recognise the value well trained graduates

and PhDs can bring to business growth and development. Gordon Brown's budget announced plans to expand R & D tax credit support to medium-sized companies. Hopefully they will take full advantage of this and employ more of our graduates and spend more on research. The total spend on R & D in the UK, as % of GDP, is 1.86 of which 47% is financed by business (2002 figures). This is some way away from the 2010 EU target of 3% of GDP spent on R & D, of which two-thirds comes from the private sector.

SfAM has recognised in our mission statement that in the UK there are skill deficiencies in applied microbiology and that we will be involved in identifying these deficiencies and will work towards implementing solutions. We have still a lot of work to do in this area but we have made a start. We ran a very successful design-a-bug competition for Primary school children a few years ago and we are sponsoring the MISAC (Microbiology in Schools Association Competition) this year. Our 'Students into Work' scheme has been very successful (see page 38) — in fact Committee increased the budget for this grant this year, to allow as many undergraduates and recent graduates as possible, the chance to gain valuable laboratory experience. We will continue to promote the importance of our subject at every opportunity and through the collective work of others, such as the Biosciences Federation.

In the meantime, I am now off to set some exam questions for a final year 'Food Microbiology and Biotechnology' paper, which I think is almost as challenging as being on the receiving end as a student.

Good luck to all those who are sitting exams in the next few weeks. I hope at least some of you will end up with a scientific career.

## References

- <sup>1</sup>Moor *et al* (2006) Mathematics and science in secondary schools. *The deployment of teachers and support staff to deliver the curriculum*. Research Brief RB708, Report for the Department for Education and Skills. ISBN 1 844786560
- <sup>2</sup>*Enthusing the next generation*. A report on the bioscience curriculum by a working group established by the Biosciences Federation. November 2005.

**Dr Margaret Patterson**  
President of the Society

**Philip Wheat** reports on the latest developments within the Society

In the March 2006 issue of the *Microbiologist* the President of the Society highlighted that we were planning to become incorporated whilst still retaining our charitable status. I can confirm that we are progressing this initiative. New governing documents to replace the existing Constitution are being produced. We have been reviewing and revising the draft Articles and Memorandum of Association, which the Society's solicitor has written. The change to become incorporated with charitable status was suggested by the Charity Commission and it should be noted that other learned Societies have proceeded down this line of organisation. The proposed changes for the Society for Applied Microbiology will ultimately have to be approved by the membership.

Another initiative which was mentioned in the last issue of the *Microbiologist* was the Society potentially taking new improved office facilities. The Society has occupied the current office since 1996. Whilst the Blore Tower is a building with plenty of character it is less than ideal as office space. I am currently assessing alternative modern office facilities which are still based in Bedford. A draft of the proposed new lease for these premises has been received and if everything goes to plan it is proposed we will be moving offices in August/September this year.



been planned or to promote future events or other initiatives. In addition, we are now issuing a monthly email bulletin detailing any activities in the coming month. I am sure Lucy Harper will provide you all with more information on this initiative.

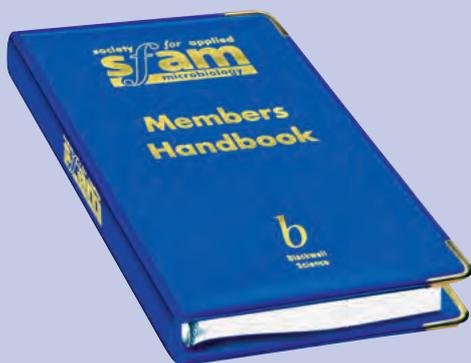
Whilst on the subject of Lucy, I am delighted to announce that the Trustees of the Society have decided to appoint Lucy on a full-time basis as the Communications Officer for the Society. Lucy's role will cover all aspects of communication. These will include being responsible for all issues relating to the website. She will also co-ordinate all areas concerned with public relations and affairs. This will include dealing with the media and if appropriate, drafting and sending out any press releases and arranging press briefings.

Planning is well underway for next year's Winter meeting. The meeting will once again be held at the Royal Society, London. It will be a one day meeting on 11 January 2007. The topic of the meeting will be '*Hospital Acquired Infections*' with a concurrent theme in the afternoon entitled '*Microbiology for Environmental Health Officers.*' With

the wide appeal of the topics the meeting is sure to be popular. Further information can be obtained from the preview on page 27 of this issue of *Microbiologist* as well as the Society office. A booking form will be available on the website shortly.

Officers and myself have attended and are proposing to attend a number of national and international conferences in the coming months. These have included Biomedica (Dublin 26-27 April), American Society for Microbiology (Orlando 22-24 May) and the International Food Technology (Orlando 26-28 June). In addition, I hope to meet many of you at the forthcoming SfAM Summer Conference (Edinburgh 3 – 6 July). I am sure the meeting will be an outstanding success. The scientific programme is topical and up to date with the latest research findings. In addition, the social programme is complete with many activities planned. All this, in the surroundings of the wonderful city of Edinburgh. I look forward to welcoming you all as delegates at the meeting.

**Philip Wheat**  
Chief Executive Officer



You should all by now have received a questionnaire so that we can produce an updated members handbook. The information will also be used to keep you informed of events which have already



# MED•VET•NET

Teresa Belcher reports on the gathering of international infectious disease experts in Malta to share research advances



**MED-VET-NET IS A EUROPEAN** Network of Excellence that aims to improve research on the prevention and control of zoonoses by integrating veterinary, medical and food science research. Comprising 16 European partners and over 300 scientists, **Med-Vet-Net** will enable these scientists to share and enhance their knowledge and skills, and develop collaborative research projects.

## Second General Scientific Meeting

The very successful second **Med-Vet-Net** General Scientific Meeting, took place in Malta between 3-6 May 2006. After nearly two years of joint activities we are now beginning to see the outputs of our collaborative efforts, many of which were presented at this meeting.

Over 180 delegates met for four days at the Dolmen Resort Hotel on this sunny Mediterranean island. Scientists from all **Med-Vet-Net** institutes were represented, and for the first time, the meeting was open to external delegates in order to share new research information and develop greater external collaborations worldwide. The meeting was opened by the Honourable Dr Louis Deguara, Maltese Minister for Health, the Elderly and Community Care.

## High-calibre presentations

The topics selected for the meeting focused on aspects of zoonoses related to epidemiology and risk, detection and control, host-microbe interactions, microbial ecology as well as new and emerging zoonoses. The emphasis of the scientific presentations was to promote current and new findings from both a clinical and veterinary perspective, and 70 scientists gave oral presentations. In addition to this, over 150 posters were also presented.

**Med-Vet-Net** was fortunate to have a number of high-calibre, international

keynote speakers attending the conference. Professor Patricia Smith from the Laboratory of Bio-Anthropology and Ancient DNA, Faculty of Dental Medicine, The Hebrew University of Jerusalem, Israel, presented a talk entitled '*The zoonotic revolution: the impact of domestication.*' Professor Scott McEwan from the Department of Population Medicine, University of Guelph, Canada discussed '*Uses and abuses of microbiological risk assessment.*' Dr Eric Fèvre from the Centre for Infectious Disease, University of Edinburgh, Scotland spoke on '*Emerging zoonoses, animal movements and disease risks.*' Professor Jean-Pierre Kraehenbuhl from ISREC and Institute of biochemistry, Lausanne, Switzerland gave a presentation on '*Host-microbial interactions at mucosal surfaces.*'

**Net**, to develop network relations with other representatives from outside of the network, widen participation to other member states, and to raise awareness of the state-of-art science inside and outside of the network. The meeting also promoted further understanding of the network's scientific aims and objectives and provided an opportunity for the development of collaborative ideas and projects.

On the final day of the conference, a session covering '*Networking for Food Safety*' was held. Dr Marta Hugas from the European Food Safety Authority (EFSA) gave an outline of the newly-formed body that is the keystone of EU risk assessment regarding food and feed safety. Dr Jan Sargent from the Public Health Agency of Canada (PHAC), spoke about the Food Safety Research and



### Further Information

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

Conference Centre, Dolmen Hotel, Malta

Professor Gadi Frankel from the Department of Biochemistry, Imperial College London, England spoke on '*Application of contemporary molecular and cell biology technologies to study host-microbe interactions.*'

## Serious science

The main aims of the annual General Scientific Meeting were to review scientific research supported by **Med-Vet-**

Response Network (FSRRN). The FSRRN is a multi-institutional, multidisciplinary team of more than 50 food-safety specialists from 18 universities, state and federal agencies and agricultural commodity stake-holder groups and is funded by the U.S. Department of Agriculture. Dr Susanna Lukinmaa from the Statens Serum Institut (SSI), Denmark explained the workings of PulseNet Europe, the molecular surveillance

network for food-borne infections in Europe. In conclusion, Dr Claire Cassar from the Veterinary Laboratories Agency (VLA), UK spoke about the new network EUUS-SAFEFOOD, which aims to develop a transatlantic strategic alliance, between food-borne zoonoses research networks in the European Union and the United States.

The Closing session saw Project Manager, Professor Diane Newell talk about the future of **Med-Vet-Net** followed by Dr Alfredo Caprioli and Dr Franco Ruggeri who outlined plans for the next Annual meeting to be held near Pisa in Italy. A summary of the meeting was given by Chairman of the **Med-Vet-Net** Advisory Panel, Professor Bill Reilly, and closing remarks by **Med-Vet-Net** Project Coordinator's Representative, Dr André Jestin.

### ...sun, sea, scenery

This year, **Med-Vet-Net** also departed from their current member countries to find the sun and sea in one of the European Union's southern-most



*Eating by the sea*

countries, Malta. Inhabited since prehistoric times, and with an excellent natural harbour, Malta has always maintained a strategic location at the crossroads of the Mediterranean. It has frequently been a key prerequisite to domination of the Mediterranean by various powers, being first colonised by the Phoenicians and then subsequently by the Romans, Arabs, Normans, the Knights Hospitallers of St. John of Jerusalem, and

the British. All have influenced Maltese life and culture to varying degrees and many delegates took the opportunity to appreciate this aspect of local history.

Several social events were organised to showcase the beauty of Malta. These activities included ocean-side finger buffet and wine reception for the first evening, a night's entertainment comprised of a short tour around Mdina, Malta's medieval capital, followed by a champagne reception on the bastions overlooking the island culminating with a dinner at Bacchus, a reception venue located in chambers built by the Knights of Malta in 1657 on the second evening. A relaxed poolside barbeque overlooking the islands of St Paul was organised back at the hotel for the final evening.

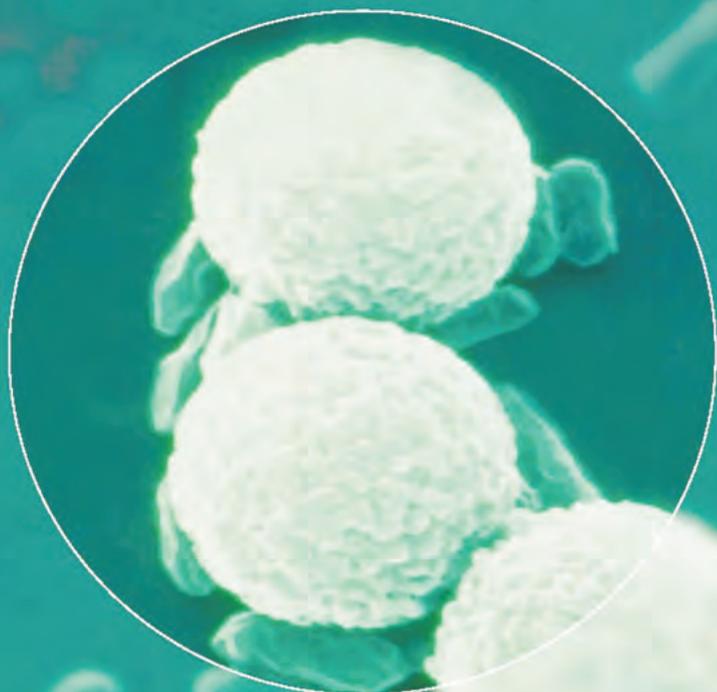
**Med-Vet-Net** gratefully acknowledges the support from FAO, Malta Tourism Authority, Pfizer and Air Malta for the running of this meeting.

**Teresa Belcher**

Med-Vet-Net Communications Director

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## New Members

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

### Australia

Dr E Bartowsky

### Denmark

Dr A Gravesen

### Egypt

Mr Mohame Darwish

### Greece

Dr M Braoudaki

### India

Dr S Reddy Mondem

### Ireland

Ms L A Deering; Mr D Hayes; Dr K Horgan;  
Ms A-K Liliensiek; Miss L Lillis; Dr R Murphy;  
Mr O Ojo; Mr P Sawulski; Miss U M Scallan

### Mexico

Dr C Wacher-Rodarte

### United Arab Emirates

Mrs Ambika Rajesh Ambika

### United Kingdom

Mr G Aboagy; Mr P J Airey; Mr M Boateng;  
Dr C D Campbell; Mrs T Cheetham;  
Miss P A Conway; Dr V Davenport;  
Dr A L Doran; Miss S Easton;  
Mr M J Garland; Mr T Gibbs;  
Dr B F Gilmore; Mr A D Glancey;  
Mr V Gohel; Mr M T Jamal;  
Dr V Javid Khojasteh; Mr C Kwang-Kuk;  
Mrs E Langley; Dr S P Law; Miss S Lee;  
Dr D Lowrie; Mrs A Newaj-Fyzul;  
Miss C Pope; Mr S N Teteyfio;  
Dr John Thomson; Mr David Williams;  
Mr J R Wingate

### USA

Mr H Chen; Dr B Green; Mr K Kauers;  
Dr M Kendall; Dr A J Miller; Dr T B Norris;  
Dr S Perkins; Dr Om Singh; Dr P Sreenivasan

## CORPORATE MEMBERS

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## Sad News

Geoffrey Talbot Banks  
(23/9/33 – 29/3/06).

We regret to inform members of the loss of SfAM member, Geoffrey Talbot Banks. Geoff will be remembered as a lecturer and fermentation technologist at the Department of Biochemistry at Imperial College, London. After a spell at Glaxo in Barnard Castle in the early 1960s, Geoff joined Ernst Chain's team at Imperial College, where he worked *inter alia* on interferon production, publishing several papers in *Nature*. He retired from Imperial College in the early 1990s, settling with his wife, Mary, in the Vale of the White Horse.



## Speakers for Schools Database

On 12 September 2002 the Biosciences Federation (BSF) Speakers Database was launched at the BA Festival of Science.

This database enables university academics to enter details of any talks or workshops that they are willing to give to local schools. The unique feature of this database is that it holds information about the location of each speaker and the distance that (s)he is willing to travel. Teachers then enter the postcode of their school and the system delivers only those speakers who would be willing to travel to that location.

The database can be found at the [biology4all.com](http://www.biology4all.com) website:

<http://www.biology4all.com/talks.asp>

This service is entirely free to use and

is meant to complement the efforts of many universities in publicising their "schools talks" programmes.

The site now has nearly 450 speakers registered. Over the last 12 months the BSF have received about 200 email requests for talks. You may however rest assured that speakers' actual email addresses and phone numbers are not accessible from our website.

If you already visit schools to give talks, or if you would like to start, then we would like to hear from you. Simply send an email to:

[pkrobinson@biology4all.com](mailto:pkrobinson@biology4all.com) with your name, email address and university postcode (for location purposes) together with your major society affiliations to enable an appropriate society logo to appear with your entry in the database.

Upon registration we will then email you a password to enable you to complete your personal details and enter the full details of your talk(s). Further details of how to join this scheme can be found at: <http://www.biology4all.com/join.asp>

## SfAM School Associate Membership



Why not recommend SfAM membership to your local school?

### Benefits

- Quarterly copies of *Microbiologist*
- Full access to the Society website
- Preferential rates at Society Meetings
- All for only £15.00 per annum!

## SfAM Anniversary Photography Competition



Have you taken a striking photograph of your beloved bugs? Do you know someone who has and you'd like to see their work published? Or perhaps you've taken a photograph while attending an SfAM conference which you think is worthy of reproduction?

The SfAM anniversary photograph competition offers you the chance to see your work become part of the society's anniversary commemorations. We are offering the 12 best photographs the opportunity to appear in this year's Christmas gift — a stunning desk calendar. The best overall photograph will also win a bottle of bubbly to help you celebrate 75 years of SfAM!

To enter this competition, please send your photographs to the Editor. The photographs can be entered into one of two categories:

1. **Scientific:** photographs taken in the laboratory of your beloved organisms.
2. **SfAM:** artistic photographs taken whilst attending a SfAM event.

Photographs in this category don't necessarily need to be scientific.



To enter, please provide high quality, JPEG images or original prints, label them with their entry category and post them to:  
SfAM Anniversary Photographic Competition, **Society for Applied Microbiology, The Blore Tower, The Harpur Centre, Bedford MK40 1TQ, UK**, before the closing date of 28 July 2006. Alternatively, email your entry to: [lucy@sfam.org.uk](mailto:lucy@sfam.org.uk) with the subject 'SfAM Anniversary Photographic Competition.'

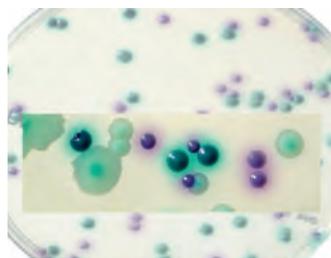


### The colour of confidence



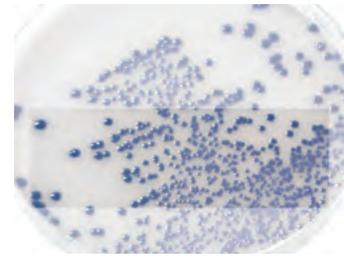
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**Biomedical Scientists** form the foundation of modern healthcare. **Ian Cocking** explores the rewards and challenges of this important work

# CAREERS

## Biomedical Scientist



**W**HEN YOU ASK THE GENERAL public 'who tests the samples Doctors and Nurses take from you in a hospital?' it usually draws a blank facial expression. The fact is, there is an 'unknown army' of healthcare professionals dedicated to the analysis of patient samples of tissue and body fluids to diagnose disease and monitor treatment.

Biomedical Scientists (BMSs) in the NHS and private healthcare form the foundation of modern healthcare. Patient treatment is based upon the results of vital tests and investigations performed by Biomedical Scientists. It is estimated that up to 70% of diagnoses of all illnesses are made on the basis of laboratory results.

There are very few hospital departments that could function without the input from Biomedical Scientists. From A&E to Intensive Care, BMSs test and analyse patient samples to ensure patients obtain the optimal health support and treatment they expect from the NHS. Biomedical Scientists are based in hospital pathology departments surrounded by a myriad of sophisticated and expensive analysers and hi-tech laboratory equipment including microscopes, automated analysers and computers. They work continuously to provide essential patient healthcare; some departments are run 24hrs a day, 365 days a year.

The work of Biomedical Scientists is diverse, complex and specialised. Within Pathology there are a network of departments working together to provide the service. These include:

- Clinical Biochemistry
- Haematology
- Medical Microbiology
- Histopathology
- Cytology
- Immunology

These departments may then be subdivided into smaller more specialised departments that include:

- Virology
- Molecular Genetics
- Blood Transfusion/Blood Bank
- Coagulation

### Career Progression and Training

I stumbled into a career as a Biomedical Scientist accidentally, but in the eight years I have spent in this area I have found the career rewarding, and both physically and mentally challenging. If you want a career that is diverse and

technically challenging on a daily basis, then this is the career for you.

My career started as a student placement within the Microbiology Department at Northern General Hospital, Sheffield. This formed one year of the four year sandwich degree course in Biomedical Sciences (BSc Hons) at Sunderland University. After graduating in Biomedical Science, I returned to Sheffield, but this time to the Royal Hallamshire Hospital (RHH), to complete my training towards becoming a State Registered Biomedical Scientist in Microbiology. At the time, this involved at least two years at the bench learning the basics of laboratory diagnosis in Microbiology and completion of a logbook of competence. A *viva-voce* by an external assessor at the end of two years determined whether you were competent to practice unsupervised as a qualified BMS. This has since been superseded by the Health Professions Council (HPC) Registration of Competence Portfolio (6-12 months to complete) to become a registered Biomedical Scientist and the Institute of Biomedical Sciences (IBMS) Specialist Portfolio (1-2 years to complete) to become a Registered Specialist in a chosen discipline(s).



To be a competent Biomedical Scientist requires life long learning. Continued Professional Development (CPD) is fundamental to maintaining knowledge and proof of Continual Professional Competency (CPC) will soon become mandatory to ensure safe practice and entitlement to stay on the HPC Register. The career is structured in such a way that CPD and higher degree/specialist qualifications are essential to career progression.

I continued to work at the RHH for a further four years, during which I completed an MSc in Pathological Sciences at Sheffield Hallam University, along with various CPD activities. After gaining my MSc and accruing sufficient experience and CPD points as a qualified BMS, I met the requirements to progress up the BMS career ladder. In 2003, I moved to Doncaster Royal Infirmary for promotion to a Senior BMS. Three years on, I now form part of an experienced laboratory team dedicated to providing a quality diagnostic microbiology service to the Doncaster and Bassetlaw NHS Foundation Trust.

### Duties of a Senior BMS

A Senior BMS is responsible for the supervision and management of other BMS staff within a sub section of a laboratory. This requires a professional attitude and a broad knowledge and understanding of the pathology discipline of your speciality. As a Senior BMS you are a point of reference for staff and ensure all procedures within the laboratory are performed according to standard operating procedure.

Laboratory testing involves interpretation of test requests using guidelines and personal judgement. This often requires the review of a patient's clinical history, current drug treatment, past exposure to disease and vaccination status. The daily duties of a BMS involve constant decision making, interpretation of test results and reflex testing from that result.

Within any laboratory, safety is paramount and as a Senior BMS you are responsible for monitoring health and safety within the department, regularly performing risk assessments on laboratory procedures in conjunction with the laboratory manager to ensure safe working practice at all times.

### Life as a Senior BMS

A normal day within the laboratory starts at 9:00am, whereby I first check my diary and emails for possible departmental meetings, training meetings or appointments with sales representatives from the various companies from which the department purchase laboratory supplies/equipment. After a few minutes (giving time for the late arrivals to try and sneak into the laboratory unnoticed) it's time to put on the white laboratory coat and enter the laboratory to liaise with the other senior

BMSs and decide how to manage the laboratory sections to ensure every section is adequately covered by a combination of qualified BMS and Medical Laboratory Assistant (MLA) staff.

Sitting at the bench it's time to start reading the 'Blood Culture' bench. 'Blood culture' is one of the few automated areas of Bacteriology, where complex analysers continuously monitor blood samples from patients with a suspected bacterial infection of the blood.

My first task involves removal of positive cultures from the analyser and preparing Gram films to determine the presence of bacteria or yeasts within the culture. Depending on the Gram reaction of the isolates, I determine which antibiotics to test against the isolate and subculture the blood culture onto appropriate agar plates for overnight culture to confirm my diagnosis.

Next, its time to examine the culture plates from the previous day to confirm initial laboratory findings. I examine the plates and identify the bacteria present and read the corresponding antibiotic sensitivity susceptibility plates to determine the antibiogram of the bacterial isolate. If I make a mistake here, the patient may be given the wrong antibiotic, resulting in treatment failure, causing a prolonged illness for the patient or worse.

I record the details onto the laboratory computer system and worksheets before passing the details on to our Consultant Microbiologist for approval.

It's 10:30 am — time for a break, but my break is interrupted by the arrival of an urgent CSF sample from a young child with suspected bacterial meningitis. In microbiology this can be a life or death situation and subsequent treatment of the patient is highly dependent on the laboratory findings. I examine the CSF sample to determine the presence of infection. The CSF has a significant high white blood cell count, but I cannot see any bacteria in the Gram film (have I missed them?). I ring the result to the requesting clinician who informs me the child has already been administered antibiotics on attendance by the General Practitioner. That may explain the absence of bacteria in the Gram film.

After a quick coffee with the other staff and putting the world to rights, I return to finish my work in the section. Next I move to other benches to check progress with other staff. My signature is required to verify confirmed isolates of *Neisseria*

*gonorrhoea*, from two people attending the hospital GUM clinic. I check the colony morphology on the culture plates, Gram films and API NH profile (commercially available biochemical test kit) and authorise the results accordingly.

Next it's the Faeces bench, and three potential cases of *Salmonella*, four cases of *Campylobacter* and a faecal smear containing *Cryptosporidium* have been detected by the BMS. I verify the results and forward them to the Consultants. Where appropriate, the significant isolates are saved and forwarded to a reference laboratory for typing. The results will form part of the national surveillance and epidemiology of *Salmonella*, which, in liaison with Communicable Diseases and Environmental Health help serve to protect the public from gastro-intestinal infections.

There's no rest as an Infection Control Nurse enters the laboratory to inform me there is a potential Methicillin Resistant *S. aureus* (MRSA) outbreak on one of our hospital wards and they are screening potential contacts. A quick check of our media supply in the fridge confirms we are low on agar plates used to detect MRSA. A quick phone call to our suppliers and they assure me more stocks will arrive by tomorrow lunchtime — crisis averted.

An important role of a Biomedical Scientist is to research and evaluate current and new diagnostic techniques to improve patient care. Currently I am evaluating a new chromogenic agar media for the rapid screening of MRSA from suspected cases. The new media is run in parallel with our existing method and compared on a like-for-like basis. I sit down at my desk to review the data so far and after a few statistical calculations and costs, the results appear favourable towards the new agar media. My next task is to present the findings of the evaluation at next months departmental audit meeting.

I manage to escape the laboratory for a lunch break. Afterwards, it's time to attend this month's 'Senior Management Meeting.' The pending Continuous Pathology Accreditation (CPA) inspection is discussed between the senior and chief BMSs, Laboratory Manager and Consultants to determine what action is required and who is responsible for carrying out that action. I am assigned the task of updating Standard Operating Procedure (SOP) documents for my

laboratory section and writing a new SOP for the implementation of a new laboratory test kit for the rapid identification of (MRSA) from routine culture agar plates.

Back in the laboratory, its time to ensure other staff are completing their tasks and help out if required. It's not long before my opinion is required. A fellow BMS has found an auramine smear of sputum containing acid fast bacilli. A review of the clinical details and patient history indicate that the smear is from a male returning from Africa after visiting family. He's attended the A&E department complaining of a persistent cough for several weeks and complains of feeling hot and sweaty during the night. A discussion ensues with the Consultant Microbiologist and it's decided to isolate the patient and begin treatment for suspected *Mycobacterium tuberculosis* (TB). I pass the details onto our Infection Control Nurses who begin to contact close contacts of the index case to be screened for possible cross infection.



Later that afternoon I have a meeting with a Sales Representative from a laboratory equipment supplier. He wonders if we are interested in a new automated method for urine microscopy. We review the literature on the system and agree on a field visit to a local hospital to see the analyser in action. The visit gives me chance to speak to the BMS staff that use the analyser and gauge their opinion.

Back in the laboratory, I answer the telephone to find a new Senior House Officer is unsure how to investigate a patient for Bilharzia. I inform him that he needs to obtain a terminal urine specimen (final passing of urine during micturition)

between 12 and 1pm the next day and send it to the laboratory marked for my attention.

The rest of the afternoon is spent processing specimens as they arrive in the laboratory. As 5:30pm approaches the daily workload of specimens is finally cleared and it's time to do some basic laboratory housekeeping. All BMS and MLA staff clean and restock laboratory benches and maintain laboratory equipment in readiness for another day at the laboratory.

## Career Opportunities

Biomedical Science is a demanding career, both physically and mentally. It requires personal expertise and this is something all BMSs expand during their career. Biomedical Science is a continually changing, dynamic profession with long term career prospects including management, research, education and specialised laboratory work.

Current opinion is to expand the role of BMS to a higher specialised level, possibly covering some of the roles currently performed by Medical Consultants. This would allow BMSs to help, in light of the shortage of Medical Consultants in the pathology disciplines.

Although in this career patient contact is minimal, the impact a Biomedical Scientist has on patient care is still highly regarded. As a BMS you have the reward of knowing your laboratory findings are contributing to patient care either in hospitals, or in the community, even when it seems a thankless task.

NHS pathology laboratories are not the only place BMSs are employed. BMSs are employed within the veterinary service, the Health & Safety Executive, universities, forensic laboratories, pharmaceutical companies and Her Majesty's Forces.

### Ian Cocking

Senior BMS, Microbiology Department,  
Doncaster Royal Infirmary

If you are considering a career in Biomedical Science then the following web sites provide further detailed information:

■ [www.ibms.org.uk](http://www.ibms.org.uk)

■ [www.nhscareers.nhs.uk](http://www.nhscareers.nhs.uk)

■ [www.hpc-uk.org](http://www.hpc-uk.org)

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## Am I eligible - can I apply?



This new award is intended to assist Society members in developing countries and Eastern Europe to visit laboratories and give lectures and training in appropriate areas of applied microbiology, or support overseas members to visit UK laboratories to receive training in appropriate areas of microbiology, or to support technology transfer in applied microbiology for which sources of funding do not exist.

Nominations for awards will normally be considered by the Society's Awards panel in March, July and November each year.

To apply, please read the guidelines below and then submit your application by email or post to the Society Office.

### GUIDELINES

1. Individual awards up to a maximum of £5000 will be considered.
2. The laboratory supporter must be a full member of the society and have held membership for at least 3 years.
3. Detailed information must be provided about the relevance of the application and the available local support.
4. Each application must be accompanied by full supporting documents.
5. A condition of the funding is that an appropriate report must be written for publication in SFAM Microbiologist magazine together with photographs where possible.
6. Applications should be sent by email or by post to the Society Office.

[www.sfam.org.uk/members/prizes.php](http://www.sfam.org.uk/members/prizes.php)

## Report on a visit to the Kwame Nkrumah University of Science and Technology (KNUST) Kumasi, Ghana



Students enjoying the open seminar

**T**HE VISIT TOOK PLACE between 24 October and 3 November 2005, during which time I gave five lectures on Health-Related Environmental Microbiology to MSc students in the Department of Theoretical and Applied Biology. KNUST:

1. Survival and persistence of pathogens in the environment
2. Zoonoses: Re-emerging infections
3. Zoonoses: Emerging disease
4. Pets and wild birds as reservoirs for disease and vectors of pollution
5. Epidemiology and environmental microbiology of *Vibrio cholerae*.

The last lecture was particularly timely as it coincided with an outbreak of cholera in Kumasi.

I also gave an open research seminar to the College of Science on '*Wild birds and the spread of disease: bird flu.*' This was gratifyingly well attended and produced some interesting questions.

The collaboration in environmental microbiological research between the departments of Biological Sciences at Lancaster University and KNUST grew out of a British Council Link programme in the mid 1990s to develop an MSc in Environmental Science at KNUST. During my visits to Kumasi I realised that there was useful health-related environmental microbiological research that could be done and obtained funding from the Society for General Microbiology and UNESCO for further research visits, microbiological equipment and consumables. At that time, Kwasi Obiri-Danso, a lecturer at KNUST, came to

Lancaster to do a PhD on the '*Seasonal variation of indicator and pathogenic bacteria in coastal and inland bathing waters.*' Lancaster City Council provided the funds for the microbiological monitoring. This proved very successful and resulted in six papers in peer-reviewed journals and eight posters at international conferences. The work has influenced the way in which regulatory bodies such as the Environment Agency view bathing waters. In particular, we showed that the time of the day that samples are taken influences compliance with the EU Bathing Water Quality Directive; and that flocks of wild birds are major sources of pollution.

Since his return to Ghana in 1999, we have continued our collaboration, publishing papers on drinking water, all of which have had a local impact above and beyond the usual scientific interest.

Dr. Elias Aklaku Integrated biological treatment and biogas production in a small scale slaughter house in rural Ghana, which has now been accepted for publication in Water Environment Research. Elias, an engineer with an eye to solving local problems, has built a small scale integrated abattoir and waste digester in Ejura, a small rural communities north of Kumasi. The digester converts waste from the animals into methane, which provides energy for singeing carcasses and for use by the wider community. It also provides clean effluent that can be used for irrigation. In addition, there have been reductions in nuisance smells, flies, dogs and vultures. The use of methane as energy has also

lessened the burden of wood collection and reduced smoke levels in houses, which WHO has identified as a major cause of death of women and children in the Developing World.

I also spent some very productive time visiting research field sites and discussing the current applied microbiology research projects being done by Kwasi and his research students. Tyhra Carolyn Kumasi is working on the ways in which the water quality of the Barekese Reservoir, the major source of water for Kumasi, is being affected by anthropomorphic changes in land-use in its catchment area. Linda Aurelia Andoh is tracing the transfer of parasites from irrigation water used on farms onto salad vegetables, both on the farm and at point of sale. Other students are doing similar work but concentrating on tracing indicator bacteria from irrigation water onto fresh produce. This work fits in well with research that we are doing in Lancaster and in the Western Balkans, funded by WaterWeb, an EU programme, on the connection between the microbial quality of irrigation water and the contamination of fresh fruit and vegetables at point of sale. Since this visit, I have returned to Ghana with Joanna Heaton, a Lancaster PhD student working on this project, and we have extended the range of bacteria being looked at in Kumasi to include *Listeria*, *Aeromonas* and *Salmonella*.

Socially, the visit was great fun. I was well looked after by Kwasi and his colleagues, both from the University and the wider community. The adventure highlight was a seven-story-high rainforest canopy walk in the Kakum National Park, Elmira, Cape Coast. However, the evenings spent in the University Senior Staff House drinking Star lager and putting the World to rights, linger longest and most fondly in the memory.

I gratefully acknowledge the Overseas Development Award from SfAM that made the visit possible.

#### Further Information

on the connection between health-related environmental microbiology at Lancaster University and KNUST can be found on the Lancaster Alumni web site :

■ <http://www.alumni.lancs.ac.uk/Upload/Content/files/203/ParaFiles/STEPS2005.pdf>

#### Dr. Keith Jones

Department of Biological Sciences, Lancaster Environment Centre, Lancaster University

### Food Safety Implications of Neurotoxin Producing *Clostridium* species



**I** ARRIVED IN READING FROM Nigeria on 1 June 2005 to begin a four month visit to the School of Food Biosciences at the University of Reading, supported by an Overseas Development Award from SfAM.

I reported to the School the following day and was greeted by Dr Bernard Mackey — an old friend whom I first met nine years ago. I was then shown round the new laboratory facilities and introduced to my project team and other members of the group. I was going to work on a project looking at the growth properties of some potentially pathogenic clostridia.

The main species of clostridia associated with foodborne illness are *Clostridium botulinum* and *C. perfringens* and much effort has been directed towards understanding the factors controlling their growth, toxin production and the resistance of their spores. Other clostridia may cause spoilage problems in cheese, canned fruit and pasteurised acid sauces, but are not considered to be dangerous. *Clostridium butyricum* and *C. barati* are normally regarded as harmless but rare strains that have acquired the botulinum neurotoxin gene have caused infant botulism or foodborne illness. *Clostridium tertium* and *C. bifementans* occasionally occur in food in high numbers, but the food safety implications of this, if any, are not fully understood. The project I was joining, funded by the Food Standards Agency, aimed to examine the factors controlling growth of these organisms in

broth and model foods and to use gene probe methods to gauge the frequency of toxigenic strains in food raw materials and the environment.

Part of the project would involve working with toxigenic strains of *Clostridium butyricum* and *C. barati* and, although I am an experienced microbiologist, I had to receive specific training on working in a containment level 3 laboratory before starting this aspect of the work. It took several weeks to complete the training and receive approval from the Safety Officer. Meanwhile, I familiarised myself with the anaerobic techniques using non-toxigenic strains to start with. It took the first couple of weeks to learn the technique of culturing strict anaerobes, and to investigate their ability to grow at different temperatures and on different types of media for optimal growth and spore production. I then examined the growth of *C. butyricum* in dairy based desserts and growth of *C. barati*, *C. tertium* and *C. bifementans* in pate at different temperatures and did some work defining pH limits in broth. The physicochemical parameters of the food material such as pH and water activity were measured. I had an opportunity to use API strips to check the identity of the test strains and also to identify *Clostridium* isolates obtained from food. I also learned methods for DNA extraction and the use of PCR for identification and detection of the toxin gene.

This research project presented an opportunity to learn new techniques in isolating and culturing strict anaerobes. The most fascinating was a very simple modification of the standard plate count method. This will be very useful to my colleagues and students back home. I wish to thank Dr Richard Sherburn and Dr Bernard Mackey who readily took on the role of supervisor. I have benefited a great deal from his wealth of experience in research. I must not fail to mention my project mate Hamid who was always ready to assist me on any issue in the lab and other members of our laboratory who rendered help whenever necessary. Words are not enough to express my sincere gratitude to SfAM for giving me this opportunity, a rare asset to those of us involved in teaching and research from developing countries.

#### Dr O.O. Aboaba

Department of Botany and Microbiology, University of Lagos, Nigeria



# Bioremediation: bugs keeping it clean

**I**N RECENT YEARS, SOCIETY has become increasingly concerned with maintaining and preserving our natural environment, with the treatment and disposal of waste one of the most important problems facing mankind.

The large-scale manufacturing, processing and handling of chemicals have led to serious surface and subsurface soil contamination by a wide variety of hazardous and toxic hydrocarbons. More and more waste is being generated and the cumulative effects of pollution have led to increased public concern, and stricter legislation for the disposal of waste. Some wastes can be re-used, but in most cases, the removal and purification of waste poses two problems: the energy input required for the process, and the problem of dealing with the remaining concentrate. In addition, many new chemicals such as polychlorinated biphenyls (PCBs) and trichloroethylene differ substantially in

chemical structure from natural organic compounds and are much harder to degrade. Additionally, other compounds such as polycyclic aromatic hydrocarbons (PAHs), produced by incomplete combustion of natural organic materials, are also toxic and those with higher molecular weights, also more resistant to degradation. There is clearly a definite need for new and more environmentally sound methods of disposal.

## The good and the bad

It is hard to imagine that the vast majority of life on this planet is microscopic and it is even harder to comprehend that we know hardly anything about these microorganisms because fewer than 2% of them can be grown in a laboratory. Generally, people tend to associate bacteria as being 'bad' – in particular those causing disease. However, in reality, many microbes enhance our well-being and greatly influence and benefit the environment

that we live in. Microbes are the major players in the synthesis and degradation of all sorts of molecules in the environment. In all habitats, microorganisms degrade dead organisms, making nutrients available for the future growth of other living things. This is a natural process, and understanding which microbes are in each ecological niche and what they are doing there, is critical for our understanding of the world. Scientists have also strived to better understand this, in order to enhance the process and ultimately use bacteria to do even more good in an environmental sense.

## What is bioremediation?

Bioremediation is the use of living organisms to clean up environments that have become contaminated with organic or inorganic substances. Bacteria and fungi 'feed' on the hazardous pollutants and are able to convert them into less toxic compounds. This is an ideal treatment method as it transforms the

pollutants rather than simply moving them to another location.

Bioremediation is both one of the oldest but also one of the newest environmental remediation technologies. It has been used throughout recorded history to turn organic waste into fertilizer by the use of compost heaps. Our sewerage wastes are treated using biodegradation in wastewater treatment plants, and the petroleum industry has relied on bioremediation for many years to clean contaminated soil.

Research has been conducted on the possible uses of bioremediation since the 1940s. Originally, much bioremediation focused on the breakdown of organic contaminants to benign end-products, and since the 1970s has been used for the *in situ* clean up of fuel contaminated soil and groundwater. Bioremediation has since developed into a popular method of pollution remediation. Many new active remediation technologies utilize bioremediation: bioventing, landfarming, bioreactors, composting, bioaugmentation and biostimulation.

## Influencing factors

Through studying natural processes, researchers have been able to determine the conditions necessary for degradation. Even though very often the actual microbes responsible for degradation are not known, it is still possible to research which pollutants they can degrade and under what conditions this best occurs. Several factors influence the success of bioremediation including the environment, contaminant type and concentration, soil type, and the condition and proximity of groundwater. For successful bioremediation, these factors have to be considered on a site-by-site basis. The challenge of effective bioremediation is to provide both the physical and chemical conditions which are most favourable to the growth of these microbes and thereby maximizing degradation of the pollutant.

The environment in which the contaminated site exists influences the type of organism that can be used. For example, in cold environments (0°-15°C) psychrophilic organisms would be effective, whereas in hot environments (>45°C) thermophilic organisms would be effective. Temperature, pH, heavy metal concentration, microflora and microbial diversity are some of the environmental factors that must be

considered. Sometimes, extreme contaminant concentrations can be toxic to the microbes and inhibit their effectiveness. Scientists need to understand the specific microbial environment and its threshold level to the relevant contaminants. In some cases, high concentrations of contaminants can be toxic to the microbe, damaging the cell membranes.

The soil type of the area must be known in order to determine whether or not *in situ* treatment is possible. In order to treat soils, microbes must have continual access to nutrients to promote growth. Soils with high clay content are more likely to restrict the flow of nutrients to the microbes. Conversely, sandy soils are well aerated and well structured, allowing nutrients and oxygen to flow and are therefore more suitable to bioremediation.

Proximity of groundwater must be taken into account when trying to treat contaminated soil alone. If the groundwater is shallow, this could be easily be contaminated and allow further movement of the pollutant.

## Helping things along

If practical, the environment may be adjusted to provide optimum conditions for breakdown of the contaminants. Treating a contaminated site can be compared with looking after a farm and growing crops: you need to provide the bacteria with optimal conditions for growth. The main limiting factors in bioremediation are the carbon source, oxygen, nitrogen, phosphorus and water. If the right conditions are provided, then the bacteria will 'eat' at the optimal rate, and decomposition is facilitated.

Over time, ageing and weathering of the soil can make the contaminants less available due to chemical oxidation reactions and the incorporation of contaminants into the organic matter. This decrease in bioavailability can be overcome by the use of chemical or bio-surfactants during the biodegradation process.

## Bioremediation technologies

Generally, bioremediation technologies can be classified as either '*in situ*' or '*ex situ*'. *In situ* bioremediation involves treating the contaminated material at the site while *ex situ* involves the removal of the contaminated material to be treated elsewhere. A common *ex situ* method is landfarming. This is when the

contaminated soil is removed from the polluted site and is treated in a 'farm'. Frequent tilling provides the crucial oxygen for the microbes to survive, essential nutrients are supplied by application of fertilizers, and the soil is irrigated to provide water. An even more stringent variation of this is a Bioreactor Landfill. These operate to rapidly transform and degrade organic waste, through the very controlled addition of liquid and air to enhance the microbial processes. These can be configured to be aerobic, anaerobic (which produces methane gas often used for energy projects) or hybrid, utilizing a combination of methods.

Another *ex situ* method includes soil washing. Here, contaminants sorbed onto fine soil particles are separated on the basis of particle size from bulk soil in a water-based system. The wash water may have a basic leaching agent, surfactant, or chelating agent added, or pH adjusted, to help remove organics and heavy metals. The wash water and various soil fractions are usually separated using gravity settling. Solvents can also be used in a similar way to separate organic and metal contaminants from the soil in a process known as solvent extraction.

Common forms of *in situ* bioremediation include air sparging and bioventing. Air sparging involves the injection of air or oxygen through a contaminated aquifer. Injected air traverses horizontally and vertically in channels through the soil column, creating an underground stripper that removes volatile and semi-volatile organic contaminants by volatilisation. The injected air helps to flush the contaminants into the unsaturated zone. Oxygen added to the contaminated groundwater and vadose-zone soils can also enhance biodegradation of contaminants below and above the water table. Bioventing uses extraction wells to circulate air through the ground, sometimes pumping air into the ground. Another *in situ* method includes soil vapour extraction where a vacuum is applied to the soil to remove volatile contaminants.

## Success stories: Exxon Valdez spill

One of the most famous cases of bioremediation was in the clean up of the Exxon Valdez oil spill in Puget Sound, Alaska in 1989 when nearly 11 million gallons of crude was spilt. At this point,

bioremediation had been studied to some degree, but the effectiveness of its application on such a large area of contamination was unknown. Scientists studied the local contaminated environment and found a large community of microorganisms which made it advantageous to try bioremediation and made it unnecessary to introduce microbes. They studied the limiting factors to the natural degradation of the hydrocarbons in the oil and found that concentrations of available nitrogen and phosphorous in seawater were the limiting factors. Tests found that the application of fertilisers containing the limiting reagents to test areas on the shoreline assisted in the breakdown of the oil, and a visible reduction in oil was seen on rocks and sand particles. Additionally,

environmentally sound remediation technique, and its use has expanded to areas such as sludges, surface waters and process waters contaminated with pesticides, metals, crude oil and industrial solvents.

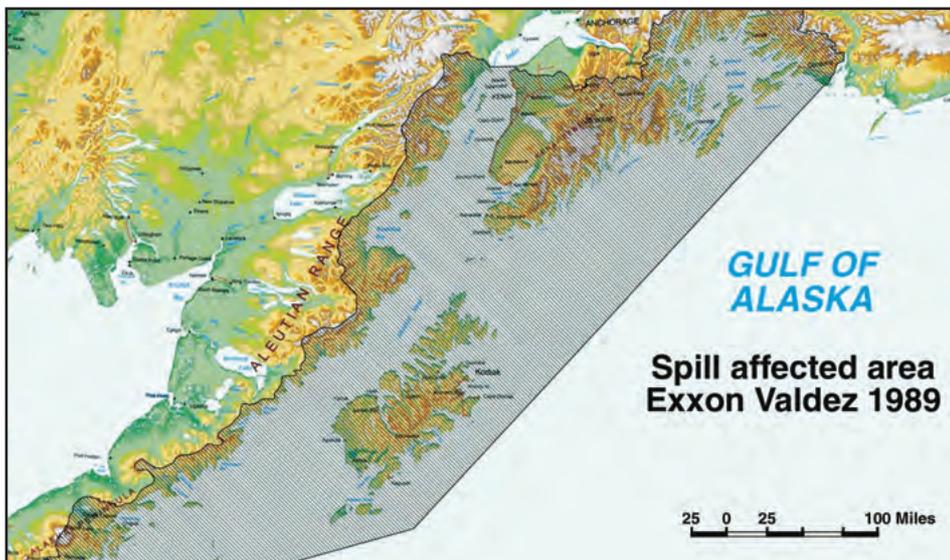
Interest in bioremediation of polluted soil and water has increased even further in the past two decades after it was recognized that microbes were able to degrade toxic xenobiotic compounds that were previously thought to be resistant. The microbial processes occurring are extremely complex, and while scientists know that microbes have the primary catalytic role in bioremediation, their knowledge of the alterations occurring within the microbial communities remains relatively unknown. Research is continuing to look at the interactive and

are extremely diverse, PAH-degrading microbes need to either have a range of enzymes capable of accepting the different PAH substrates, or have enzymes with broad substrate specificity. Consequently, some of the more complex PAHs may only be partially catabolized or not transformed at all. There is also evidence that microorganisms have the capacity to evolve catabolic systems for mineralization of xenobiotics or newly introduced synthetic compounds.

Genetic engineering is being studied as a way to increase the biodegradation capabilities of microbes. In order to design improved, contaminant-degrading microbes, scientists need to understand the current metabolic processes and even create new metabolic routes. This is indeed an exciting and growing area, which will require the combined effort of expertise from microbiologists, biochemists and geneticists as well as chemical and environmental engineers.

Additionally, combining chemical, physical and biological treatments may also improve the extent of degradation. For example, wood-rotting fungi have evolved biocatalytic systems that can make compounds more suitable for microbes to transform. Other agents such as ozone, potassium permanganate or ferrate can also promote initial redox reactions.

In light of new regulations requiring cleanup of many polluted sites and considering the expense of other remediation methods, bioremediation is definitely considered the best option for many situations in the future. Limiting factors in biodegradation can be overcome by optimizing growth conditions, improving activities of the natural soil flora, isolating or engineering better strains and designing microbial consortia of suitable organisms.



tests of the tidal waters and the absence of algae growth concluded that excess levels of nitrogen and phosphorus were not reaching the ocean.

Overall, the clean up was regarded as a successful example of the possibilities of bioremediation. It was estimated that under natural conditions it would be five to ten years before natural conditions were achieved, whereas, with bioremediation, and the provision of optimal conditions, this would be possible in two to five.

### Current research activities and the Future

Bioremediation is a continually growing field, and research is progressing to further the cost-effectiveness and expand application possibilities. It is becoming recognized as a powerful,

interdependent roles played by individual species in these communities, and to characterize key enzymatic reactions that participate in contaminant transformation. This knowledge will assist in the engineering of biocatalysts with improved substrate specificities and reaction rates.

There has also been significant research into degradation in anaerobic environments, focusing on the catalytic mechanisms that facilitate the anaerobic catabolism of pollutants. Anaerobic degradation systems require terminal electron acceptors such as iron III, manganese oxide or nitrate to replace the function of oxygen in aerobic systems.

The partial transformation of contaminants, known as 'cometabolism' is also an area of research that is of much interest. For example, as PAH molecules

### References

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- <http://www.clu-in.org/techfocus/> - Hazardous waste cleanup information
- <http://www.bact.wisc.edu/> University of Wisconsin

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**75th Anniversary Conference  
1931 - 2006**

**Living together:  
polymicrobial communities**

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



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

There will be sessions on:

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

# Programme

## Monday 3rd July

Arrive and Register: Apex International Hotel, Edinburgh.

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

Professor Max Sussman. Lecture Theatre, The Royal Museum of Scotland

**19.00-20.00: Drinks Reception.**

From **20.00: Evening at leisure / Quiz night (optional)**

## Tuesday 4 July

### Session 1. Physiology and functionality of polymicrobial communities.

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

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

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

**10.45-11.15** Coffee/ posters.

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

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

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

**13.00-14.00** Lunch/Trade Exhibition

### Session 2. Influencing polymicrobial communities.

**14.00-14.35** Combating polymicrobial communities: learning from Nature.  
Dr Jeremy Webb, University of Southampton, UK

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

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

**15.45-16.15** Tea/posters

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

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

**17.30-19.00** Trade Show

## Wednesday 5th July

### Session 3. The gut microflora

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

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

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

**10.45-11.15** Coffee/ posters.

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

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

**12.25 -13.30** Lunch.

### Session 4.

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

**19.30-20.00** Drinks reception

The Hub, The Royal Mile.

**20.00 - late: Conference and 75th Anniversary Dinner**

## Thursday 6th July

### Session 5. Bioremediation

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

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

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

**10.45-11.15** Coffee/ posters.

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

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

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

**13.00-14.00** Lunch and Close

# BOOKING FORM and INVOICE

**SFAM SUMMER CONFERENCE 3 - 6 JULY 2006**

## Living Together: polymicrobial communities

Only ONE person per form please. If additional forms are required please photocopy this one

CLOSING DATE FOR REGISTRATIONS: Friday 9 June 2006. A LATE BOOKING FEE of £30.00 will be applied to all bookings made after this date.

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	Full Members	Student, Honorary, Associate & Retired Members	Student Non - Members	Non - Members
<b>Whole Conference Rate:</b> inclusive of registration fee, coffee breaks, lunches, Society dinner and accommodation in the Apex hotel for the entire conference	£500.00	£250.00	£500.00	£700.00
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<input type="checkbox"/> I wish to attend the Society Dinner on Wednesday evening (This is included in the Whole Conference Rate but costs £50.00 for delegates who elect to pay the Day rate). Please note that numbers are limited:	£ <input type="text"/>
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## January Meeting 2007 ▼

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- How and why do enteropathogens make you ill?
- Norovirus and Hepatitis A — an important cause of foodborne illness?
- How do foodborne pathogens emerge?

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The full programme for this meeting will be available soon on the website at:  
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Scientist, Craig Venter now plans to make microbes produce clean energy

# MAKING HISTORY



Alun Anderson reviews the controversial career of Craig Venter

**C**RAIG VENTER, The maverick who led the private-sector human genome sequencing, now has plans for clean energy. He says he never aimed to “privatise” the genome and thinks science and commerce can be best friends. If you had led a team that sequenced the human genome, then became the world’s first biotechnology

billionaire (on paper, at least), and had just finished sailing around the world on your own 95-foot yacht, what would you do next? How about creating life from scratch, saving the Earth from global warming and, as a by-product, ending western dependence on imported energy? That’s what Craig Venter, the scientist whose private company raced the rest of the world to

sequence the human genome, is apparently up to - and I’ve come to meet him in his London hotel to hear more. If the stories were about anyone other than Venter, I would assume they were just dreams. But he has a record of going where no one else would dare, which is why he is described as a maverick, pirate, opportunist, egoist and the ‘bad boy’ of science. And now

he has raised a lot of money for a new company looking at novel sources of energy, and has just recruited a top US department of energy official, Aristides Patrinos, to join him. Patrinos is well connected to those who set US energy policy and is said to have had a hand in President Bush’s recent state of the union commitments to finding new energy sources.

Venter is pacing his hotel room, blue-eyed and barefoot, as though still on the deck of his yacht. He was up until 3am trying to out-drink the editor-in-chief of *Nature* and coffee is in plentiful supply. Before hearing about his energy plans, I want to get his side of the story of the race for the human genome and his views on private versus public funding for science. I know he feels he has been unfairly attacked by the British press, including *New Scientist*, the magazine at which I was editor-in-chief when the human genome project was going at full speed.

But first some background on how Venter got that bad boy reputation. Back in 1998, the vast publicly funded consortium to sequence the human genome had been rumbling along for years, first under the leadership of James Watson, the co-discoverer of the structure of DNA, and then under Francis Collins, who discovered the gene for cystic fibrosis. The planned completion date was 2005.

Then, in May 1998, all hell broke loose. Venter announced he was going to set up a privately funded company—later named Celera—to sequence the entire human genome, using radical new methods at a fraction of the cost of the public programme and in only a couple of years. The reaction from the public consortium was a mixture of rage that anyone would dare to compete with biology's equivalent of the Apollo programme and fear that Venter's company might not only beat it to the sequence but also obtain commercial rights over the use of the data. There was also much anxiety that governments might think twice about continuing their funding at all. Venter had not helped matters by suggesting that the global consortium of distinguished scientists should leave the human genome to

him and 'do the mouse genome' instead. What followed was the scientific drama of the century. Sequencing the human genome was a great scientific goal that was expected to give a huge boost to biology and medicine. Many of the scientists involved were brilliant overachievers who had their eyes set on a possible Nobel prize. But although everyone wanted to win, no one wanted to admit he had entered into anything as unseemly as a race.

A former head of Georgetown University, an institution founded by Jesuits, once told me, 'scientists are the only people more hypocritical than us Jesuits.' Just as a candidate for Pope would not go around saying 'vote for me,' but would quietly murmur about his sole wish being to serve God, the scientist publicly claims his 'god' to be the progress of truth. This progress can be achieved only by the sharing of results: everyone stands on everyone else's shoulders. But, of course, personal advancement only comes from beating everyone else. In the world of science, competition always has to wear the mask of co-operation.

Scientists on the public consortium wanted to win, but under the banner of co-operation and 'the principle of free access to genetic information,' as John Sulston, who ran the British part of the programme, put it. Every bit of the data should be freely available to all scientists and none of it used to give anyone a commercial edge or, even worse, patented so that others would have to pay a fee to use it.

Sulston worked at the Sanger Centre (now the Wellcome Trust Sanger Institute) in Cambridge. Thanks to hundreds of millions of pounds of funding from the Wellcome Trust, he

had the biggest genome centre in the world, and he was the only player with no need to fear the US government cutting his funds if a private alternative seemed viable. He stuck firmly to his total opposition to the commercial programme.

Venter insisted both to his colleagues and to Congress that the privately funded genome sequence would be made publicly available. But no one quite believed him. When Venter phoned Sulston to suggest collaboration 'to get the sequence out for everyone,' Sulston was tempted, as he records in his book *The Common Thread*, but refused, telling himself that, wherever Venter had been, 'the decisions came down to profit.' For Sulston, a clergyman's son, that was not acceptable.

Collaboration never happened, however beneficial it might have been. Further peace meetings ended in recrimination and, as the race sped up, barrages of press releases from both teams confused the media about who was really ahead. President Clinton stepped in as peacemaker and the two teams agreed to stop fighting in public. A few months later, on 26th June 2000 and only two years after the race began, Clinton, flanked by Venter and Francis Collins, announced that both sides had reached the finishing line together. A simultaneous announcement was made by Tony Blair, although inexplicably he failed to mention the Sanger Centre. The race had officially ended in a dead heat, although at that stage the teams had finished only drafts and much work was still needed.

But even with the race long over, the dust has never entirely settled. To put the genome together at such speed, Venter's team had employed radically new

methods and untried mathematical algorithms, and the quality of its work has been repeatedly attacked and defended. Sulston claims that Venter's team was merely a 'distraction' and that the public programme did everything important. Yet the public programme rapidly changed its strategy in response to Venter, ruthlessly ditching smaller, less efficient labs in the consortium, and buying many of the new fast machines to get to the finish line years earlier than planned, which suggests that Venter provided a kick to the consortium's backside.

Of course, en route Venter had annoyed lots of scientists as well as some of the business people in Celera, appearing to lean too much towards business for the scientists and too much towards free publication for the business people. Back then, everyone was caught up in the tech bubble madness of the times. In little over a year, some six biotech companies raised a total of \$4bn in various stock offerings. That was something I wanted to ask Venter about. The promise was that 'genomics' would deliver a quick way to create new drugs and even 'personalised medicine,' with drugs tailored to individual genetic characteristics. Most of these things have proved very hard to realise, and patenting genetic information has provided no guarantee of a workable drug or a profit.

The human genome sequence is a great advance for basic research, but it has been much slower to deliver for the common good. The long slog of development and clinical trials continues much as before, and the boom times for biotech stocks, including Celera, are long over. I was interested in Venter's views on this. But first I wanted to know how he began to mix science and commerce. 

AA: The human genome project turned you into a figure of controversy. But you were arguing with Jim Watson over gene patenting long before that.

CV: Back in 1992, I was a government researcher at the National Institutes of Health (NIH). I developed a method for labelling genes quickly and the NIH tried to file patents on hundreds of genes. I was attacked by Watson and others even though the so-called 'Venter patents' were filed by NIH, not me, and I would never have gotten any money from them. The irony is that, thanks to Watson attacking me, everyone heard about my work and I started to get big offers from biotech companies. One of them offered me a \$5m signing fee, and when I turned it down because I wanted to keep doing science, people saw it as a negotiating ploy. But I eventually did a deal with private investors. I said, if you allow me to start my own not-for-profit institute in parallel with a for-profit institute, and they are kept independent of each other except for funding, I will do it. So I got \$70m over ten years to fund my lab called the Institute for Genomic Research (TIGR) with the intellectual property rights going to the for-profit institute (Human Genome Sciences). The scientific founders of biotech companies are mostly just looking for new ways to get their science funded, and when the company comes under pressure to make products, the founder gets the chop. I was trying to avoid that fate by coming up with a model that would allow me to do basic science and reap commercial benefits.

AA: The human genome project had started small under James Watson, but by the time you entered the fray it had grown into a global project spread among many labs around the world.

CV: Initially I had the notion that the best way to sequence the human genome was to build a single industrial-style centre. But the project took a different turn and became a giant international co-operative. It planned to take \$5bn of public money and distribute it around the planet. Every scientist and government in the world was involved and it became a political project with people fighting to control the money. New ideas were shut out. At

genome.' Everyone wanted to keep going without trying anything new. The reaction was something like: 'Very nice Mr Ford, you developed the automobile, but you know our horses and buggies are just fine, thank you.' The idea to turn these techniques into a privately funded project did not come from me but from the equipment-maker Applied Biosystems (ABI). It developed a new instrument that is particularly powerful when combined with the

AA: That's what led to the launch of your company, Celera, and Watson's notorious remark that you 'wanted to own the human genome the way Hitler wanted to own the world.' In Britain, John Sulston thought the same, writing that, 'Craig was aiming to gain total control of the information contained in the genome for commercial gain.'

CV: They got it all wrong. I said to ABI that if I do set up this company to sequence the human genome, I will need a guarantee that I can publish it. The response was, OK we'll give you the \$300m, but if you want to publish the results you'll have to come up with a business plan. So I did. I knew that the 3bn-letter human genome was worthless without the computing infrastructure and the software tools to do something with it. So my business model was to give the sequence away for free and sell the necessary software tools and the computer infrastructure. How is that against the public interest?

AA: At least the stock market liked the idea.

CV: It was insane. At one point the market capitalisation of Celera reached \$24bn, making it bigger than any British company. The model could not justify that value. But I could not say that in public as it was against SEC rules and would have caused a market crash. The irony was that Francis Collins and others in the public programme were hyping the human genome, describing it as the most important thing since the moon landing. I was trying to downplay it because you can go to jail for hyping the stock! I still became the world's first biotech billionaire, but only on paper—as head of the company, I could not sell my stock.

AA: You clearly have hurt feelings about the press you



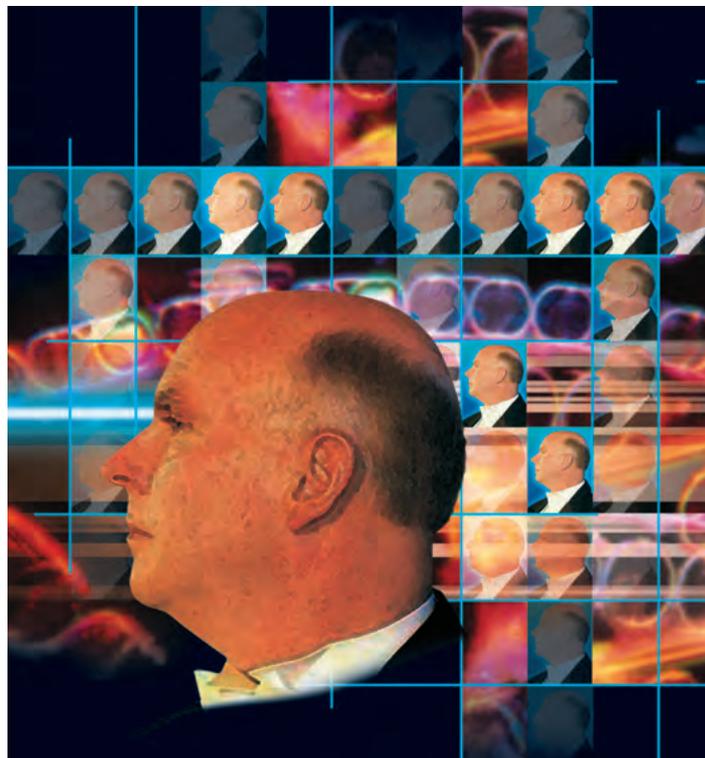
the institute I had set up, meanwhile, we were trying to develop new technology to sequence faster. It had taken scientists 13 years to sequence the genome of the bacterium *E. coli*, but using the new 'shotgun' methods I developed with Hamilton Smith (a Nobel prize-winner who works closely with Venter), we did the genome of *Haemophilus influenzae* in four months. Instead of people saying, 'Wow, here is a faster way to go,' I just heard, 'Oh that could not possibly work on the scale of the human

shotgun sequencing and offered to put up \$300m to create a new company to sequence the genome using it. It was not an altruistic move. They saw that this would help to sell a lot of these new instruments, which it did. I looked at the prototype and worked out that we could probably sequence the human genome in two or three years. I told Ham Smith that I was going to leave and try to sequence the human genome. He replied, 'I don't think it will work, but I'm going with you!'

got in Britain, which often followed Sulston's line: 'there's one bunch of people who are doing this for the benefit of humankind, and another bunch who are trying to do it for their personal gain.'

CV: A study of the global press coverage of the human genome by a German university found that the coverage in Britain was uniquely biased, and there was always this mano-a-mano battle between me and Sulston. He was one of the players who made good contributions, but there is nothing unique about him except that he was railing about how evil commercialisation was. But look at it this way: the public effort consumed about \$5bn of public assets. Celera, by contrast, used its own money to sequence the genome for \$100m and gave it to the public for free. When you look at the amount of medicine or education that \$5bn could have purchased, I think you can come up with quite a left-wing argument for letting the private sector play its part. There are important issues here for government and political leaders. Nations take pride in their scientific accomplishments, but if we want to make national heroes out of people then it can lead to disaster. Look at what happened in Korea where the government pressured one stem cell researcher to beat the world and he ended up faking data. We should be asking whether the best decisions are being made about the use of public money, and how to evaluate science as a whole. At the same time as those hundreds of millions of dollars from the Wellcome Trust were going to the human genome project here, a lot of scientists lost funding for their work. Many left Britain during that period. We live in a zero-sum game where there is a

finite amount of money. If one thing is funded, another is not. In the 'we must race against Celera' fervour, no one in Britain really stopped to ask if this made sense. Why didn't we just combine these projects and make them better, faster and cheaper? No one would answer those questions in Britain because national pride ordained that you had to support Sulston. You have to look at the whole system, not just individual morals. Scientists can be working for



altruistic reasons, people funding the science can be doing so for altruistic reasons, or they can be doing so out of financial greed, but the greed is only satisfied if the science is good and leads to a drug that actually treats disease and makes a body better. The system overall is oblivious to individual motivation. Investors in Celera wanted to make money. But when we asked employees why they wanted to work there, they wanted to be part of history. People like to make a difference. Celera published

its results in the journal *Science* on the same day as the public project published its results in *Nature*, with the sequence freely available to researchers in a database, and conditions only for those with commercial aims.

AA: Then, just 11 months after that day of joint publication, you suddenly decided to leave Celera, the company you had founded. Why?

CV: I was fired.

AA: You were fired?

CV: Yes. I hadn't gotten along with the head of the parent company from the beginning. I had sequenced the human genome, I had a great customer database of all the top universities and companies. I had experience in raising huge sums of money and credibility in both the scientific and business communities. I decided to raise the money for a new company. I now have TIGR, my original institute, and the new Venter Institute, both not-for-profit laboratories funded through the Venter Science

Foundation into which I put most of my stock from Celera. I didn't make much personally but my foundation got a windfall. I have over 500 people working for me at the two research institutes. Then recently I raised \$30m to start a new company called Synthetic Genomics. Its goal is to build on our basic research and find new commercial energy alternatives that do not cause global warming and will end the massive dependence on imported energy. The problem with trying to find novel sources of energy is that governments in the US, Britain and the rest of Europe aren't supporting that kind of research. Our governments should be spending 10 per cent of their budgets on trying to find new energy sources that don't add carbon to the atmosphere. It is vital for national security—we are fighting all these wars over energy sources instead of developing independent sources. It is also a priority for the health of the planet. Climate change is real and if we don't find alternatives to burning coal and oil we are going to be in trouble.

AA: You are thinking about using bacteria to produce fuels like hydrogen. But you need to create a new life form to do it?

CV: People have been looking for naturally occurring organisms to produce hydrogen or methane. But it does not make sense that anything would evolve naturally to produce commercial amounts of methane or hydrogen. Organisms would use the energy themselves. But if we can design synthetic cells then we can make them put all their energy into pathways that will drive methane or hydrogen production. DuPont is a world leader in using modified bacteria on an industrial scale to transform renewable natural 

resources into products that would normally have to come from oil. They are building a \$100m plant in Tennessee that will turn corn sugar into propanediol, a key polymer for making plastics, but it has taken them over five years to modify a bacterium so that it will turn the sugars into the polymer efficiently. Most of the work involved shutting down existing pathways in the bacterium so that the sugar would end up as the polymer and not be used for something else. The result showed that huge gains in efficiency are possible.

My approach starts from a different place. I think that if we can build a cell from scratch with only the very minimum of processes it needs to survive, we won't need to go through this long process of modifying an existing bacterium to shut down all the pathways you don't want. Instead, you can add to this 'minimal cell' the pathway you need in order to make a specific product. I have been trying to understand the minimum a cell needs to survive for the past ten years, as part of a basic research project; the fact that it has commercial and social applications is wonderful. We sequenced a bacterium called *Mycoplasma genitalium* which has the smallest genome of any known free-living organism and have modified it to make it simpler and find the minimum set of genes an organism needs.

At the same time, we've been finding ways to build genomes artificially. The first time a team built a simple virus genome from scratch, it took three years. Using new techniques we did it in two weeks. The genome of the simplest bacterium is around 60 times bigger so it is much more difficult, but we are moving towards creating the first very simple living organism.

AA: And that ties up with the project that has taken you right around the world in your yacht.

CV: Yes, I'm just back from a 40,000-mile trip around the globe on *Sorcerer II*. I bought the yacht in New Zealand and converted it, adding microscopes and sampling gear. All across the oceans we have been extracting unknown micro-organisms from sea water, sending them to my laboratory in Maryland and sequencing their DNA. The new discoveries are stunning. We only have the data from the first quarter of the expedition but we have already found 1.2m new genes—more than in the whole history of molecular biology. Among them are almost 800 genes involved in converting sunlight into energy.

AA: So the oceans could provide a huge stock of new genes that you could slot into your 'minimal cell,' like reprogramming a computer to do different things?

CV: That's the idea.

AA: To do all this you are once again running both non-profit and for-profit organisations. That means you have to be scientist and businessman, what Francis Collins described as a 'Faustian' situation that doesn't work. Isn't there a risk that you'll hear those words 'you're fired' again?

CV: This time I'm making sure to keep control of it. We have picked people as investors who all share the long-term view that this is a national and international priority. I want to change the world. A bacterium that makes gasoline from sugar in an academic lab doesn't change the world unless someone develops it commercially. If, all of a sudden, oil and energy could be produced locally and we were no longer reliant upon oil, global politics would be transformed. Sugar cane

becomes a major source of energy—all of a sudden Latin America and Cuba look very attractive. It changes the security landscape and it changes how much carbon we are putting into the atmosphere. There is a unique opportunity here, so let's try it. This is my third company and I think this will be the one that really works. Basic research and commercialisation sit well together. They are not separate worlds: in fact, the more they get together, the better for society. People make a distinction between business skills and science skills, but if you can run a science programme and inspire people you can lead a business.

AA: You have set yourself big goals. Is part of that conditioned by your experience in the Vietnam war?

CV: I left home when I was 17 to surf and explore life. Instead, I got drafted into the war so I turned 21 in Vietnam. Being faced with the death of thousands of people your own age and younger, and trying (as a medical orderly) to save as many as you could are tough lessons for someone at the end of their teens. Everybody that was there had their lives changed. Many people changed for the negative and did not recover from the war, but I was changed in such a way that I wanted to go on and make a difference. I thought it was wrong, politically, that we were in Vietnam, but none the less, 30 years later, we are back in an almost identical situation.

AA: Many people say you have a very big ego. After all, the DNA you sequenced was mostly your own.

CV: You have to have a strong sense of self to get things done. I believe in myself and my team and our ideas and making history.

Will Venter succeed in

making history? He's already made quite a bit of it through his enviable scientific publication record. That's quite separate from the notoriety which he acquired at Celera. But the impression you get on meeting Venter is that he always needs to move on to the next project. If his audacity works, and he and Hamilton Smith succeed in creating a synthetic bacterium, then they will start a vast new field. The capacity to build genomes opens up so many possibilities, both in understanding life and in using bacteria as chemical factories. There will be new worries too: the technologies will make biological weapons easier to make. Whether bugs can provide the next energy supply is controversial. There are plenty of simple creatures that make hydrogen as a by-product of their usual metabolic processes but can anyone make them do so efficiently enough to drive a switch to a non-polluting hydrogen economy? Venter is certainly right about one thing. Governments aren't spending enough to find alternatives, or even to implement the solutions they already have.



**Alun Anderson**

Alun Anderson is a freelance writer and former editor-in-chief of *New Scientist*. This article first appeared in the April 2006 issue of *Prospect* magazine ([www.prospect-magazine.co.uk](http://www.prospect-magazine.co.uk)) and is reproduced with the kind permission of the author and the magazine

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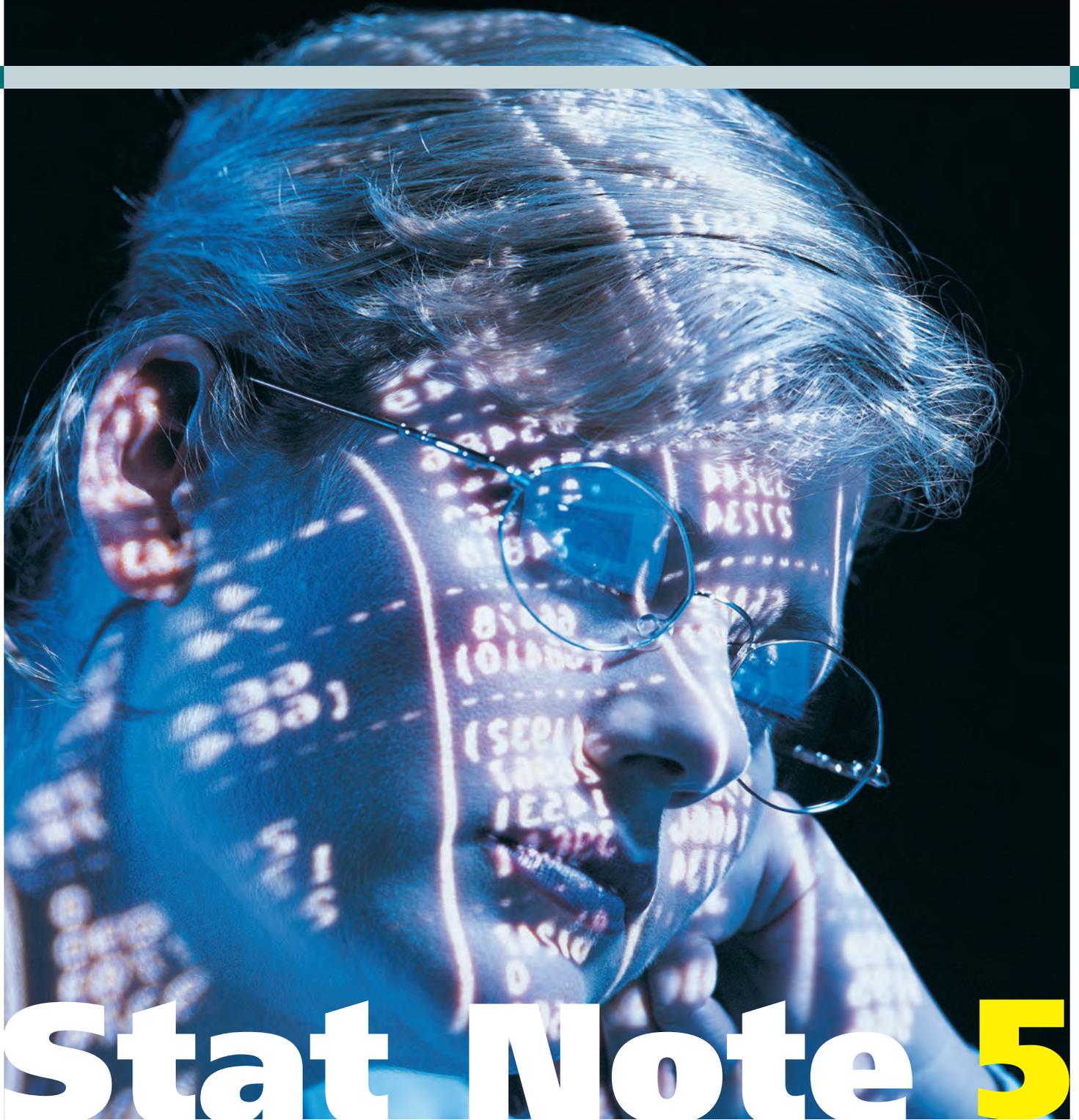
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# Stat Note 5

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

## is one set of data more variable than another?

**T**HERE MAY BE occasions when it is necessary to test whether the variability of two or more sets of data differ.

An investigator, for example, may wish to test whether a new treatment reduces the variability of a particular microbial response compared with an older treatment. In addition, an

important assumption for the use of the 't' test (Hilton & Armstrong, 2005) or analysis of variance (ANOVA) (Armstrong & Hilton, 2004) is that the variability of the different groups being compared is similar, i.e., that they exhibit *homogeneity of variance*. Replicate measurements within a control and a treated group, however, often exhibit different degrees

of variation and the assumption of *homogeneity of variance* may need to be explicitly tested. This Statnote describes four such tests, *viz.*, the variance-ratio (F) test, Bartlett's test, Levene's test, and Brown and Forsythe's test.

### The scenario

We return to the scenario first described in Statnote 3

(Hilton & Armstrong, 2005). A hypothetical experiment was carried out to investigate the efficacy of two novel media supplements (S1 and S2) in promoting the development of cell biomass. Three ten-litre fermentation vessels were sterilised and filled with identical growth media with the exception that the media in two of the vessels was supplemented with ten ml of

either medium supplement S1 or S2. The vessels were allowed to equilibrate and were subject to identical environmental / incubation conditions. The vessels were then inoculated with a culture of *Bacterium x* at an equal culture density and the fermentation allowed to proceed until all the available nutrients had been exhausted and bacterial growth had ceased. The entire volume of culture media in each fermentation vessel was then removed and filtered to recover the bacterial biomass, which was subsequently dried and the dry weight of cells measured. This experiment was repeated 25 times and the dry weight of biomass produced in each of the three groups recorded in Table 1.

## The variance-ratio test

If there are only two groups involved, then their variances can be compared by a two-tail variance ratio test (F-test) (Snedecor & Cochran, 1980).

## How is the test done?

The larger variance is divided by the smaller and the resulting F ratio compared with the value in a table of the variance ratio to obtain a P-value, entering the table for the number of degrees of freedom (DF) of the numerator and denominator. This test uses the *two-tail probabilities* of F because we are testing whether or not the two variances *differ* rather than whether variance A is greater than variance B. Hence, this calculation differs from that carried out during a typical ANOVA, since in the latter, it is whether the treatment variance is *larger than* the error variance that is being tested (Armstrong & Hilton, 2004). Published statistical tables of the F ratio (Fisher & Yates, 1963; Snedecor & Cochran, 1980) are usually in the form of one-tail tables. Hence, the 2.5%

**Table 1.** Dry weight of bacterial biomass under unsupplemented (US) and two supplemented (S) growth conditions (S1 and S2) in a sample of 25 fermentation vessels.

US	S1	S2	US	S1	S2	US	S1	S2
461	562	354	506	607	556	518	617	714
472	573	359	502	600	578	527	622	721
473	574	369	501	603	604	524	626	722
481	581	403	505	605	623	529	628	735
482	582	425	508	607	644	537	631	754
482	586	476	500	609	668	535	637	759
494	591	511	513	611	678	542	645	765
493	592	513	512	611	698			
495	592	534	511	615	703			

**Variances:** US = 463.36. S1 = 447.88. S2 = 18695.24

**Variance-ratio test comparing US and S1:**  $F = 463.36/447.88 = 1.03$   
(2-tail distribution of F,  $P > 0.05$ )

probability column has to be used to obtain the 5% probability.

## Interpretation of the results

When the unsupplemented and S1 data are compared (Table 1), a value of  $F = 1.03$  was obtained. This value is less than the F value in the 2.5% column ( $P > 0.05$ ) and consequently, there is no evidence that the addition of the medium S1 increased or decreased the variance in replicate flasks.

## Bartlett's test

If there are three or more groups, then the different groups could be tested in pairs using the F-test

described above, but a better approach is to test all the variances simultaneously using Bartlett's test (Snedecor & Cochran, 1980).

## How is the test done?

If there are equal numbers of observations in each group, calculation of the test statistic is straight-forward and a worked example is shown in Table 2. If the three variances do not differ from each other, then the ratio M/C is a member of the chi-square ( $\chi^2$ ) distribution with  $(a - 1)$  degrees of freedom (DF), where 'a' is the number of groups being compared. If the groups have different numbers of observations in each (unequal 'n'), then the

**Table 2.** Comparison of the variances of three groups with equal observations ( $v = 25$ ) in each by Bartlett's test.

Group	Variance	ln (variance)
Unsupplemented	436.36	6.1385
S1	447.88	6.1045
S2	18695.24	9.8360
Total	19606.48	22.079

$M = \sqrt{[a (\ln s^{*2}) - \sum \ln s^2]}$  where  $s^{*2}$  is the mean of the variances, 'a' the number of groups,  $v = DF$  of each group, and  $\ln =$  logarithms to base e.  
Hence,  $M = 102.62$   
 $C = 1 + (a + 1)/(3av) = 1.018$   
 $\chi^2 = M/C = 102.62/1.018 = 100.8$  (DF =  $a - 1$ ,  $P < 0.001$ )

calculations are slightly more complex and are given in Snedecor and Cochran (1980).

## Interpretation of the results

In the worked example in Table 2, the value of  $\chi^2$  was highly significant ( $P < 0.001$ ) suggesting real differences between the variances of the three groups. The previous F-test suggested, however, that the variance of the unsupplemented data was similar to that of the growth medium S1. Therefore, it is the effect of the growth medium S2 that has substantially increased the variance of bacterial biomass. Hence, if these data were to be analysed by ANOVA (Armstrong & Hilton, 2004), the assumption of homogeneity of variance would not hold and it may be necessary to transform the data to logarithms before analysis to stabilize the variance. Data transformation is described in more detail in Statnote 4 (Hilton & Armstrong, 2006).

The use of the  $\chi^2$  distribution to test the significance of M/C is questionable if the DF within the groups are less than five and in such a case, there are special tables for calculating the significance of the statistic (Pearson & Hartley, 1954). Bartlett's test is used less today and may not normally be available as part of a statistics software package. This is because the test is regarded as being too 'sensitive' resulting in too many significant results especially with data from long-tailed distributions (Snedecor & Cochran, 1980). Hence use of the test may raise unjustified concerns about whether the data conform to the assumption of homogeneity of variance. As a consequence, Levene (1960) developed a more robust test to compare three or more

variances (Snedecor & Cochran, 1980).

### Levene's test. How is the test done?

Levene's test makes use of the absolute deviation of the individual measurements from their group means rather than the variance to measure the variability within a group. Avoiding the squaring of deviations as in the calculation of variance results in a measure of variability that is less sensitive to the presence of a long-tailed distribution. An ANOVA (Armstrong & Hilton, 2004) is then performed on the absolute deviations and if significant, the hypothesis of homogeneous variances is rejected.

### Interpretation of the data

A Levene's test on the data in Table 1 using STATISTICA software, for example, gave a value of  $F = 52.86$  (DF 2,72;  $P < 0.001$ ) confirming the results of Bartlett's test.

More recently, Levene's test has also been called into question since the absolute deviations from the group means are likely to be highly skewed and therefore, violate another assumption required

for an ANOVA, that of normality (Armstrong and Hilton, 2004). This problem becomes particularly acute if there are unequal numbers of observations in the various groups being compared. As a consequence, a modification of the Levene test has been proposed by Brown and Forsythe (1974).

### Brown-Forsythe test. How is the test done?

This differs from Levene's test in that an ANOVA is performed not on the absolute deviations from the group means but on deviations from the group medians. This test may be more accurate than Levene's test even when the data deviate from a normal distribution. Nevertheless, both Levene's and the Brown-Forsythe tests suffer from the same defect in that to assess differences in variance requires an ANOVA, and an ANOVA requires the assumption of 'homogeneity of variance,' which some authors consider to be a 'fatal flaw' of these analyses.

### Conclusion

There may be circumstances where it is necessary for microbiologists to compare variances rather

than means, e.g., in analysing data from experiments to determine whether a particular treatment alters the degree of variability or testing the assumption of homogeneity of variance prior to other statistical tests.

All of the tests described in this Statnote have their limitations. Bartlett's test may be too sensitive but Levene's and the Brown-Forsythe tests also have problems. We would recommend the use of the variance-ratio test to compare two variances and the careful application of Bartlett's test if there are more than two groups.

Considering that these tests are not particularly robust, it should be remembered that the homogeneity of variance assumption is usually the least important of those considered when carrying out an ANOVA.

If there is concern about this assumption and especially if the other assumptions of the analysis are also not likely to be met, e.g., lack of normality or non additivity of treatment effects (Armstrong & Hilton, 2004) then it may be better either to transform the data or to carry out a non-parametric test on the data.

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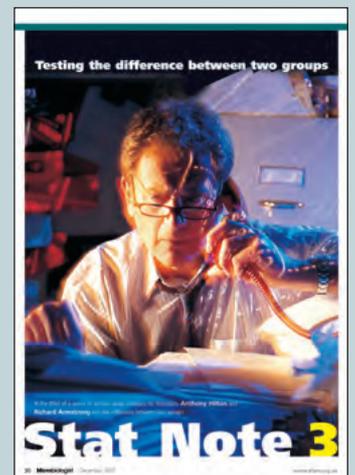
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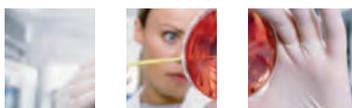
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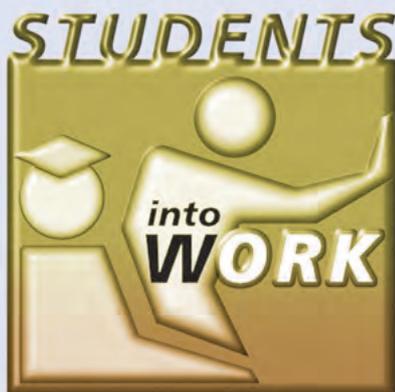
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## Summer Project at Food Microbiology, Queen's University of Belfast. Gemma McClatchey reports on her project

**D**URING THE SUMMER, I WAS fortunate enough to be given the opportunity to work in the Food Microbiology branch of the Food Science Department at the Queens University of Belfast.

Being a Microbiology student about to enter my final year, this experience was invaluable to me as it was an ideal opportunity to develop my research and laboratory skills. Furthermore, it also highlighted the applications and importance of microbiology research. It enabled me to gain hands-on experience of microbiology within a research institute setting.



My assigned project was designed around previous research into the diversity of *Arcobacter* species in foodstuffs in Northern Ireland. *Arcobacter* belongs to the family *Campylobacteraceae* and are found in a range of animal origin foodstuffs. As yet *arcobacters* have not been regarded as significant food-borne pathogens, rather they can be thought of as emerging food-borne pathogens.

Previously, 104 cultures of *Arcobacter* had been stored in cryovials after isolation from a range of foodstuffs as part of previous PhD research that had finished in 2002. The main aim of this investigation was to study the

abnormalities noted in the previous work when using a genetic fingerprinting assay known as AFLP (Assorted Fragment Length Polymorphism). A mathematics software package, BioNumerics 4, was used to determine the genetic relatedness of the food-borne *Arcobacters*. The package operates through analysing AFLP profiles by clustering isolates into distinct groups according to their genetic banding pattern. Values of 90% homology and above were used as an indication of identical isolates. AFLP profiles generated during the research had shown a significant proportion of isolates gave patterns with an unusually high number of bands.

The aim of my project was to examine the isolates to see if the unusual AFLP patterns were due to mixed cultures (species or genotypes) being analysed. Stored isolates were to be resuscitated then streaked to purity and AFLP analysis conducted. The resulting profiles would be compared to the AFLP profiles gained previously.

Ten isolates were randomly chosen to be studied in detail and five pure cultures were obtained from each isolate by repeated streaking to single colonies. AFLP profiles were prepared and analysed from all fifty cultures. For eight out of the ten original isolates studied, subcultures produced acceptable AFLP profiles showing over 90% profile homology with the original isolate, when analysed using BioNumerics. This indicates that they were identical and can be used as a measure of colony purity. Only one isolate gave results suggesting that the original analysis was based on a mixed culture. Multiple band profiles that had appeared in the initial AFLP profiles from the previous research were still present in the AFLP profiles generated in my research, suggesting that they are probably characteristic of some wild-type *Arcobacter* AFLP profiles. Two isolates did not produce acceptable profiles and these may be untypable by this specific method. The research has generated ideas for further research with *Arcobacters* and this is exciting as they continue to emerge as food-borne pathogens.

I thoroughly enjoyed my placement and I feel that I have acquired a range of

important transferable skills. The development of independent working is a large part of doing a project such as this and is certainly a skill that is essential in the area of scientific research. I found this to be a challenging experience but also highly stimulating and rewarding. It has been of great benefit to me and I would highly recommend it to any student who has the opportunity to take part in such a scheme. I would like to extend my gratitude to both my supervisors Dr Madden and Mrs Moran for their professional advice and also to Carmel Kelly and Sarah Hamill who also assisted me. I would particularly like to thank the Society for Applied Microbiology for enabling me to have taken part in such a valuable and rewarding experience.

Gemma L McClatchey

### The aerobic and low oxygen (O<sub>2</sub>) growth of *Pseudomonas aeruginosa*.

Chris Wright reports on his project



**A**FTER COMPLETING MY Applied Microbiology Honours degree at the University of the West of England I wanted to gain more experience in a relevant subject area and give myself a potential advantage in looking for a job. The SfAM 'Students into Work' grant gave me the opportunity to work in a microbiology research laboratory.

The first part of my nine week project was to investigate the aerobic and low oxygen (O<sub>2</sub>) growth of *Pseudomonas aeruginosa* using a previously refined biofilm model. The second part was to utilise environmental scanning electron microscopy (ESEM) to attempt to visualise the biofilms produced in both the aerobic and low O<sub>2</sub> systems.

The biofilm mode of growth of bacteria represents the major form of growth of bacteria. The process begins with bacterial attachment to inert surfaces and formation of interactions with other bacteria and the surface. A highly organised 3D structure is formed, often composed of multiple species of bacteria and extracellular polymeric substance (EPS). Both the structure of the biofilm and the bacteria themselves contribute to the well documented phenomenon of resistance. The EPS matrix creates concentration gradients of O<sub>2</sub>, nutrients and even antibiotics. This can affect the efficacy of some antibiotics, such as gentamicin, which rely on oxygen for active uptake into the bacterial cell. As well as this, EPS can function as a molecular sieve trapping antibiotics. Bacterial physiology is also a factor in biofilm resistance. As considered above, nutrient gradients exist within the EPS creating regions of slow growing or stationary phase cells which in turn will affect the efficacy of antibiotics. This growth as a biofilm can have serious and costly consequences for our use of medical devices, such as central venous catheters and other indwelling prosthetic devices, should they become colonised with microorganisms. As well as forming biofilms on inert substrata, such as medical devices, *P. aeruginosa* is an important opportunistic pathogen in cystic fibrosis (CF). In CF patients, a decrease in pulmonary function caused by a loss of membrane ion channels and a resultant incapacity to clear bacterial cells, leads to colonisation by opportunistic pathogens. Once it has colonised, *P. aeruginosa* is responsible ultimately for approximately 90% of deaths in CF patients.

The first part of my project was to establish aerobic biofilms of *P. aeruginosa* using an established protocol. This protocol involved the Sorbarod *in vitro* continuous flow system of biofilm growth. Biofilms were cultured using a chemically defined medium (CDM) in which iron was the growth limiting factor. The medium also

contained potassium nitrate which could be utilised by *P. aeruginosa* as an alternative electron acceptor if oxygen was unavailable. Biofilms were continually perfused with CDM over the course of several days. Viable counts were taken throughout to determine the numbers of bacteria being released from the biofilms and also the final biofilm population.

A section of the first part of my project was to modify the aerobic system to run under low oxygen conditions. Biofilms initially established under aerobic conditions were subsequently perfused with an anaerobic gas mixture which created a low oxygen environment within the Sorbarod and biofilm. As with the aerobic system, viable counting was performed throughout every experiment, and a final viable count of the Sorbarod biofilm population taken. After reaching a steady state biofilm, both the aerobic and low oxygen biofilms were challenged with ciprofloxacin to assess the efficacy of the antibiotic under aerobic and low oxygen conditions. During the antibiotic perfusion into the system, viable counts were taken hourly to monitor the log drop and recovery of the biofilm.

The second aim of the project was attempt to visualise the biofilms produced using ESEM. This was achieved using a control biofilm which was dissected and hydrated with sterile water. Under low oxygen conditions, possible changes in EPS production by *P. aeruginosa* could produce visual differences in ESEM images. Furthermore, a change in EPS production could have an effect on the antimicrobial susceptibility of the biofilm population.

Working in the microbiology lab at UWE Bristol has been a great experience and it has also made me realise that I definitely want to do a PhD in the near future. The project itself required good time management skills and forward planning with the large scale media preparation and organisation of the biofilm system. I would like to thank my supervisor, Dr Shona Nelson, and the microbiology researchers and technical staff at UWE for all their help and support. Finally I would like to thank SfAM for providing sponsorship for the summer project which I have thoroughly enjoyed.

Chris Wright

## The use of starter cultures in the production of 'Akamu'—a Nigerian Fermented Maize Porridge used as a weaning food.

Patience Chisa Obinna-Echem reports on her project

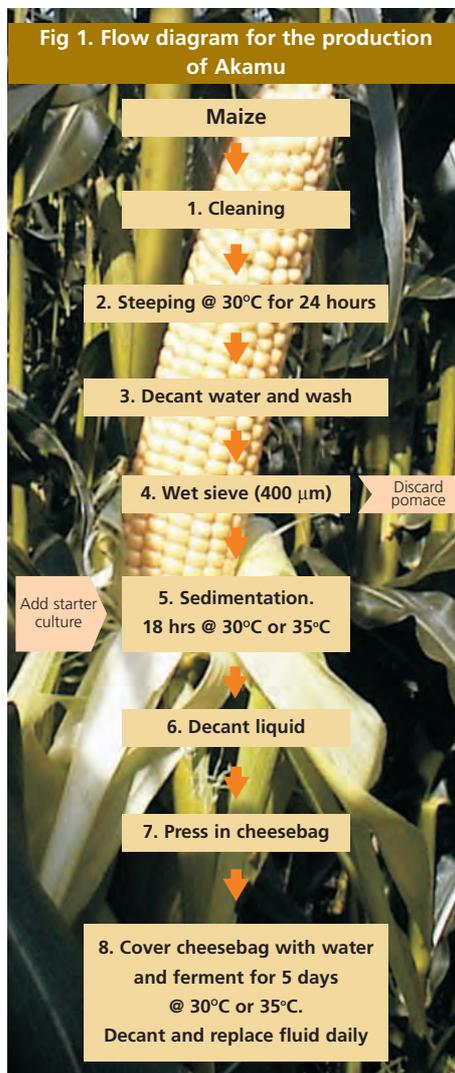
**I** WISH TO EXPRESS MY profound gratitude to the Society for Applied Microbiology for giving me the opportunity to gain experience of working in a microbiology research environment.

I joined the research group of Professor Peter Brooks and Dr Jane Beal at the University of Plymouth who are working on lactic acid fermentation of cereal based diets for pigs and poultry. Spontaneous lactic acid fermentations are widely used to produce cereal based weaning food throughout Africa and parallels can be drawn between the two processes. Therefore we decided that I would do some work on the use of starter cultures in the production of a maize based weaning food using the traditional process.

The research was aimed at the determination of the effect of Lactic acid bacteria (LAB) on the safety and bacteriological quality of 'Akamu' a fermented maize weaning food. Akamu is a traditional lactic acid fermented cereal-based starchy meal, made from maize, sorghums or millet and it is a most popular weaning food for children in Nigeria and other West African countries.

In Nigeria, diarrhoea is ranked as the second major cause of morbidity and mortality in children and up to 70% of all diarrhoeal episodes are caused either by contamination of food or drinking water (Motarjemi *et al.*, 1993). Weaning foods have been shown to be frequently contaminated with enteropathogens from various sources especially when stored at ambient temperature under poor hygienic conditions and public sanitation, hence exposing the infants to the risk of diarrhoea. However, if the food contains sufficiently high levels of lactic acid (> 75 mmol/L) contamination with enteropathogens can be prevented (Beal *et al.*, 2002).

Starch slurries extracted from maize (Fig 1) were inoculated with; *Lactobacillus plantarum* (LP), *Pedococcus acidilactic* (PA), *L. reuteri* (LR) with an uninoculated sample as a control. The slurries were fermented at 30°C and 35°C for five days. The pH, short chain fatty acid and ethanol levels, coliforms, yeasts and LAB counts and the sensory properties of the fermented slurries were analysed. There were no statistically significant differences in lactic acid, acetic acid or ethanol production between the treatments over the first three days of fermentation.



However, in all samples the liquid decanted on the first day of fermentation (Fig 1 - step 8) contained 70% of the lactic acid produced, *ca* 60 mmol/L compared with *ca* 25 mmol/L remaining in the slurry. Although the numbers of LAB remained high in the remaining slurries, lactic acid levels did not recover and were significantly reduced to <15

mmol/L in both the decanted liquid and the slurries by day three. In the control samples coliform bacteria were detected on day three in the slurries incubated at 30°C and on day one in slurries incubated at 35°C. In the slurries inoculated with starter cultures the coliforms were detected on day five at 30°C and day three at 35°C. Lactic acid bacteria numbers were *ca* 10<sup>8</sup> cfu/g in all slurries including the controls. All of the slurries, inoculated and controls, had high yeast counts *ca* 10<sup>6</sup> cfu/g. In the sensory analysis slurries inoculated with LP were the least liked in terms of sensory attributes and general acceptance.

In this study the inoculation of maize with starter cultures of LAB did not appear to have any great benefit compared with the control slurries. In all cases initial lactic acid concentrations of > 75 mmol/L were achieved. However, in the traditional Akamu making process the benefits of high lactic acid concentrations are lost as most of the acid generated by LAB is discarded with the decanted liquid after the first 24 h fermentation. My study has raised a number of questions regarding the safety of the traditional way of making Akamu as well as questions regarding the dynamics of lactic acid production and interactions between lactic acid bacteria and yeasts in such systems.

This research has broadened my knowledge on the safety quality of food processing method as well as fermentation and the activities of LAB. The practical research experiences gained will in no doubt be of great benefit in any food production and research sector. It has also heightened my interest in this area and I hope to be able to secure some funding so that I can return and continue this research as a PhD student.

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## Bacterial Degradation of Technical Compounds and Formulated Mixtures

**T**HE WORLD MARKET FOR bioremediation — the use of biological processes to treat contaminated areas, employing microbiology, biotechnology and engineering techniques — is increasing greatly. It gained approximately U.S. \$580 million between 1994 and 2000 to a total of approximately \$1050 million. Furthermore the cost of using appropriate bioremediation systems is around eight times less than incineration and two and a half times less than soil washing per ton of contaminated soil. Thus bioremediation can be seen to be not just a more environmentally friendly technology for contamination removal but commercially and economically attractive too.

Microbial degradation is one of the processes of biodegradation. 'Biodegradation' itself is often used to encompass several further terms for different processes such as biotransformation, biotransformation, biotransformation and biotransformation; the connection usually being the end result of observed loss of original xenobiotic to other product(s) usually with a reduction in toxicity. Here biodegradation may be through the indigenous population, selective isolates, or a supplemented population.

A search of publications regarding the biodegradation of compounds will yield colossal numbers, but upon further inspection almost all of these relate to either a technical or active ingredient, or a specific formulation as would be found or used in the (appropriate) environment. In many cases the active ingredient is described as the compound of study, whereas it is actually a formulation containing the compound that is used. Additionally the matter measured (directly or indirectly) may not be the technical compound but just as a constituent ingredient of the formulation. This may not mean complete remediation of a contaminated site from a technical compound since formulation components (which may or may not pose a risk) may remain. A good example of this is with polycyclic aromatic hydrocarbons (PAHs) in creosote, as presented below. Formulations themselves can have an affect on the biodegradation of compounds, increasing stability, potency of effect, adsorption and bioavailability.

However this is not to say that information is not available on the degradation of xenobiotics in technical or formulated forms.

To very briefly illustrate the 'disparity' between technical compound (presuming no impurities for clarity) and formulation biodegradation, a modern insecticide and a well known timber treatment product, both of which can give and have given environmental problems, are described.

Synthetic pyrethroids (SPs) are insecticides now found in many applications, both household and agricultural. The largest agricultural usage is in the treatment of flies and ticks on sheep and cows; in the UK sheep dips are one formulation. The SP cypermethrin is used as the active ingredient in many sheep dips and its degradation pathway (as a technical compound) is known. The fate in the environment is more of a problem because of the SP's chemical nature and its traceability. Incidents of SP based sheep dip pollution have occurred where aquatic life has been wiped out in rivers and lakes. The formulations are (generally) designed to increase wool uptake and maintain the stability and potency of the SP after treatment. Biodegradation has now been studied with the technical cypermethrin and cypermethrin sheep dips, in both laboratory and field applications. Without referring to individual and specific results, degraders isolated from technical cypermethrin cultures do appear to degrade SPs in a sheep dip formulation, but not as well as degraders isolated using sheep dip cultures (with both isolations from non-SP containing media from the same sources). However the degraders isolated from sheep dip break down the technical SP to approximately the same extent as the SP in a formulation. This may not seem unexpected but is interesting because the SP active ingredient only constitutes 5-10% of a sheep dip formulation. Testing different sheep dip formulations produces similar results. For bioremediation of used waste sheep dip the addition of previously isolated SP degraders significantly increases the rate of SP loss only by a little. This may in part be due to another potential problem — that of the exact environment where the

contamination is.

PAHs are the main constituent of creosote (the rest being mostly various phenolics), which is used in the treatment of timber to provide greater longevity; now banned or restricted in use in most of Europe. The carcinogenic and polluting nature of creosote and many PAHs are well documented as are many cases of biodegradation and the bioremediation of contaminated sites. However in biodegradation studies it is often PAH loss that is measured, and this is often extrapolated to creosote (not completely unreasonable given the chemistry). Whilst not wholly inaccurate, problems can arise with this approach, most obviously if only PAH degradation is taken as a sign of total creosote degradation in land destined for another use (which does not happen in reality); but the approach is widely used in microcosm studies. A problem does arise though when trying to isolate creosote degraders. The toxic mixture requires heavy dilution relieving a good deal of selective pressure, but using PAHs at concentrations nearer and at those of use results in isolates with greater potential for use. In contaminated sites it is not unusual to find low bacterial concentrations whilst PAHs remain, but be able to find PAH degrading isolates and stimulate degradation through the use of PAHs as carbon sources in the laboratory (energy models standardised).

One additional affect of formulations and technical compounds is upon the microbial community. Increasingly researched upon with new tools and capabilities introduced by advances in molecular and biochemical biology, the microbial ecology of contaminated sites and the changes that occur during bioremediation are attracting great attention. However, at this next 'level of resolution' in bioremediation studies disparity between the affects of a technical compound and a formulation are likely to be even greater making general studies more difficult or their results less encompassing. In possible contradiction to this though is a result presented here where the SP active ingredient appeared to be the greatest selecting factor for bacterial isolates despite only constituting 5-10% of a formulation.

To summarise, the biodegradation of technical compounds and formulations that contain the technical compound, may be unique but this does not mean that one is not useful nor mutually exclusive to the

other. Using a technical ingredient may be the only way to isolate a degrader, and using a formulation may be the only way to isolate a degrader that can be used in other formulations; similar formulation uses are likely to have similar chemistries after all. Equally, measuring a technical compound may be the only way to monitor the loss of a formulation, but it is the formulation that is almost always referred to as the pollutant.

It should be remembered that in the environment it is highly unlikely that one will find a technical compound that is not in a formulation. The technical ingredient may be the major pollutant but simply removing it alone is not removing the whole contamination.

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**Dr Russ Grant**  
University College Dublin

## Shining a light on Biofilm Heterogeneity

**M**OST SPECIES OF BACTERIA will readily colonise surfaces and grow as a community of cells embedded in an extra-cellular polysaccharide matrix, commonly referred to as a biofilm.

Biofilm cells are physiologically distinct from their planktonic counterparts and this mode of growth is associated with increased resistance to many antimicrobial regimes. This has implications in the industrial, food and medical settings, as biofilm formation can

act as a reservoir for infection where previously decontaminated surfaces can be re-colonised when cells slough off the biofilm structure and disperse to these areas again.

Microarray studies have confirmed beyond any doubt that *Salmonella* biofilm cells are distinct from their planktonic counterparts at the transcriptional level, and it has been found that nearly 10% of the genome can be differentially expressed between the two populations (Hamilton, 2005). Various studies have shown that many genes are required for biofilm formation, and the genetic mechanisms underlying different aspects of the biofilm phenotype have been extensively studied over recent years. However, while it is apparent that genes are differentially regulated in biofilm cells relative to their planktonic cousins, much less is known about the spatial and temporal expression of these genes at a cellular level within the biofilm structure. One of the most intriguing features of biofilms is their heterogeneity. Whether differential gene expression plays a role in the development of this heterogeneity or whether heterogeneity is caused by environmental factors is currently unclear. It has been suggested that the development of some features of the biofilm structure are simply due to stochastic environmental factors such as the distribution of nutrients on the surface, rather than a programmed genetic response to the environment. In both scenarios differential gene expression between biofilm cells is likely to occur, either as part of a genetic program of development, or as a result of differing environments within the biofilm structure. So how do we find out what is going on in these structures?

There is an increasing body of literature citing the use of Green Fluorescent Protein (GFP) to determine the spatial aspects of gene expression. One of the first papers describing the use of GFP to investigate the spatial aspects of differential gene expression in the biofilm structure was by Kievit *et al.*, 2000. They found that genes involved in quorum sensing (a cell-to-cell signalling mechanism dependant on bacterial cell-density) were expressed at a higher level in the cells located near the substratum than at the top of the biofilm. Several studies have followed on from this. A recent example is the discovery that two genes involved in quorum sensing are preferentially expressed at a higher level

in the 'stalks' of the mushroom type structure characteristic of *Pseudomonas aeruginosa* biofilms, as opposed to the 'caps' (Lequette and Greenberg, 2005). It is this type of information that could prove vital in the development of new prevention strategies. It is common practice to impregnate surfaces with antimicrobials to prevent biofilm contamination, such as is seen with many medically relevant surfaces.

However, if these substances target processes occurring in the upper parts of the structure, they may only stimulate the uppermost regions of the structure to slough off, leaving the base layer untouched. Compounds that disrupt regulation near the base of the biofilm would be much more effective at actually removing the biofilm from the surface and preventing reattachment. Knowing that certain genes involved in quorum sensing, for example, are expressed near the base of the biofilm may facilitate the development of compounds that target this process. Recent advances in molecular techniques coupled with technological improvements have made investigations into this aspect of biofilms even more exciting. These include the development of reporter constructs using a bright variant of GFP that give a more accurate representation of gene expression, (Scholz *et al.*, 2000; Hautefort *et al.*, 2003), and highly sensitive microscopy capable of detecting much lower levels of fluorescence than was previously possible. Together these have shown promise as a suitable method to elucidate the genetic regulation at the single cell level within the biofilm structure. This could lead to a level of understanding about the development and maintenance of the biofilm lifestyle that was previously unthinkable.

As our knowledge of the genetic regulation involved in the formation of biofilms increases, so too will our ability to develop better control strategies to minimise the economic and health costs incurred by their formation on industrially and medically important surfaces. While there are many techniques that can be used to dissect the underlying mechanisms involved in this process, the use of a reporter such as GFP has one feature that sets it apart from the others. We know that it is ideal for providing valuable spatial and temporal information of gene expression *in situ* and in a non-destructive manner. But perhaps the best part about using GFP in this way is that it

enables a very visual approach to exploring this area further, and for me, this fascinating world can become truly captivating when you can actually see something happening before your eyes.

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Michael Pogson

## To treat or not to treat your RNA samples with heavy duty DNase for quantitative RT-PCR assays: a necessary step for high GC content bacterial strains?



**Q**UANTIFICATION OF GENE expression by real time quantitative reverse transcription polymerase chain reaction (qRT-PCR) is becoming a widely used technique replacing more time-consuming and less sensitive ones such as Northern Blotting, RNase protection assay or conventional RT-PCR.

However, several limitations need to be considered when real time qRT-PCR is used, particularly normalisation, template quality, operator variability and the reverse transcription step itself. These and other features related to qRT-PCR for quantification of gene expression were addressed in an intensive six days practical course organized at EMBL, Heidelberg during summer 2005. Together with a group of 21 students, I had the chance to learn the latest developments in this technique from a group of knowledgeable experts in the field. From the beginning of the course, it was clear to me that the 18 hour trip was worth it, not only scientifically, but also because a particularly friendly environment prevailed among the student's group. This and the fact that the instructor's team was a group of old friends, created an atmosphere where having fun and learning were mixed.

Besides the well structured practical and theoretical lectures, short sessions were organised, so that each student had the chance to explain his/her research project and their experiences regarding qRT-PCR. Nearly all the projects were related to issues concerning eukaryotic gene expression. My work on the other hand is focused on gene expression in bacteria with high GC content in their genome. Therefore, the major problem I was having with my qRT-PCR experiments, i.e genomic DNA contamination of my RNA samples, seemed at glance a trivial difficulty to most of the students. Despite this, everyone engaged in an interesting troubleshooting session that allowed me to come back to the lab with a couple of ideas on how to solve this problem. First of all I realized that most of the in-column techniques commercially available for RNA isolation are standardized for eukaryotic cells and for some classical prokaryotes mainly *E. coli*. However, when it comes to other prokaryotes, particularly those with a resistant cell wall, these isolation methods need further standardisation. Since bacteria have a smaller genome than eukaryotic cells, the

number of bacterial cells to be used for RNA preparations needs to be much larger than the amount of cultured human or animal cells. Therefore the content of genomic DNA is larger, too. After performing some RNA preparations and RT-PCR assays, I found that good quality RNA is more important than to have a large amount containing some DNA contamination. For this, the lysis procedure is the critical step. An inefficient lysis step would release some RNA but certainly would release more DNA molecules resulting in a DNA contaminated RNA sample. It is not wise to pursue qRT-PCR assays until an acceptable lysis procedure has been established.

The next critical step to consider is the DNase treatment of your sample. In spite of the hesitancy some researchers may have, this step is absolutely necessary if real time qRT-PCR is being performed, especially when prokaryotic RNA is used. Furthermore, in solution DNase treatment of the RNA sample is strongly recommended even after in column DNase digestion, since no commercial kit can guarantee a DNA free preparation (Bustin, 2002; Peters *et al.*, 2004). DNase I is the commercially available enzyme for this purpose and this poses a challenge for those working with high GC content organisms such as *Brucella*. Genomic DNA contamination is persistent in *Brucella* RNA isolates regardless of the DNase I concentration used, incubation time or number of treatments. Recently, a modified DNase I, 600 times more efficient than the previous existing one, was made available and seems a good choice to solve this problem. In cases as those described before, it is more realistic to think in terms of minimization of genomic contamination than of getting only background signal in the minus RT controls. It is the prerogative of the researcher to decide, according to the context, an acceptable minimisation level. However, it is important to keep in mind that any significant DNA contamination would produce misleading and non reproducible results. Other alternative methods such as use of restriction endonuclease cocktails (Ashkenas *et al.*, 2005; Dougherty *et al.*, 1993) or introduction of a non-homologous sequence at the 5' end of the cDNA during reverse transcription have been used to overcome this problem (Aguena & Spira, 2003). Nevertheless, the efficiency of DNA removal of these techniques was

assessed by visual inspection of samples run on agarose gel electrophoresis. Certainly, this does not guarantee negligible Ct values during real time RT-PCR.

In conclusion, the answer to the question raised in the title of this report is yes, not only DNase treat your bacterial RNA samples but also perform a careful validation of the assay before any interpretation. Ideally, the data originated from qRT-PCR should be analyzed together with data gained by examining other events or molecules relevant to the investigation. By these means, the biological relevance of the qRT-PCR data is reinforced.

I am grateful to Dr. Peter Green for encouraging me to apply for a President's Fund award and thank the SfAM for awarding me this grant to attend the qRT-PCR course in Heidelberg.

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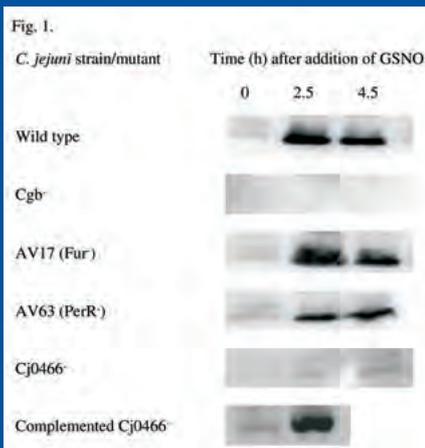
## Campylobacter jejuni and nitric oxide

**T**HE MOST COMMON laboratory-confirmed bacterial cause of gastrointestinal infection in England and Wales continues to be *Campylobacter*.

In 2004 alone, 42,146 cases were reported to the Health Protection Agency. In reality this figure is known to be much higher as many cases are not presented to the GP. Severity of the disease in humans varies from a few days to several weeks and causes a significant economic impact through loss of working time. Symptoms include headache, fever, diarrhoea and abdominal pain. This acute phase is normally self-limiting, however complications may include reactive arthritis and paralysis caused by Guillain-Barré syndrome. Most cases of infection are sporadic and epidemiological studies suggest the route of transmission is usually through the consumption of contaminated poultry meat. Raw milk and milk from bottles pecked by birds, sewage, untreated water and contact with pets with diarrhoea have also been reported to cause infection. Cross contamination of foods in kitchens is also likely to be a significant risk if hygiene standards are poor.

Nitric oxide (NO) has become one of the most intensively studied molecules. Before 1987 its role in human health was primarily as an irritant in air pollution. In 1998 the Nobel Prize in Physiology or Medicine was awarded to the scientists who discovered production of NO in the body and identified its key role in intra- and intercellular cell signalling. However, over- and under-production of NO contributes to numerous human diseases such as rheumatoid arthritis and Alzheimer's disease. NO is a simple molecule with an unpaired electron making it a highly unstable and reactive free radical. It can yield nitrite (NO<sub>2</sub><sup>-</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) in aqueous systems and reacts rapidly with the superoxide radical forming peroxyxynitrite anion (ONOO<sup>-</sup>). It has numerous beneficial roles such as a vasodilator and is the key bactericidal component of macrophages. Consequently pathogenic bacteria have evolved a number of activities that detoxify NO and its redox products and are able to modulate gene expression in response to it.

Fig 1.



Cj0466 (NssR) mediates the inducible expression of Cgb in response to reactive nitrogen species. GSNO was added to growing cultures of wild type *C. jejuni*, *cgb* mutant, AV17 (Fur<sup>-</sup>), AV63 (PerR<sup>-</sup>), Cj0466<sup>-</sup> and complemented Cj0466. The expression of Cgb was detected at the times specified using anti-Cgb antibody. Bands corresponding to a protein of 16 kDa were detectable in all strains except *cgb*<sup>-</sup> and Cj0466<sup>-</sup>.

*Campylobacters* are likely to encounter elevated levels of NO during infection and must therefore respond to this bactericidal activity. Invasion of the epithelial mucosa plays an important role during *Campylobacter* infection and NO production from macrophages and enterocytes forms a key component of the inducible defence. Possibly as a consequence of these mechanisms NO synthesis is markedly increased in patients with infective gastroenteritis (Forte *et al.*, 1999). *Campylobacters* may also be exposed to NO in the stomach since the chemical generation of NO can occur here as a consequence of microbial nitrite production in the mouth.

Current research at the University of Surrey has investigated the mechanisms underlying survival of *Campylobacter jejuni* during oxidative and nitrosative stress. Following publication of the *C. jejuni* NCTC 11168 complete genome sequence (Parkhill *et al.*, 2000) two novel haemoglobins were identified (Elvers *et al.*, 2004). Bacterial haemoglobins can be classified into three broad groups, the flavohaemoglobins for which the most fully understood is Hmp of *E. coli*, the single domain globins first identified in *Vitreoscilla* and the truncated globins two of which have been identified in

mycobacteria. *C. jejuni* contains both a single-domain globin and a truncated globin. The single-domain globin Cgb (*Campylobacter globin*) has significant homology to the heme domain from *E. coli* Hmp and other flavohaemoglobins, however it does not possess the binding sites for FAD and NAD(P)H seen in these two-domain globins. The role of Cgb was investigated by comparing oxidative and nitrosative stress resistance in the wild-type and *cgb* mutant. The *cgb* mutant was found to be hypersensitive to a nitrosating agent (*S*-nitrosoglutathione; GSNO) and sodium nitroprusside (SNP) and NO releasing agent spermine NONOate, yet sensitivities to peroxide, organic peroxides and superoxide were

superfamily. Mutant strains defective in *perR* and *fur* showed unaltered inducible *cgb* expression and have been eliminated as major regulators for *cgb* (Fig. 1). However, when the gene encoding Cj0466 was mutated, inducible expression of Cgb was almost totally abolished in the corresponding mutant (Fig. 1) suggesting a prominent role for this regulator in NO-responsive *cgb* expression (Elvers *et al.*, 2005). Complementation of the NssR-mutant by insertion of an intact copy of the gene into an isolated pseudogene in the chromosome fully restored GSNO-inducible Cgb expression (Fig. 1). As a result, Cj0466 was designated as NssR (Nitrosative stress sensing Regulator). Microarray experiments were conducted

Fig 2.

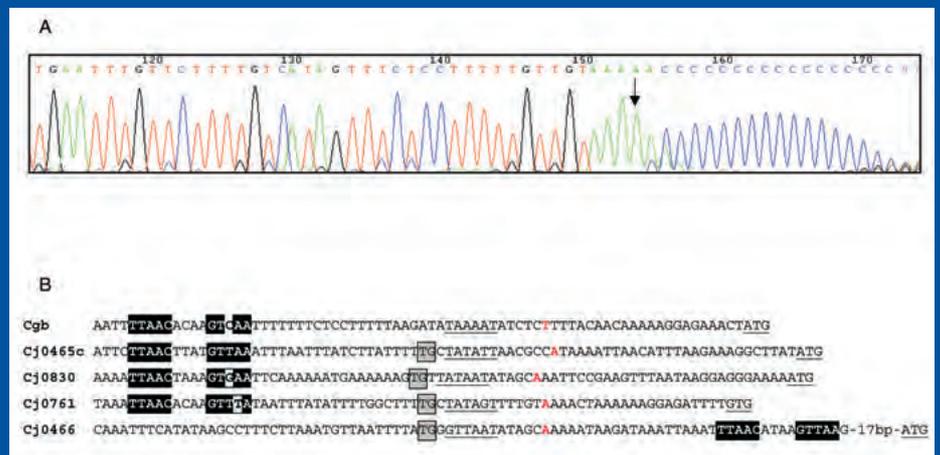


Fig. 2a. The sequence of the *cgb* PCR product from 5' RACE indicating the transcription start site.

Fig. 2b. Transcription start sites (bases in red) of promoters upstream of *cgb*, Cj0645c (truncated haemoglobin), Cj0830, Cj0761 and *nssR* (Cj0466) as determined by 5'RACE. Proposed -10 sequences are underlined. Putative NssR binding sites are boxed and nucleotides identical to the consensus TTAAC-N<sub>4</sub>-GTTAA shown in negative print while TG residues characteristic of extended -10 promoters are in grey boxes

equivalent to the wild-type. Construction of a reporter gene based on arylsulfatase and Western analysis using a polyclonal antibody prepared from purified Cgb showed that Cgb expression was strongly and specifically induced after exposure to nitrosative stress and absent in the *cgb* mutant (Fig. 1). This suggested that there was a novel capacity for NO-related stress sensing in this foodborne pathogen.

Further analysis of the genome sequence revealed a number of potential regulators, which by analogy with other bacteria, might sense NO and mediate the NO-responsive expression of Cgb in *C. jejuni*. These included Fur, PerR and Cj0466, a member of the Crp-Fnr

in order to define the extent of the regulon influenced by NssR. Eight genes were found to be over two-fold upregulated in response to nitrosative stress and these included *cgb*, the truncated globin, and six other genes of unknown function. Of these *cgb*, the truncated globin and two of the other genes were dependent on NssR. Consistent with NssR being a Crp-Fnr superfamily member, a Fnr-like binding sequence (TTAAC-N<sub>4</sub>-GTTAA) was found upstream of Cgb, the truncated globin and two of the other genes (Fig. 2). Site-directed mutagenesis confirmed that this *cis* acting motif mediates the nitrosative stress inducible expression of *cgb*.

I would like to express my thanks and appreciation to the Society for Applied Microbiology for their generous award from The Presidents Fund, together with a University Research Support Fund, which has allowed this work to be presented both orally and as a poster at the 13th International Conference on Campylobacter, Helicobacter and Related Organisms, Australia, September 2005.

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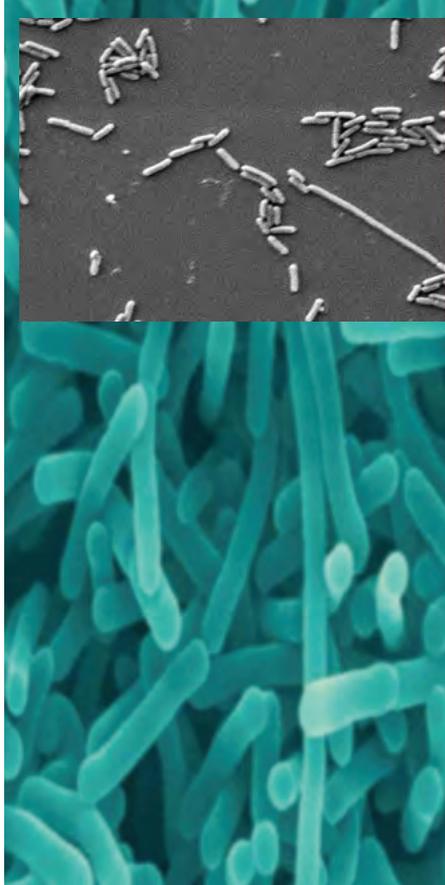
## Morphological alterations of *Listeria monocytogenes*

**L**ISTERIA MONOCYTOGENES is a causative agent of both sporadic and epidemic food-borne illness and has emerged as an important human and animal pathogen in the 1980's.

Human listeriosis is quite rare but with a high mortality rate (30%). *L. monocytogenes* is ubiquitous in nature and is resistant to diverse environmental conditions such as low pH, high sodium chloride concentrations, low oxygen level,

and is able to grow at very low temperatures (2-4°C) (Farber *et al.*, 1991). It poses a particular threat to the food processing industries owing to its ability to form biofilms, which serve to protect individual cells from operations designed to remove or inactivate microbial contaminants from food processing environments. The ability to adjust to such adverse conditions is probably important for survival and growth of the pathogen in contaminated food products, and within host organisms.

Filamentation of *Listeria monocytogenes* at pH 9.2



The resistance of *Listeria monocytogenes* to stresses is important in the food industry, where mild or inadequate treatments could allow the survival and subsequent growth of small numbers of contaminating cells, leading to the subsequent development of sufficient cell numbers to constitute infectious doses in susceptible individuals.

As addressed by Leistner (1995), food borne pathogens are frequently stressed during food processing, distribution and storage. Compelling evidence that has been gathered over the last years which

shows for many food-borne pathogenic bacteria, exposure to sub-lethal environmental stress hardens these bacteria, and as a consequence, stress-adaptive strains have increased resistance to normally lethal levels of the same (homologous) or different (heterologous) inimical stresses (Gahan, 1996). Extensive research has been carried out on some of these stresses, including acid, oxidative, low and high temperature stress in *L. monocytogenes*.

Bacteria are commonly exposed to alkaline stress in the food processing environment, particularly because of the alkaline nature of the detergents and disinfectants used to clean processing machinery and surfaces. They are also exposed to mild alkaline pH values in certain foods, in the phagolysosome during their pathogenic cycle and in parts of the intestine due to the alkaline nature of the pancreatic secretion. However, the focus of research on alkaline stress has largely been directed toward gram-negative microorganisms such as *Escherichia coli* and *Salmonella*. Less is known about gram-positive foodborne pathogens such as *L. monocytogenes*, although the alkaline responses of Gram-positive cells are likely to differ from those of Gram-negative cells, because of differences in their cell wall structure.

Knowledge concerning the mechanisms used by gram-positive bacteria for adaptation and growth at alkaline pHs comes mainly from studies of alkaliphilic strains of *Bacillus* species, although some information on *Listeria* is available. Taormina *et al.*, (2001) confirmed that *L. monocytogenes* has the ability to survive in alkaline media under refrigeration and ambient temperatures, and reported enhanced thermotolerance due to alkaline stress. The extent of cross protection of *Listeria* against heat (56°C) increased in proportion to the length of exposure time, but not the severity of alkaline challenge. It is likely that, as is the case with other bacteria, e.g. *E. coli*, the alkaline stress response of *L. monocytogenes* is transient. Thus transfer of stressed cells into a non alkaline environment, leads to reversion to the original levels of tolerance to a secondary chemical or physical assault (Taormina *et al.*, 2001).

A number of rod-shaped bacterial species, including some pathogens, form elongated filamentous cells when exposed to marginal growth conditions, suggesting that such bacteriostatic conditions inhibit

septation, leading to extensive cell elongation and filamentation. Filamentous cells of *Listeria* have been reported in a range of marginal growth conditions such as at 42.5°C (Rowan *et al.*, 1998) and at aW 0.94 (Jorgensen *et al.*, 1995).

My study using Scanning Electron Microscopy (SEM) has shown that sublethal alkaline stress induced cell elongation, filamentation and formation of atypical chains in *Listeria monocytogenes* 10403S and its *sigB* deficient mutant. Morphology of some members of the population was significantly altered above pH 9.0. In buffered media there was a relationship between the duration of alkaline treatment and the extent of morphological change. In non-buffered media, changes in cell morphology were less pronounced. Filamentation occurred in parent and *sigB* deficient mutant strains, suggesting that the process of filamentation is *sigB* independent. Sublethally stressed cells regained normal morphology and size within three hrs of transfer into neutral (pH 7.4) conditions.

Such changes in morphology may be an adaptation mechanism of the bacterium and might be important in the ability of this pathogen to survive alkaline challenge occurring during phagocytotic ingestion, and the application of alkaline detergents during cleaning of food production environments. The filamentation of major pathogen especially those with a low infectious dose such as *Listeria monocytogenes* is of particular concern, since, in such cases, the filamentous growth of even a single contaminating 'unit' in food, could result in bacterial numbers that exceed the infectious dose, on subsequent septation and division. In addition, plate count techniques to enumerate bacteria during filamentation, fail to reflect the increase in cell biomass (and future infective potential) that is occurring. The development and presence of filamentous forms, reduces the implicit relationship between colony forming units and biomass, leading to underestimation of the number/concentrations of potential infectious units in foodstuffs, and incorrect prediction of the levels of risk posed to consumers.

Finally, I would like to thank the SfAM for giving me the opportunity to present and discuss this work at the 15th European Conference of Clinical Microbiology and Infectious diseases (ECCMID) in Copenhagen, Denmark.

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## Biochemical and Structural Characterisation of a Pectate Lyase from *Clostridium acetobutylicum* ATCC 824

**P**ECTATE LYASES ARE polysaccharide lyases that catalyse the cleavage of the glycosidic bond of pectic polysaccharides via a  $\alpha$ -elimination reaction, resulting in the formation of a double bond at the newly formed non-reducing end. Pectin is a major structural component of the primary plant cell wall and is highly concentrated in the middle lamella between the plant cells, it has a complex structure consisting of a backbone of partially methyl-esterified

galacturonic acid (smooth regions) residues linked by  $\alpha$  1-4 glycosidic bonds with alternating rhamnogalacturonan branches (hairy regions).

The architecture of the plant cell wall is well adapted to resist attack from plant pathogens, it is a highly insoluble complex composed of cellulose, hemicellulose and pectins. The main role of these cell wall polymers is related to cell expansion and mechanical strength. The cellulose microfibrils are coated with hemicellulose and immersed in a pectin matrix.

Pectate lyase activity was first identified in 1962 in cultures of *Erwinia caratovora* and *Bacillus sp*; these enzymes are secreted by both pathogenic and saprophytic microorganisms leading to the maceration of plant tissue. (Star, M.P. and Moran, F., 1962). The best studied microbial pectate lyases are those produced by *Erwinia chrysanthemi*, a plant pathogen which causes severe maceration of parenchymatous tissue in various dicot plants. The bacterium enters the plant through wounds produced by either insects or harvesting, then multiplies in the intracellular spaces secreting large amounts of pectinolytic enzymes.

Pectate lyases belong to the polysaccharide lyase group of carbohydrate active enzymes and are grouped into families based upon sequence similarity. (Coutinho, P.M. and Henrissat, B., 1999). It is likely that all pectate lyases secreted by pathogenic and saprophytic bacteria share a common enzymatic mechanism. Major advances in the understanding of these enzymes catalytic mechanism has been made through the recent structure determination of Pel1C and Pel9A from the plant pathogen *Erwinia chrysanthemi* and Pel10A from *Cellvibrio japonicus*. (Kita, N. *et al.*, 1996; Charnock, S. *et al.*, 2002; Jenkins, J. *et al.*, 2004).

*Clostridium acetobutylicum* is a gram positive, spore forming, anaerobic bacterium which resides in soil and lives opportunistically on decomposing plant matter. This bacterium secretes an array of carbohydrate active enzymes: it has been predicted that six genes encode putative polysaccharide lyases, sixty four genes encode putative glycosyl transferases, seventy one genes encode putative glycosidases and transglycosidases and twenty two encode putative carbohydrate esterases. Open

reading frame CAC1968 belongs to polysaccharide lyase family 9, this gene has been cloned and the protein has been expressed and purified to homogeneity via immobilised metal affinity chromatography and gel filtration.

Preliminary biochemical assays show CAC1968 is active against polygalacturonic acid, has a pH optimum of 7.0, a calcium chloride optimum of 0.2mM.

For structure determination a selenomethionyl protein preparation of CAC1968 was required to facilitate multiple amorphous diffraction data collection. However CAC1968 lacks any internal methionines, therefore a methionine mutant; I44M, was created. An appropriate location for this mutation was identified via alignment of CAC1968 with another family 9 pectate lyase, Pel9A from *Erwinia chrysanthemi*. (Jenkins, J. *et al.*, 2004).

Native CAC1968 and the I44M mutant were crystallised in the P3<sub>1</sub>2<sub>1</sub> space group with unit cell dimensions of a=56.2827Å, b=56.2827Å, c=120.0700Å, each containing one molecule in the asymmetric unit. Data was obtained to a resolution of 1.8 Å.

In addition two inactive mutants: K209A and K209R were created and co-crystallized with trigalacturonic acid to identify key enzyme substrate interactions to unravel the catalytic mechanism. These crystals have been subjected to x-ray crystallography and diffraction data has been obtained and is awaiting analysis.

A greater understanding of these enzymes will inform the design of new anti-microbials directed against phytopathogenic microbes, facilitate the further exploitation of these enzymes in the fruit and vegetable industry and aid in the conversion of waste lignocellulose biomass.

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## Sterilisation of medical devices in hospitals

**H**IGH-LEVEL DISINFECTION of hospital items is a key measure in the prevention of nosocomial infections.

Hospital items fall into three different categories; critical, semi-critical and non-critical items. Critical items are those devices that carry a high risk of infection if the item is contaminated with microorganisms or bacterial spores.

Therefore any item that enters sterile tissue or the vascular system must be sterile. Items in this category include catheters, needles, surgical instruments and surgical implants. Semi-critical instruments such as endoscopes require high-level disinfection to destroy all microorganisms, but not high-levels of spores. Semi-critical items do not need to be free of spores as they only come into contact with intact mucous membranes which are generally resistant to infection by common bacterial spores. Non-critical items are those that do not come into contact with skin such as bedpans and as

a result the items are simply cleaned and low-level disinfectants used to reduce levels of bacteria.

The sterilisation of critical items is important requiring the removal of all forms of microbial life, including spores, to avoid patient to patient transfer of infection. This is especially pertinent as there is an expanding practice of re-using critical items such as pace-makers and narrow-lumened heart catheters. In the United States laparoscopes and arthroscopes (critical items) that enter sterile tissues are often not sterilised between different patients and only undergo high-level disinfection (Rutala *et al.*, 1991). This seems inadequate as many of the high-level disinfectants available for clinical use are not effective sporicidal agents. The medical devices themselves also offer an obstacle, due to their design which often includes long and narrow lumens and sharp angles. The presence of organic matter on medical devices represents a further problem as this limits the effectiveness of sterilisation especially when the narrow lumens contain a high organic load (Alfa *et al.*, 1996). The prior cleaning of medical devices is therefore paramount to the effectiveness of sterilisation techniques.

High-level disinfectants such as those used in the USA for certain critical items, are not successful sporicidal agents as a result of a number of factors. Bacterial endospores are the most resistant living structures known, as they have no metabolism and as a result can withstand a wide range of environmental assaults including heat, UV and solvents. Many of the high-level disinfectants can not tackle this resistance, especially in the presence of organic matter, and are therefore often not sporicidal. It is also known that for a high-level disinfectant to be sporicidal higher concentrations and often elevated temperatures are needed than for bactericidal activity, which would inevitably increase the cost of the treatments. The need for complete sterilisation of critical items is paramount to avoid the transmission of disease between patients.

Steam sterilisation is thought to be the most effective sterilisation method as it is non-expensive, non toxic and sporicidal. Autoclaving occurs at high temperatures, most commonly 121°C. Heat sensitive devices however require alternative methods of sterilisation as they can not survive the high temperatures and pressures of the autoclave. The use of

Ethylene oxide for sterilisation of critical items has been used extensively, however a ban on chlorofluorocarbons in 1995, which were used as a stabilising agent in combination with ethylene oxide (Rutala *et al.*, 2001) reduced its use. At low temperatures ethylene oxide has very limited sporicidal activity, however this can be augmented as the temperature is increased. The use of alternative stabilising agents such as carbon dioxide and hydrofluorocarbons has allowed this chemical to continue to be used to sterilise critical items.

Since the observation by Koch over 100 years ago that *Bacillus anthracis* could survive boiling there has been a tremendous amount of research on the mechanisms of spores and their resistance. Through continued research, the strict following of guidelines for the sterilisation of critical and other medical devices, and effective staff training programmes this may help to reduce the incidence of disease including those attributed to the presence of spores.

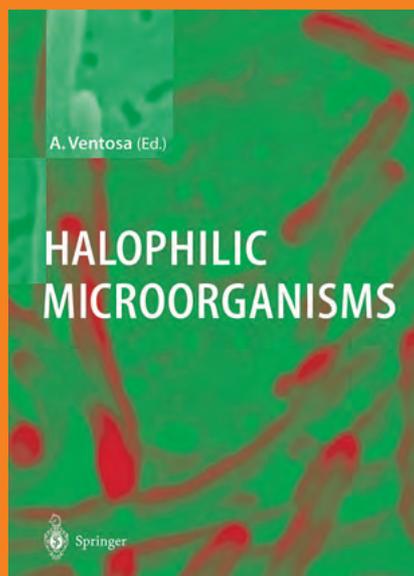
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Jennifer Shackelford

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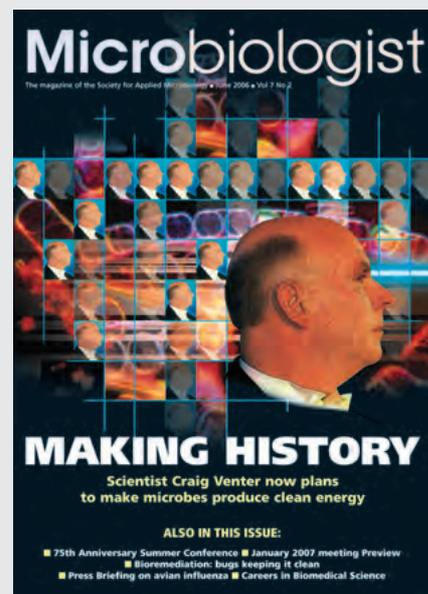
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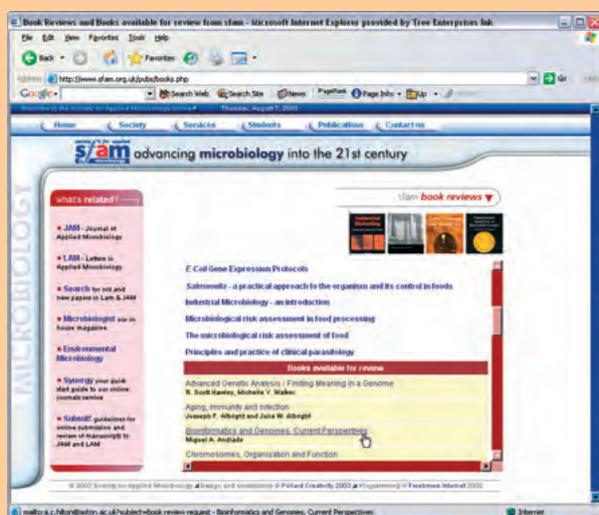
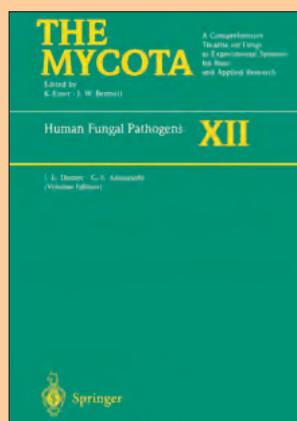
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## Principles of Virology

S. J. Flint, L. W. Enquist, V. R. Racaniello, and A. M. Skalka  
 Publisher: ASM Press  
 ISBN No.: 1-55581-259-7. \$109.95  
 Reviewed by: **Wes P. Kuhne**

My first introduction, in detail at least, to the field of virology was with the 2nd edition of *The Biology of Animal Viruses* by Frank Fenner, *et al.*, while doing my graduate studies. *Principles of Virology* takes a more student-reader friendly approach. Many texts in this and other disciplines of microbiology have a mixed and erratic style. It is apparent from the text that the authors went to extraordinary lengths to ensure a uniformity of style and presentation. While of some substance in its detailed approach to the field of virology, the text will be well suited for upper class ranking in undergraduate courses and graduate work. The major determinant of this is the heavy emphasis on the molecular biology of viruses and the host.

Two sections of the text demonstrate the authors' application of current information. First, the section on Molecular Biology of viruses takes the approach of studying the virus at this level – the integration of biochemistry, molecular genetics, and cell biology. The information presented is based on the latest research in the fields at the time of publication. Following a thorough tour of viral genomes and structure, the reader is immersed into the core of viral-host interaction with chapters on RNA virus genome replication, reverse transcription, DNA templates, DNA virus replication, and viral pre-mRNA. Additionally, the citation of various websites gives the reader some initial guidance in furthering their studies. Secondly, the final 2 chapters on the control and evolution of

### Erratum

The previous issue of *Microbiologist* contained an error in the contact details of T2 scientific, publishers of the '*Microbiology of Drinking Water*' CD which was kindly reviewed by Alan Godfree. The correct email address is: [t2scientific@ntlworld.com](mailto:t2scientific@ntlworld.com) and the correct website address is: [www.t2scientific.co.uk](http://www.t2scientific.co.uk)

You can also make contact with T2 Scientific using the Contact Form on the website

viruses and viral diseases bring in a unifying approach to the entire text. While the concept of evolution of infectious diseases is not new, its application in such a text takes the reader from a reductionist approach of studying virology to a much larger macrobiologic approach. These final 2 chapters help the reader put the wealth of information found in this text into a usable viewpoint that should be readily adaptable to taking laboratory theory and applying the information in the real world.

As mentioned above, the authors did an excellent job unifying the writing styles of the various contributors. A conglomeration of different styles is often the downfall of many texts as they pose a constant shift in how to approach the information. Such is not the case here. Additionally, the authors make good use of color diagrams and artistic renderings of experimentally determined events. These are often tied to good clear electron micrographs and computer translation of experimentally derived electronic information.

This approach is not limited to the main body of the text (molecular biology), but carries over into the section on pathogenesis. Many general texts present viral pathology as a subject relegated to the realm of the medical text book and only cover the topic with a courteous passing. This not the case here as the authors have allocated a good portion of the book's topics to dissemination and virulence, host defense and immune response, patterns of infection, the ever present chapter on HIV, and oncogenesis.

While the authors do not present any new information to the field of virology, that is not the purpose of any textbook really. The authors have done an outstanding job doing what they set out to do. That is, prepare a text that presents a wealth of information in a uniform, readable, and utterly useful format.

The text is well worth the investment by undergraduate, graduate, researchers, and anyone needing a great, readable reference in the field of virology on their book shelf.

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## Revenge of the Microbes: How bacterial resistance is undermining the antibiotic miracle

Abigail A Salyers and Dixie D Whitt  
2005. ISBN 1-55581-298-8. £20.00  
Reviewed by Bengü Said

This book is not, as one could be forgiven for assuming from the title, a work of science fiction. Instead it is a factual text and an 'attempt to write a book for the general public about antibiotics and resistance to them.' In writing for the general public the authors have also written a book, which will be of interest to a wide-range of people, including students or professionals who may require an introductory or basic knowledge of antimicrobials. This is a

timely book on an issue that is very much in the news with numerous recent headlines concerning 'superbugs' and hygiene in hospitals.

Despite the serious subject matter, the text is often light-hearted and humorous and gives the reader not only the scientific facts but also deals with them in the modern social, economic and political context. The book is written mainly from an American perspective but does retain a broadly international outlook. As they say 'The need for new antibiotics is not a US problem. It is an international problem.'

The book is primarily about antibiotics and bacterial resistance but the authors also devote chapters to antiseptics and disinfectants and to compounds that act against viruses, fungi and protozoa. The authors explain terms, such as antibiotic, antiseptic, and disinfectant, in clear simple language in their introductory chapter and return to these in more detail in later chapters. The narrative progresses through a brief history of antibiotics, how bacteria adapt and develop strategies for circumventing the

action of antibiotics, the declining efficacy of antibiotics and where this trend may lead. They identify a potential crisis in antibiotic availability, with the appearance of microbes resistant to the antibiotic vancomycin, our last defence against strains of *Staphylococcus* and *Streptococcus* species.

The core chapters of the book describe the resistance strategies of bacteria: these usually involve a single resistance protein, an enzyme that inactivates the antibiotic, or an altered antibiotic target that is no longer affected by the antibiotic. More technical information on the actual structures of antimicrobial agents mentioned in the text and a description of how resistance is measured in clinical laboratories are placed in the appendices. At the end of each chapter in this book there is an 'issues to ponder' section and these are often thought provoking and intended to stimulate further discussion. In addition the book also has a useful index so it can be dipped into or chapters can be read as stand alones to get brief but comprehensive information. In such a concise text the authors can perhaps be forgiven for the occasional over-simplification and those who wish to learn more can always turn to more traditional scientific texts, such as those in the suggested reading section.

In their final chapters the authors suggest responses to the resistance problem. They give options including, more prudent use of antibiotics to reduce the selection pressures that encourage resistant bacteria to emerge, or directly targeting and inactivating bacterial mechanisms for resisting antibiotics. It is indeed hard to contemplate a world without antibiotics and the public health impact this would have — 'The bugs have won! Here eat this root' scenario.

Fortunately, the authors remain optimistic that we can still act to preserve the antibiotics we already have. 'The keys to future success in saving antibiotics are knowledge and the willingness of the public to take an informed interest not only in preserving the efficacy of the antibiotics we have now but also in ensuring that future development of new antibiotics continues.'

So they conclude that 'there is plenty of hope' that the current 'detente' we have with the microbes will continue. After all they were here before us and they are not consciously malevolent, in fact many of them are vital to our existence.

## Essential Fungal Genetics

David Moore and LilyAnn Novak Frazer  
Springer-Verlag, New York. 2002  
357p + xi, 59 figs.  
ISBN 0-387-95367-1. €79.95

**Reviewed by:**  
**Marcel Gutierrez-Correa**

*Essential Fungal Genetics* is a concise but complete description of the genetics of fungi in a textbook that covers traditional genetics, such as mutation, segregation and recombination, while including the latest molecular biology tools. Ten authoritative chapters treat important aspects including genome interactions, wild types and mutants, segregation genetics, recombination analysis, mechanisms of recombination, the physical genotype, mapping the fungal genome, fungal phylogeny and evolution, and fungal differentiation and morphogenesis. Although the authors' goal is to integrate 'fungal genetics into current teaching by complementing major textbooks used in courses in general genetics, general organismal biology, and general mycology', I found it valuable as a textbook by itself for biology, microbiology and biotechnology advanced undergraduate majors and, perhaps, for graduate students in the fields of plant pathology (phytopathology), microbiology and industrial biotechnology. While reading the book, I thought that a specific course of fungal genetics is lacking in many schools, particularly in this century when fungal biotechnology is highly relevant.

As stated before, this book has included both classical and molecular techniques together that makes for a satisfying read. From chapters 3 to 8 the reader has an account of the above with the advantage that there are numerous interesting examples ranging well beyond the normal model species used in standard texts. In this sense, this book differs from others on fungal genetics in that the examples chosen to illustrate various features tend to favour basidiomycetes and the less-often referenced fungi. I particularly enjoyed chapter 1 (Why Study the Genomes of Fungi?), chapter 2 (Genome Interactions), and chapter 10 (The Genetics of Fungal Differentiation and

Morphogenesis). In the first chapter, there is a succinct, but useful overview of the origin, phylogeny and evolution of fungi that is then very well explained in chapter 9. In the second, the reader will find the necessary information related to fungal sexuality, which is actually very complex but treated in a fairly understandable form. The last chapter is perhaps the most outstanding as it covers very new and important topics in fungal genetics. Also, this chapter is developed as a typical textbook issue that not only informs but motivates the reader to search for further information, pushing one into the fascinating world of functional genomics and into systems biology. The book presentation omits reference citations so that readers can go undisturbed through the text. However, lists of important publications and websites 'worth a visit' and historical publications 'worth knowing about' are given at the end of each chapter.

But humans are far from perfect and so is their work! Core chapters 3 to 8 are written in quite a dense style, often demanding careful re-reading and there are fewer diagrams than are necessary for the understanding of tough topics. This is important for a textbook, particularly as young people are being raised on highly diagrammatic texts plenty of colored pictures and schemes. As classical and molecular fungal genetics are difficult subjects even for mature scientists, less dense style and more colorful illustrations would help to attract the attention of young scientists. Basidiomycetes are vindicated by the book but zygomycetes are again forgotten since really very few examples from this industrially important group are considered. Also, the adhesion process in filamentous fungi related to differentiation and gene expression is missing in the discussion. Finally, I think that the revision notes at the beginning of each chapter are too numerous and would be better if short summary sections were considered. I am afraid that the price of the book will discourage students from purchasing it.

Despite these minor reservations, I will certainly use this book to reinforce my advanced undergraduate Molecular Microbial Genetics course which was in need of this source. For professionals searching for a summarised and up-to-date basic information on fungal genetics this book is a highly valuable choice.



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### Publications

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### Meetings

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