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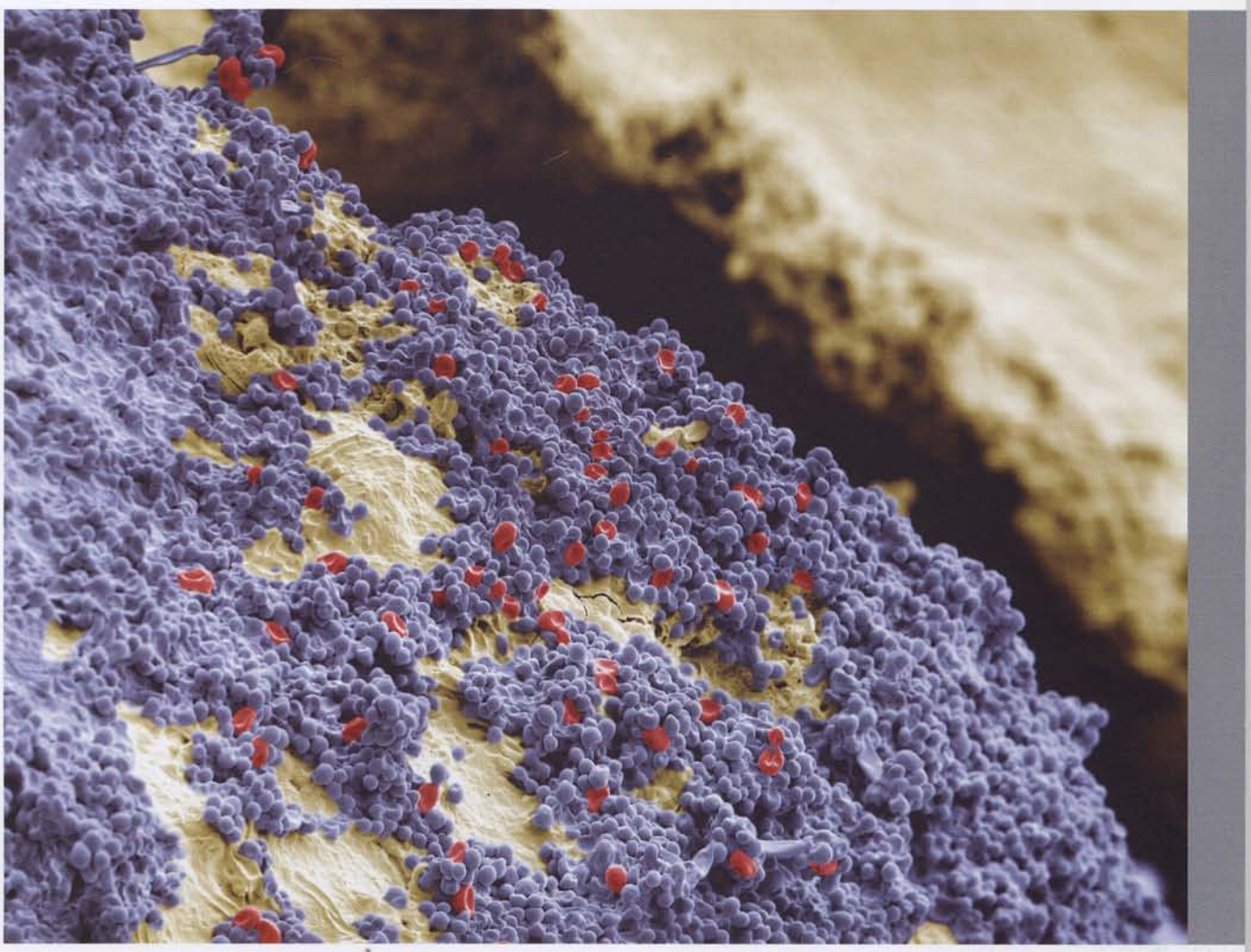

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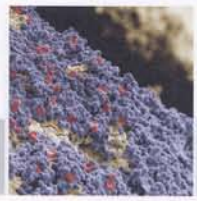

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other items

articles

Cover image Coloured SEM of bacteria on the surface of a human tooth. *Mona Lisa Production / Science Photo Library*

Editor Dr Gavin Thomas Editorial Board Dr Sue Assinder, Dr Pauline Handley, Professor Iain Hagan Managing Editor Janet Hurst Assistant Editor Faye Jones Design & Production Ian Atherton Contributions are always welcome and should be addressed to the Editor c/o SGM Headquarters, Marlborough House, Basingstoke Road, Spencers Wood, Reading RG7 1AG Tel. 0118 988 1800 Fax 0118 988 5656 email mtoday@sgm.ac.uk web www.sgm.ac.uk Advertising David Lancaster, McMillan-Scott PLC, London Office, 10 Savoy Street, London WC2E 7HR Tel. 0207 878 2316 Fax 0207 379 7118 email david@mcmslondon.co.uk Regular feature images pp. 3 *SGM*; 33 *Imperial College London/SPL*; 35 *Mauro Fermariello/SPL*; 37 *Stockbyte/Photolibrary.com*; 39 *Taxi/Getty Images*; 41 *Simon Lewis/SPL*; 43 *Tek Image/SPL*; 46 *Digital Vision/Getty Images*

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As Editor I have the privilege of announcing these enhancements to MT, but it is **Ian Atherton** and **Janet Hurst** who convert them into reality. Ian deserves the credit for the new layouts; many thanks to him and the other members of the team at Marlborough House for their enthusiasm and exquisitely good taste in recreating MT for 2005! I would also like to add my thanks and best wishes to **Janice Meekings**, who has recently retired after being Assistant Editor on MT for many years. Welcome also to **Faye Jones**, who has been helping with the magazine for a while, but is now taking on an enhanced role.

Feedback on the new design will be very welcome (**e** mtoday@sgm.ac.uk).

Gavin Thomas

IFR Prize

Catherine Burgess, a PhD student at University College Cork, was awarded the Institute of Food Research Science Communication Prize for her offered paper entitled *Lactococcus lactis, a 'vitamin factory' of the future?*. This was delivered in the symposium on *Lactic acid bacteria* at the Society's recent meeting at Trinity College Dublin. Catherine received a cheque for £100 and a certificate.

SGM Council

November meeting highlights

Honorary Membership

Sir John Skehel FRS, Director of the National Institute for Medical Research, Mill Hill, London, was elected an Honorary Member of the Society.

SGM Prizes & Lectures 2004

The following were awarded.

The Fleming Lecture to **Dr Adrian Whitehouse**, University of Leeds, for excellence in research on the molecular biology of gammaherpesviruses.

The Colworth Prize Lecture to **Professor Rick Titball**, Dstl Porton Down, for outstanding contributions to applied microbiological research relating to *Clostridium* toxins.

The Fred Griffith Review Lecture to **Professor David Ellar**, University of Cambridge, for long-standing distinguished research relating to the biochemistry and molecular biology of *Bacillus* spp.

The Peter Wildy Prize in Microbiology Education to **Professor Joanna Verran**, Manchester Metropolitan University, for excellence in teaching and curriculum development in microbiology.

A more detailed description and appreciation of the prize winners' work will appear in *Microbiology Today*. Their lectures will be delivered at SGM meetings in 2005.

SGM Industrial Liaison Officer

Council has now agreed a job description for the new position of Industrial Liaison Officer (ILO), and a search committee was formed to prepare a shortlist of interested candidates for the next Council meeting in February. Another industrial members' forum will be held at the Heriot-Watt meeting, and it is hoped that the new ILO will be in post and able to participate.

Scientific publishing

At present intense discussions are ongoing among publishers of scientific journals about the different financial models for publishing scientific papers ('subscriber-pays' vs 'author-pays'). With a stable of four excellent journals, SGM Council has been following the arguments very closely. It was reported that in 2004, a total of 11,700 pages was published in SGM journals.

Ulrich Desselberger, General Secretary

Treasurer-elect

The Society's Treasurer, **Peter Stanbury**, is due to stand down in September 2005 at the end of his 7 year period of office. **Dr Colin Harwood**, University of Newcastle-upon-Tyne, has accepted Council's invitation to be his successor. Until he takes on the role next year, Colin will be shadowing Peter and attending all the meetings and discussions relevant to the SGM's financial affairs. A biography of the new Treasurer will appear in a future issue of *Microbiology Today*.

Nominations 2005

Three members, **Alistair Brown**, **Pauline Handley** and **Keith Jones** retire from Council in September 2005. Nominations are invited from Ordinary Members to fill these vacancies. All nominations must include the written consent of the nominee and the names of the proposer and seconder. Members submitting nominations should indicate the main area of microbiological interest of their nominee, who must have been an Ordinary Member of the Society for at least 2 years. Nominations should be sent to the SGM General Secretary, Dr Ulrich Desselberger, c/o SGM Headquarters to arrive **no later than 30 April 2005**.

Grants

For 2005 only

IUMS Congress Grants

Small grants to members to support attendance at the IUMS Congresses, San Francisco, 23–28 July 2005 (see p. 27).

Closing date **18 February 2005**

Prokaryotic Development Meeting Grants

Small grants to members to support attendance at the Joint ASM/SGM Meeting, 13–16 July 2005, University of British Columbia, Vancouver, Canada (see p. 33).

Closing date **25 March 2005**

Student schemes

Postgraduate Student Meetings Grants

Grants cover travel and accommodation expenses for attendance at one SGM meeting each year. Applicants must be Student Members resident and registered for a PhD in an EU country.

Closing date for Heriot-Watt Meeting **1 April 2005**

President's Fund

Limited grants to young microbiologists making short research visits, attending courses, or presenting work at scientific meetings. Open to Society members resident and registered for a PhD in an EU country or in their first postdoctoral position in an EU country.

Closing dates for Research Visit grants: **29 April 2005** and **14 October 2005**

Vacation Studentships

To enable undergraduates to work on microbiological research projects for 6–8 weeks in the summer vacation before their final year. Applications must be from SGM members on behalf of named students.

Closing date **25 February 2005**

Elective Grants

To enable UK/Ireland medical, dental and veterinary science undergraduates to work on microbiological research projects during their elective periods.

Closing date **29 April 2005**

Student Society Sponsored Lectures

These cover the travel and other expenses of up to two speakers on microbiological topics per Society each year at student society meetings.

SGM has a wide range of grant schemes to support microbiology. See **www.sgm.ac.uk/grants** for details. Any enquiries should be made to the Grants Office, SGM, Marlborough House, Basingstoke Road, Spencers Wood, Reading RG7 1AG (**t** 0118 988 1821; **e** grants@sgm.ac.uk).

Check out the current schemes, to ensure that you don't miss any deadlines.

Education

Education Development Fund

Small grants to members for developments likely to lead to an improvement in the teaching of any aspect of microbiology relevant to secondary or tertiary education in the UK. Up to £1,000 is also available for science promotion activities.

Seminar Speakers Fund

Small grants to cover the travel and other expenses of up to two speakers on microbiological topics in annual departmental seminar programmes.

Meetings

Technician Meeting Taster Grants

Offer up to £200 to enable eligible technicians to sample an SGM meeting.

Retired Member Grants

Cover accommodation and the Society Dinner at one SGM meeting per calendar year.

SfAM/SGM Short Regional Meeting Grants

Contribution of up to £2,000 towards the costs of running a regional microbiology meeting.

Overseas schemes

Watanabe Book Fund

Funding for members permanently resident in a developing country to purchase microbiology books for their institutional libraries. Closing date **14 October 2005**.

UNESCO-IUMS-SGM Fellowships

Funding for young microbiologists from developing countries to either (1) pursue, or complete, part of an on-going research programme in a laboratory in a developed country or (2) attend a workshop or specialist scientific meeting. See website for closing dates. Applications must be sent to **Prof. Daniel O. Sordelli**, Departmento de Microbiologia, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155-Piso 12, (1121) Buenos Aires, Argentina (**e** sordelli@fmed.uba.ar).

International Development Fund

The following awards have been made for 2004. The Fund exists to provide training courses, publications and other assistance to microbiologists in developing countries.

Prof. Johan Thevelein, Catholic University of Leuven – up to £5,000 to support a practical course on Yeast Molecular Physiology in Brazil.

Dr Ron Dixon, University of Lincoln – up to £2,690 to support staff development training in Malaysia.

Prof. Nick Russell, Imperial College London – up to £2,500 to enhance microbiology teaching at the University of Jayawardenepura, Sri Lanka.

Dr Keith Jones, Lancaster University – up to £3,350 to train Balkan microbiologists in water microbiology.

Dr Iruka Okeke, University of Bradford – up to £2,550 to support a workshop on the diagnosis of bacterial enteric pathogens in Nigeria.

Dr Simon Cutting, Royal Holloway University of London – up to £1,030 to support a course in molecular biology techniques, Vietnam.

Applications for 2005 are invited. Closing date **14 October 2005**

International Research Grants

The following awards have been made for 2004. The grants allow scientists to travel to or from the UK and Republic of Ireland to carry out a defined piece of research in any field of microbiology.

Dr Claude Sabeta, Onderstepoort Veterinary Institute, to work at the UK Veterinary Laboratory Agency

Dr Stefan Vilcek, University of Veterinary Medicine, Slovakia, to work at the Moredun Research Institute

Dr Wael Hozzein, Cairo University to work at the University of Newcastle

Dr David Lamb, University of Wales, Swansea to work at Vanderbilt University Medical School

Dr Nicholas Johnson, Veterinary Laboratories Agency to work at the Federal Research Centre for Virus Diseases of Animals.

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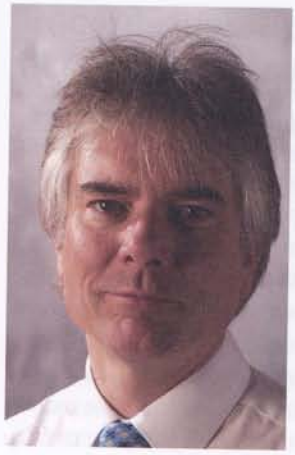

Colworth Prize Lecturer

Professor Rick Titball will deliver his prize lecture, entitled *Gas gangrene, an open and closed case*, on Tuesday 5 April 2005 at the Society's meeting at Heriot-Watt University. The Colworth Prize Lecture is awarded for an outstanding contribution to applied microbiological research.

Rick Titball is currently Group Leader for Microbiology and a Senior Fellow at the DSTL, Porton Down, UK. He is also an honorary Professor at the London School of Hygiene and Tropical Medicine and a visiting Professor at the Universities of Glasgow, Plymouth and at Birkbeck College. Aside from brief forays into virology, he has worked mainly on mechanisms of virulence of bacterial pathogens. His work over the past decade or so has exploited this information to devise pre-treatments and therapies for diseases caused by candidate biowarfare or bioterrorism agents.

Fleming Lecturer

Dr Adrian Whitehouse will deliver his prize lecture, entitled *Understanding the latent-lytic switch in gamma-2 herpesviruses* on Wednesday 6 April 2005 at the Society's meeting at Heriot-Watt University. The Fleming Lecture is awarded for outstanding research by a microbiologist in the early stages of their career.

Adrian graduated from Sheffield University, and obtained a DPhil in molecular virology at Oxford in the laboratory of Professor Bob Possee. In 1994 he took up a postdoctoral position with Professor Alex Markham in the Molecular Medicine Unit, St James's University Hospital, where he was awarded a MRC Non-clinical Fellowship in 1998. He joined the School of Biochemistry and Microbiology, University of Leeds, as a Lecturer in 2002.

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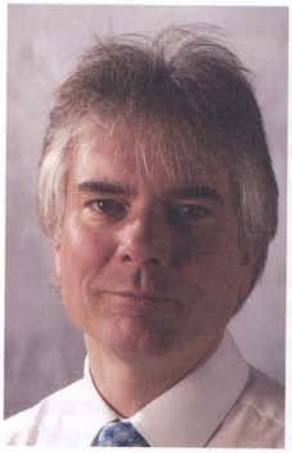

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Staff

Goodbye to MT and all that...

Janice Meekings, a long-serving member of the *Microbiology Today* team, took early retirement at the end of 2004. Janice (along with other magazine stalwarts, Ian and Janet) started at the SGM in 1990 where she worked as an editorial assistant on *JGV*. With previous publishing experience she was persuaded to join the *Quarterly* (as it was then called) staff and moved from 'downstairs' (journals) in Marlborough House to 'upstairs' (administration) and from part-time to full-time hours. She took over the day-to-day administration of the magazine and the book reviews and has been chasing up authors and reviewers firmly but tactfully ever since. She has also sub-edited all the text – thousands upon thousands of words have been under her fingers on the keyboard – helped to find illustrations, proof read all the copy and carried out the numerous sundry tasks that comprise magazine production. For a few years Janice also ran the advertising procurement, until we were able to pass on this thankless chore to an agency. Her creative input and ideas for content were also invaluable.

In her time the magazine has evolved from a dreary mono publication with few illustrations and content focusing on Society activities, into the colourful general interest magazine packed with features that we see today. This process has taken place under the guidance of a series of Council officers, whom Janice got to know very well. In the early days the copy was typeset and the pages laid out from galley proofs. Gradually the production was taken in-house and now Ian does all the DTP and we print from pdfs. Janice's contribution to the current success of *Microbiology Today* has been considerable and we are all most grateful for her efforts. Not to be forgotten are her other duties in putting together and setting a range of other materials such as the meetings programmes, posters, calendars, journal sales leaflets and the annual report. Another particularly dreadful job has been proof reading the biannual address book of members.

Although a 'backroom' person at SGM, who was happy to stay in the office, many people in the Society will know Janice through her various roles, particularly the book reviews, but also as the voice of a very helpful relief telephonist. Always public-spirited, she was also the safety officer for a while and kept the staff on their toes.

Janice will be sadly missed at the SGM, but we wish her and husband Barry every happiness in retirement. They are hoping to move away from the frenetic Thames Valley and down to the more peaceful air of Cornwall. In appreciation of her stalwart efforts over the years Council and staff gave Janice a high-spec food processor. Typically she did not want a farewell party, but we were able to make a surprise presentation at the staff Christmas lunch. We are hoping Janice will visit us soon and bring some of the goodies made with the amazing new machine.

Goodbye Janice. *Microbiology Today* won't be the same without you.

Newcomers

A warm welcome to two new staff editors who joined the SGM in January, **Claudette Doe** and **Pauline Stevenson**. Claudette has a DPhil in Biochemistry and has been working as a post doc in the Microbiology Unit at Oxford University, investigating the components and mechanisms of DNA recombination in the fission yeast, *Schizosaccharomyces pombe*.

Following some years carrying out microbiological research at the National Institute for Biological Standards and Control, Pauline has worked as a production editor, first on learned journals published by the Physiological Society, and more recently on FRAME (Fund for the Replacement of Animals in Medical Research) publications.

Prizes

Lister Research Prizes 2005

www.lister-institute.org.uk

Applications are now invited from young clinical and non-clinical scientists who wish to pursue their personal research interests in biomedical science as effectively as possible. Prizes will be allocated on the basis of independent research-based proposals. Proposals are assessed on the grounds of scientific quality and potential and are not constrained by diseases or disciplines. Each Prize Fellow will receive £150,000 to spend as they choose in support of their research over 3 years.

Infectious disease watch

OIE papers

www.defra.gov.uk/animalh/int-trde/euint/index.htm

Defra has announced a new page on its website which allows interested parties to view, and comment on, documents produced by the Office International des Epizooties (OIE). This organization makes an important contribution to international animal health issues.

Training in infection

www.trainingininfection.org.uk

This new resource for trainees in infection provides information on societies, courses, conferences, textbooks, funding and other relevant websites such as the National Electronic Library for Health (www.nelh.nhs.uk) and the National Electronic Library of Infection (www.neli.org.uk).

Foresight

Detection & Identification of Infectious Diseases

www.foresight.gov.uk

Defra is sponsoring a new Foresight Project which aims to produce a challenging and long-term vision for the detection and identification of infectious diseases in plants, animals and humans. It will take account of evolving risk of diseases, changing user requirements for detection and identification, and cutting edge science. The project will inform policy at national and international levels. You can register an interest on the website.

Fife superlab

A new £6 million research laboratory, the Scottish Structural Proteomics Facility, based at St Andrews University, opened in December. Teams will work on computer-based drug design while others screen thousands of compounds as the starting point for drug discovery. The dual approach is unique in the UK and hopefully will lead to faster development of cures for dangerous infectious diseases.

Healthcare risk management

The Centre for Hazard and Risk Management at Loughborough University is offering a postgraduate certificate/diploma/MA course on this subject starting in March 2005. It is aimed at senior managers in healthcare units. For information contact Joyce Bostock (**e** j.g.bostock@lboro.ac.uk).

Mycoplasmology

16th International Congress, Cambridge, 9–15 July 2006

For information contact **j.snook@vla.defra.gsi.gov.uk**

Recent developments in open access for journal articles

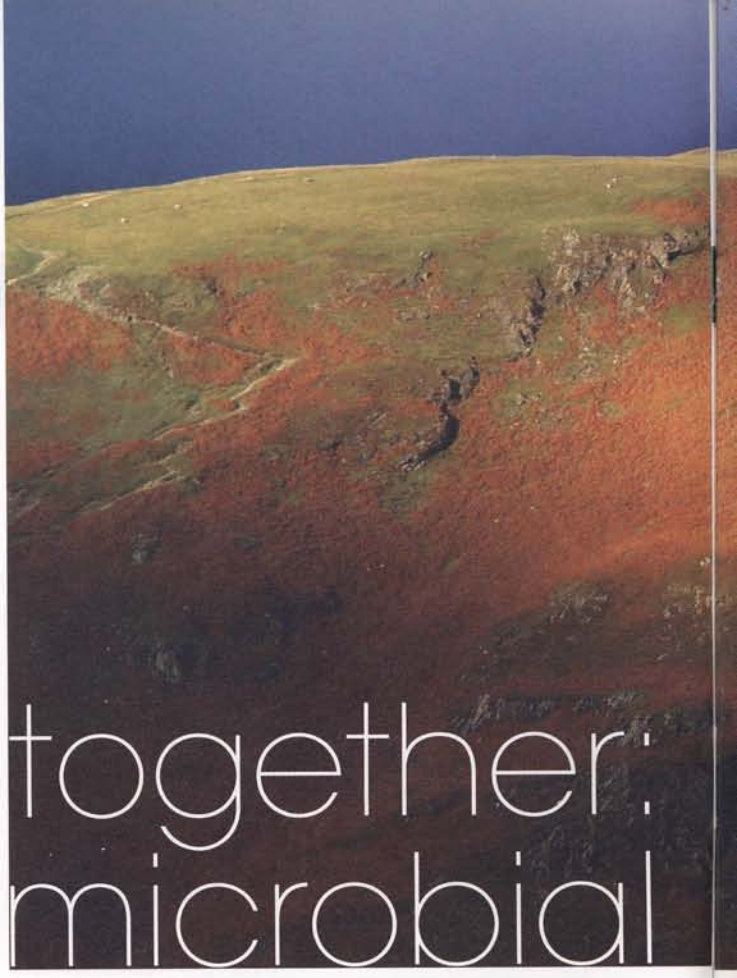
The House of Commons Science and Technology Select Committee published the report of its inquiry into Scientific Publications in July 2004. This supported the idea that authors should make their articles freely available online on their institutional repositories (something that SGM and many other publishers already allow), and that the government should make funding available for construction of such repositories. The Committee did not come out at this stage in favour of government support of open access, author-pays journals, but suggested that funds be made available for further experimentation with the model. They recognized the challenges to the traditional subscription model, and noted that learned societies used their surpluses from journal publication for general benefits such as support of students, subsidized conferences and educational activities.

In response, the government said it was unwilling to interfere with the operation of the market by subsidizing one particular business model. In this, the Committee felt that the government had been heavily influenced by the Department of Trade and Industry, on behalf of the commercial publishing industry.

Meanwhile across the Atlantic, the National Institutes of Health created quite a stir, by demanding that in future, articles describing NIH-funded research should be deposited on PubMedCentral within 6 months of publication, and be made freely accessible. Similar proposals emanated from the Wellcome Trust here. These moves elicited a range of responses from publishers: many learned societies felt that they had not been fully thought through, and risked causing collateral damage to society activities and the integrity of the publishing process through the law of unintended consequences. However, NIH and Wellcome are powerful bodies: NIH is acknowledged as a funder on about 10 % of articles published in SGM journals, and Wellcome on 4 %. Both bodies are prepared to make funds available towards the costs of early open publication; whether these would be sufficient to defray any loss of subscription income remains to be seen. There will have to be lots more discussions in various fora, right up to Capitol Hill, before the final shape of things becomes clear.

SGM Publications Committee and the Editorial Boards will continue to monitor developments and consider how best the Society's journals should respond.

Ron Fraser, Executive Secretary



Living together: microbial

This issue of *Microbiology Today* focuses on microbial communities. Scientists are only just beginning to unravel the complexities of the interactions between micro-organisms living together. **Hilary Lappin-Scott** and **Sarah Burton** explore some of the latest research in this fascinating area.

Traditional microbiological research has focused on simple systems, often monocultures, as these offered the most suitable means of characterizing and identifying micro-organisms. In nature microbes do not live in isolation; they live in communities where their activities and impact on their environment, such as the soil, gut flora and dental plaque, remain to be fully elucidated. During the pre-molecular era, research into the complexities of natural microbial communities, including genetic diversity and gene expression, was hindered by the lack of suitable techniques. With the advent of the modern molecular toolbox the intricacies of community interactions and dynamics are becoming apparent.

For example genetic composition and environmental pressures play important roles. Moreover, some of the consequences of this communal style of existence may have a profound impact on cell functions (e.g. population density dependent communication, also known as quorum sensing).

Biofilms

The impact of the physical nature of the surrounding environment on microbial communities has been highlighted by the study of biofilms – cells associated with surfaces. Adherence has been found to have a gross effect on cellular survival, growth and removal from these surfaces, which has important implications for healthcare (e.g. control of infections), industry (e.g. control of pipeline fouling/corrosion) and natural microbial ecosystems.

Genomics

Until recently there was a great investment of resources in species definition, characterization and phylogenetics. Current developments in bacterial genomics have resulted in

communities

an enormous growth in DNA sequence analyses and accompanying evolution of database management. This explosion of information has allowed a greater appreciation of the diversity of microbes within commonly encountered natural communities (e.g. soil). Research methods are no longer dependent on the traditional culture of these microbes and the bias that is inherent within this. Many organisms that cannot be cultured in the lab are being revealed.

Community genome function

In comparison there has been limited progress in our understanding of the spatial distribution within communities. One of the major challenges which remains is to understand real-time genome functions of whole communities. Advances in technology, for example the introduction of fluorescence *in situ* hybridization (FISH) and developments in proteomics, have enhanced research capabilities and allowed the development of analysis of whole-community genome expression. This invites research into natural community functions, encompassing all their complexities, to give a more realistic understanding of *in situ* environmental community function.

Methods have been recently developed for the direct profiling of complete natural microbial populations by hybridization of native rRNA recovered directly from environmental samples to microarrays designed to identify major microbial groups (Phylochip). Currently, there is much excitement about high-throughput DNA microarrays that allow simultaneous hybridizations of thousands of genes and have the potential to elucidate whole-community gene expression.

Identification of functional portions within microbial communities

Nascent molecular methods are now used for the detection and identification of the organisms within microbial communities with particular activities (e.g. biodegradation of environmental pollutants). This approach employs the use of stable isotope probing (SIP). Specific isotopic (heavy) substrates are made available to the microbial community and the fate of the substrate isotopic constituents tracked via incorporation into the nucleic acids by *de novo* synthesis during replication. Ultracentrifugation is used to achieve separation of the heavy and light fractions of DNA. Subsequent sequencing of the 'heavy'

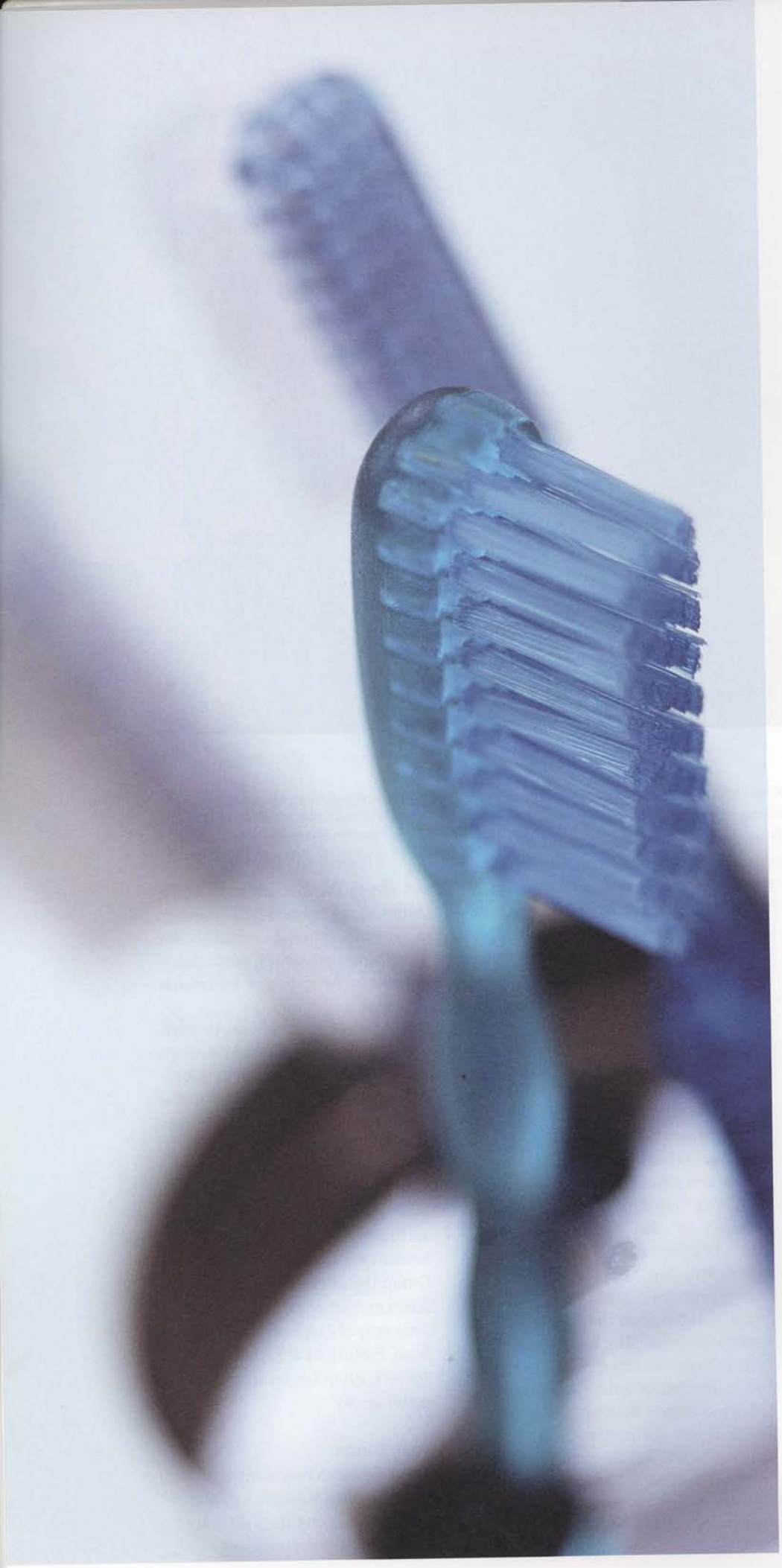
▲ Landscapes may be seen as environments of complex microbial communities. *Freefoto SEM* of bacteria on a sponge. *Visuals Unlimited*

DNA allows the identification of the functional organisms. This method has already proved valuable for determining the active community constituents in soils and freshwater sediments.

The molecular toolbox is now expanding and will aid the development of a holistic approach to understanding the complexities of microbial communities from genome to whole-community function.

Hilary Lappin-Scott
SGM Scientific Meetings Officer and former Convener of the Environmental Microbiology Group, Department of Biological Sciences, Hatherly Laboratories, University of Exeter, Prince of Wales Road, Exeter EX4 4PS, UK (**t** 01392 264674; **e** h.m.lappin-scott@exeter.ac.uk)

Sarah Burton
Senior Research Fellow in the Environmental Microbiology and Ecology Research Group at Exeter University (address as above)



Every time we clean our teeth, the oral biofilm is disrupted and has to reform in a few hours.

Paul Kolenbrander and his colleagues believe that molecular signalling between the bacteria in plaque is crucial to the success of this process.

◀ *Imagesource / Photolibrary.com*

Let it flow but don't let go: saliva and colonization

After our teeth are cleaned, bacteria reappear on the tooth enamel in the form of mixed-species communities, which are again disrupted when we next floss and brush. This article focuses on biofilm formation during the interval between typical personal oral hygiene procedures, roughly 8–12 hours. Saliva bathes the tooth surfaces carrying bacteria to and from the enamel. To be successful in the first step of colonization of the tooth, bacteria must possess constitutively expressed cell-surface-associated polymers whose function is to recognize and bind structurally complementary molecules in a pellicle formed by saliva on the enamel surface. Human oral bacteria also possess cell-surface-associated polymers that mediate cell–cell binding between genetically distinct cells. This cell–cell interaction is called coaggregation. Oral bacteria display a propensity to coaggregate and each strain coaggregates with a specific set of partner strains. Consequently, the potential exists for a network of coaggregation-mediated multi-species communities. Initial adherence of bacteria to the saliva-coated enamel substratum, coaggregation and cellular growth are considered the three primary mechanisms integral to bacterial colonization of a clean enamel surface.

Colonization of enamel

Four hours after cleaning, enamel surfaces in the mouth will harbour a variety of mixed-species communities, most of which contain between two and ten cells. Such communities formed on a retrievable, initially sterile enamel piece carried in the mouth of a volunteer can be visualized using fluorescently labelled antibodies reactive with specific bacterial surface molecules or by using fluorescent dyes that stain all bacteria on the enamel. One particularly useful antibody (generously supplied by J. Cisar) reacts with several types of streptococcal receptor polysaccharides, which in turn are recognized by a complementary protein adhesin borne on type-2 fimbriae of certain actinomyces. Streptococcal receptor polysaccharides are also recognized by *Veillonella* spp., and veillonellae form a natural metabolic food chain with streptococci: the streptococci ferment carbohydrates to lactic acid and the veillonellae ferment lactic acid to other end-products. Veillonellae are unable to obtain energy from carbohydrates directly, and they establish mixed-species communities with streptococci on the enamel (Fig. 1). Streptococci containing any of three of the six known receptor polysaccharides are visible throughout the specimen area, and often veillonellae are seen near these bacteria. The cells that react with the antibody against streptococcal receptor polysaccharide could represent several species including *Streptococcus gordonii*, *Streptococcus oralis*, *Streptococcus mitis* and *Streptococcus sanguinis*, each of which have strains that produce coaggregation-mediating receptor polysaccharides. *Veillonella* and *Actinomyces* are only two of many genera that recognize the streptococcal receptor polysaccharides; other genera include *Haemophilus*, *Eikenella*, *Prevotella*, *Capnocytophaga*, and certain other *Streptococcus* spp. Members of these genera compete for coaggregation sites on streptococci bearing these receptors. The success of these genera during initial colonization of the tooth surfaces might be determined by their competitive attachment to the most successful early colonizers, the streptococci.

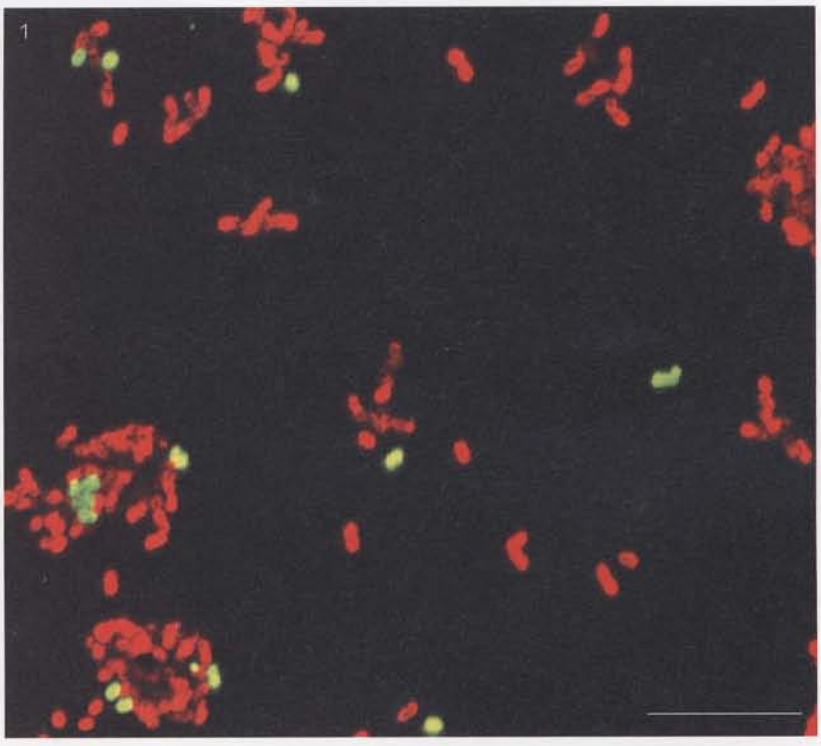
▼ Fig. 1. Mixed-species colonies formed *in situ* on an enamel surface. Red cells are labelled with antibody against streptococcal receptor polysaccharide. Green cells are labelled with an antibody against whole cells of *Veillonella atypica* PK1910. Bar, 10 μm. *Rob Palmer*

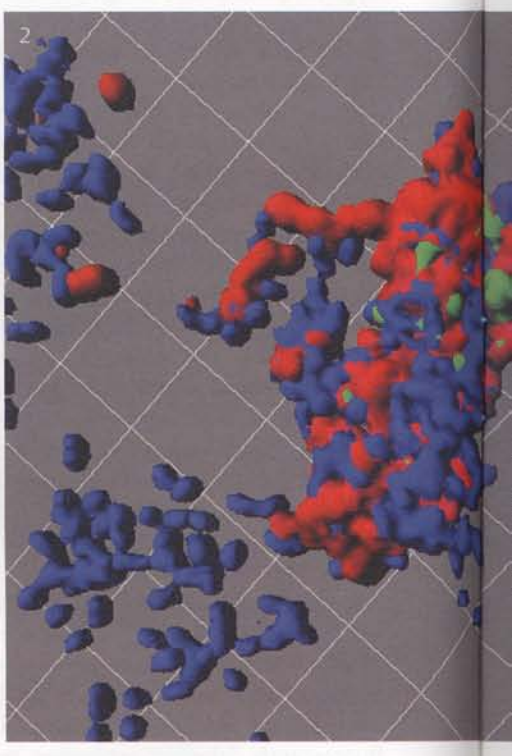
▼ Fig. 2. Three-dimensional rendering of confocal scanning laser microscopic data showing a dual-species biofilm community formed in a flowcell with saliva as the sole nutritional source. Blue cells are *Streptococcus gordonii* stained with Syto-59, red cells are *Veillonella atypica* stained with fluorescently labelled anti-*V. atypica* antibodies and green cells are *S. gordonii* expressing GFP. GFP is produced only by streptococci that are in direct contact with veillonellae: juxtaposition is required for communication in this flowing environment. *Rob Palmer and Paul Egland*

Salivary flow vs bacterial colonization

The colonization and organization of bacterial communities on enamel are not random processes. When developing a model of oral microbial communities, one consideration in choosing the species for study is their temporal appearance on enamel following oral hygiene procedures. We chose to focus on initial colonizers and included *Streptococcus gordonii*, *Actinomyces naeslundii* and *Veillonella atypica* as well as a fourth organism, *Fusobacterium nucleatum*, which is one of the most numerous bacterial species in dental plaque. These four species interact physically and metabolically with other members of the consortium. Examples include *S. gordonii* coaggregating with *A. naeslundii* as well as *V. atypica* signalling *S. gordonii* to modulate lactic acid production. *F. nucleatum* and *S. gordonii* possess LuxS, which is the enzyme that catalyses the production of 4,5-dihydroxy-2,3-pentanedione, a precursor of the multi-species signal autoinducer-2. Other observations also support the critical role of juxtaposition of interacting cells and bacterial communication in proliferation of bacteria on a saliva-conditioned surface. For example, when inoculated individually with saliva as the sole nutritional source, each of the four species bound to saliva-coated glass in a flowcell, but only *S. gordonii* grew as a biofilm. However, all four species persisted in the flowcell when they were inoculated together. Interestingly, when the four species were first coaggregated before inoculation into the flowcell, both *V. atypica* and *A. naeslundii* showed enhanced growth not visible when the four organisms were inoculated sequentially, suggesting that juxtaposition may be a key element in successful colonization.

In the mouth, where saliva secretion creates a flowing environment, juxtaposition of bacteria would be critical for interspecies communication mediated by small-molecule signals. Based on the observations that a number of bacteria, including human oral bacteria, produce a molecule that induces auto-





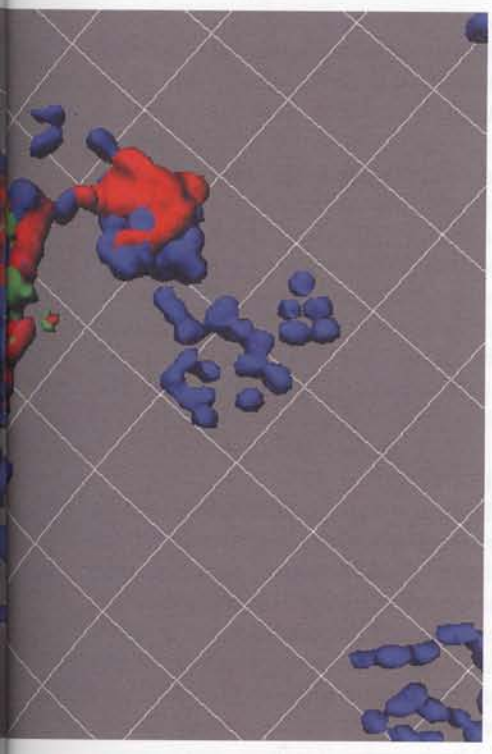
inducer-2-dependent bioluminescence in *Vibrio harveyi*, B. Bassler proposed that autoinducer-2 mediates interspecies bacterial communication. Although little is known about signalling across species barriers that does not involve *V. harveyi*, the broader concept of interspecies signalling among oral bacteria is amenable to study.

Diffusible signals

The initial colonizers of the tooth surface are an excellent group for the study of interspecies signals because their spatio-temporal colonization is not random, and these mixed-species communities must reform every 8–12 h (the cycle between brushings). Streptococci are the predominant bacteria in initial dental plaque with actinomyces and veillonellae also contributing. We hypothesize that these organisms and others communicate by molecular signals.

The *S. gordonii* and *V. atypica* pair is interesting because of the previously described metabolic interaction. We postulated that veillonellae might signal streptococci to produce more lactic acid. Considering that these bacteria live in a flowing environment, it is advantageous that they coaggregate and are therefore in close contact. Juxtaposition would facilitate exchange of lactic acid in the same way that it also facilitates transmission and reception of molecular signals. To monitor the expression of an amylase gene that could participate in the conversion of glucose polymers to glucose and lactic acid, a reporter plasmid containing *gfp* (encoding green fluorescent protein, GFP) under control of the streptococcal amylase gene promoter was introduced into *S. gordonii*. Within 4 h of exposure to veillonellae the streptococci produced GFP. GFP expression was not seen in the absence of veillonellae. When cells were placed in a flowing environment, direct contact between streptococcal and veillonellae cells was required for amylase promoter activity (Fig. 2). However, when the two species were separated by a dialysis membrane in a closed vessel, the signal diffused through the membrane and induced GFP production in all streptococci.

The colonization and organization of bacterial communities on enamel are not random processes



It is important to consider the flowing environment when investigating the role of molecular signals in the natural ecosystem of the oral cavity. To attain the necessary concentration to induce a response, a diffusible signal might only be effective when the producing and receiving species are in juxtaposition. Signals exchanged within and among initial oral bacterial communities may be coordinated to optimize beneficial interactions, and thus biofilm development, over the very short time period between brushings. Understanding the interactions among initial colonizing bacteria might allow us to predict and control the nature of biofilms at later stages of plaque development.

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Further reading

Egland, P.G., Palmer, R.J., Jr & Kolenbrander, P.E. (2004). Interspecies communication in *Streptococcus gordonii–Veillonella atypica* biofilms: signaling in flow conditions requires juxtaposition. *Proc Natl Acad Sci U S A* **101**, 16917–16922.

Kolenbrander, P. E., Andersen, R.N., Blehert, D.S., Egland, P.G., Foster, J.S. & Palmer, R.J., Jr (2002). Communication among oral bacteria. *Microbiol Mol Biol Rev* **66**, 486–505.

Palmer, R.J., Jr, Gordon, S., Cisar, J.O. & Kolenbrander, P.E. (2003). Coaggregation-mediated interactions of streptococci and actinomyces detected in initial human dental plaque. *J Bacteriol* **185**, 3400–3409.

Rickard, A.H., Gilbert, P., High, N.J., Kolenbrander, P.E. & Handley, P.S. (2003). Bacterial coaggregation: an integral process in the development of multi-species biofilms. *Trends Microbiol* **11**, 94–100.

Eavesdropping on bacterial conversations

One of the surprising discoveries in recent years has been that many bacteria have sophisticated systems of communication. Small molecules that co-ordinate gene expression are released into the environment and enter other bacteria in the vicinity. This cell-to-cell signalling is usually referred to as quorum sensing – to indicate that it is a density-dependent process that allows concerted activity of a population. Our interest developed from a study of biofouling of surfaces in the sea. We found that it is impossible to understand the attachment of large biofouling plants and animals without considering the bacteria that grow on those same surfaces. From those initial questions, we have discovered a complex story of how a seaweed utilizes quorum sensing molecules to select a suitable surface for attachment and growth. In other words, the seaweed appears to eavesdrop on the cell-to-cell communication of bacteria by exploiting quorum sensing molecules.

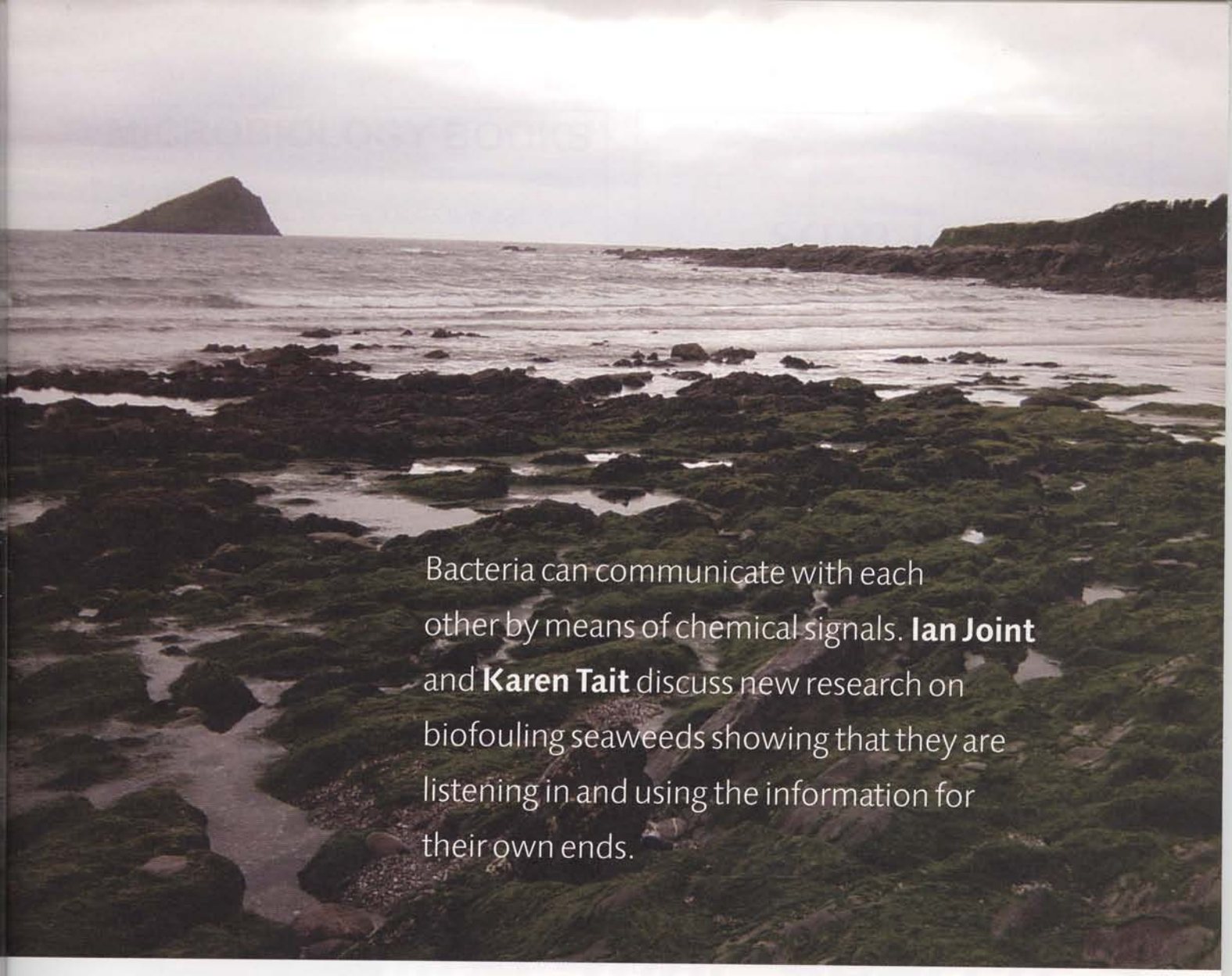
Marine biofouling

The growth of animals and plants on structures in the ocean is a billion dollar a year problem. Biofouling organisms increase the roughness of surfaces and so increase drag; ships consume more fuel to maintain speed and large structures such as oil platforms have to be engineered to account for the increased stresses imposed by biofouling organisms. A great deal is known about the biology of large biofouling organisms and how they are dispersed, but there has been relatively little research to link these animals and plants to the microbiology of the surfaces. So far, treatments to prevent biofouling have relied on toxic chemicals, but the most effective substances are no longer acceptable because of detrimental impacts on the environment.

Our work has focussed on the green seaweed *Ulva*, which is a common biofouling organism and a prominent seaweed on the shore throughout the world (Fig. 1). *Ulva* reproduces by releasing motile zoospores that swim away from the parent, attach to a surface and develop into a new plant. The numbers of zoospores released are truly immense and thousands swim away from the tip of a mature plant each day. Look in a rock pool in summer, on a shore where *Ulva* grows, and it will be bright green with the zoospores that the seaweed has released.

What is quorum sensing?

The best understood system is that of Gram-negative bacteria and involves *N*-acylhomoserine lactones (AHL) as signal molecules. By moderating gene expression in the whole population in response to cell density, microbial population behaviour can be considered to be analogous to that of a multicellular organism. Quorum sensing systems are very widespread and modulate many physiological processes in bacteria that are associated with humans, plants, animals, soils and marine and fresh waters; it is increasingly seen as



Bacteria can communicate with each other by means of chemical signals. **Ian Joint** and **Karen Tait** discuss new research on biofouling seaweeds showing that they are listening in and using the information for their own ends.

▲ Fig. 1. *Ulva* is a common green alga in intertidal rocky shores. *Paul Williams, University of Nottingham*

central to the success of bacteria. The importance can be judged from the fact that between 5 and 25 % of the genes within bacterial genomes sequenced to date are controlled by quorum sensing regulatory networks. It seems to be particularly important to the process of bacterial infection of both plants and animals, because mutants defective in quorum sensing exhibit greatly reduced virulence. The maintenance of bacterial biofilms, which are complex and dynamic populations, is also under the control of quorum sensing.

The evidence for eavesdropping on bacteria

Our initial observation was that more *Ulva* zoospores settled on surfaces colonized by bacteria than on clean surfaces. Also, the numbers of attached zoospores increased with the size of the bacterial population. Moreover, we proved statistically that this was not a random process and that zoospores attached directly to bacterial cells (Fig. 2). This suggested a specific selection mechanism – but what cues might the zoospores be using? It is known that settlement is enhanced by many factors, such as surface roughness and extracellular polymeric substances (EPS), but the response appeared to be more specific and was a property of certain types of bacterial cells. Quorum sensing molecules were an obvious candidate mechanism but they had not previously been shown to be important for eukaryotic cells. In the event, we were able to prove unequivocally that AHLs are exploited by zoospores in the selection of sites for attachment.

The evidence was based on four types of experiments. Using biofims of the marine bacterium *Vibrio anguillarum*, we showed that the wild-type attracted zoospores, but mutants that were defective in AHL production did not attract. Zoospore settlement assays were also carried out using *Escherichia coli* expressing recombinant AHL synthase genes; again, settlement was only enhanced when AHLs were produced. Synthetic AHLs were also found to attract zoospores. Finally, the attraction was inactivated when *V. anguillarum* expressed a recombinant gene from *Bacillus* that inactivates AHL molecules. Clearly, there is little doubt that *Ulva* zoospores exploit AHLs when they select a surface for attachment.

However, zoospores are not attracted to all bacteria and we have a number of isolates that inhibit attachment. The

The growth of animals and plants on structures in the ocean is a billion dollar a year problem

production of AHLs is also not constant during the growth phase of a bacterial biofilm and the relationship between quorum sensing, biofilm age and composition has proved to be very complex. But what benefit might a seaweed get from attaching to certain bacterial species? The answer is not yet clear. It is known that bacteria are essential for the normal development of seaweeds; that is, certain bacteria have to be present for normal morphology. However, there does not appear to be a close correlation between the species of bacteria that attract zoospores and those that are essential for normal morphological development. This interaction is complicated and is the subject of other research.

The wider significance of quorum sensing molecules in community ecology

Although in the context of higher organisms, bacteria are usually thought of only in terms of disease, beneficial interactions also occur. Symbiosis is widespread and is involved in processes as diverse as nitrogen fixation in the rhizosphere and light generation in deep-sea fishes. Quorum sensing is essential for maintaining these interactions between host and bacteria. The case of *Ulva* eavesdropping on bacterial communication is a novel example of cell-to-cell communication. We do not know if this phenomenon of communication across the prokaryote/eukaryote boundary is widespread. We suspect it might be common and it will be interesting to discover how many organisms have evolved mechanisms to exploit bacterial communication for their own benefit.

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Further reading

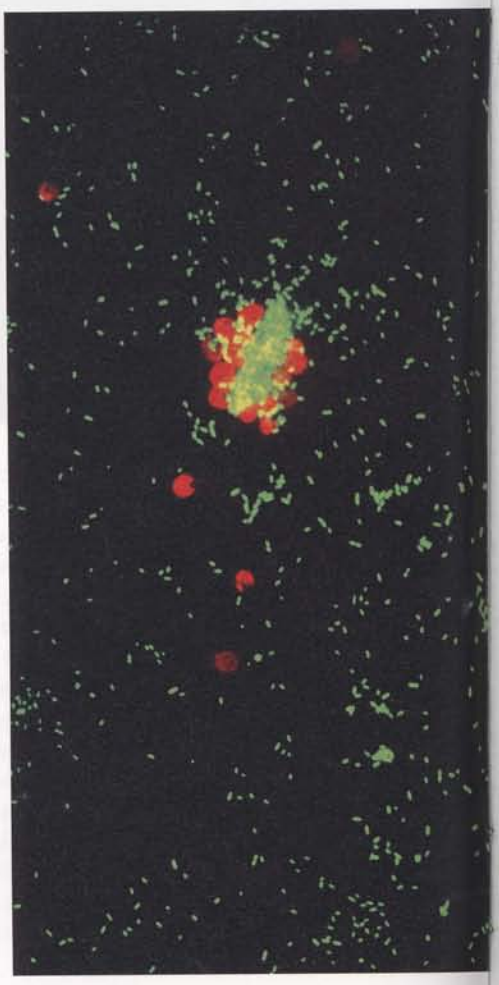
Swift, S., Downie, J.A., Whitehead, N., Barnard, A.M., Salmond, G.P. & Williams, P. (2001). Quorum sensing as a population density dependent determination of bacterial physiology. *Adv Microb Physiol* **45**, 199–270.

Joint, I., Callow, M.E., Callow, J.A. & Clarke, K.R. (2000). The attachment of *Enteromorpha* zoospores to a bacterial biofilm assemblage. *Biofouling* **16**, 151–158.

Joint, I., Tait, K., Callow, M.E., Callow, J.E., Milton, D., Williams, P. & Cámara, M. (2002). Cell-to-cell communication across the prokaryote–eukaryote boundary. *Science* **298**, 1207.

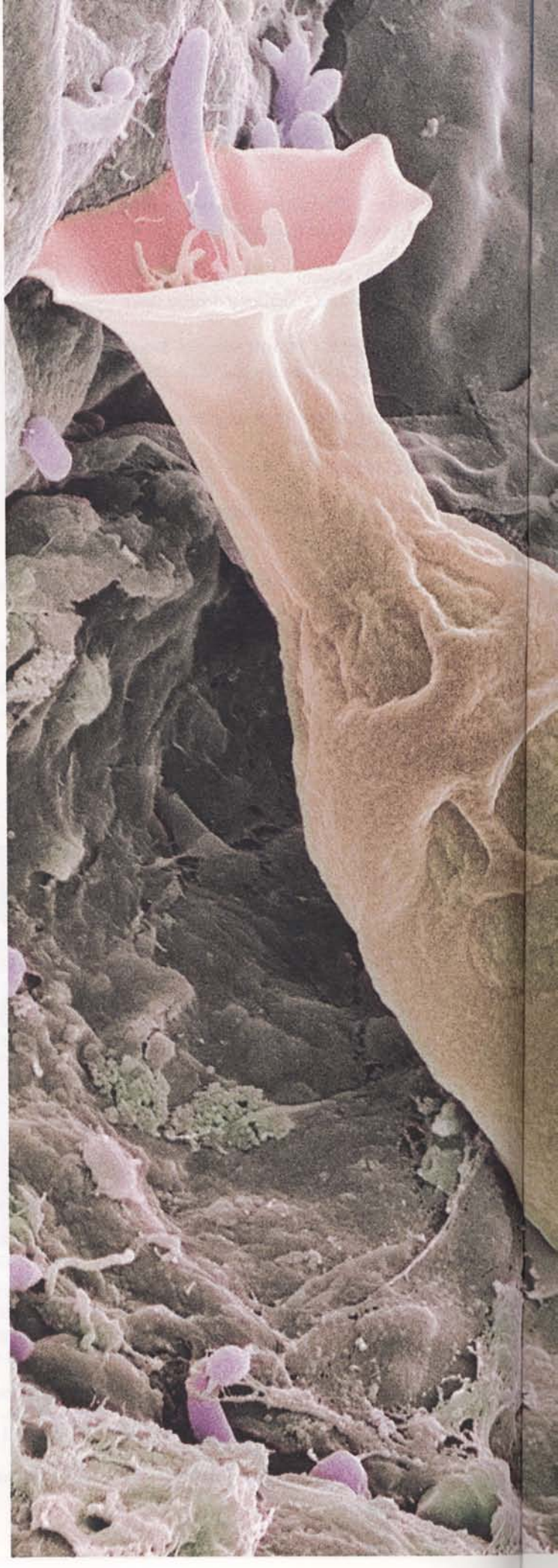
Matsuo, Y., Suzuki, M., Kasai, H., Shizuri, Y. & Harayama, S. (2003). Isolation and phylogenetic characterization of bacteria capable of inducing differentiation in the green alga *Monostroma oxyspermum*. *Environ Microbiol* **5**, 25–35.

Tait, K., Joint, I., Daykin, M., Milton, D.L., Williams, P. & Cámara, M. (2005). Disruption of quorum sensing in sea water abolishes attraction of zoospores of the green alga *Ulva* to bacterial biofilms. *Environ Microbiol* **7**, 229–240.

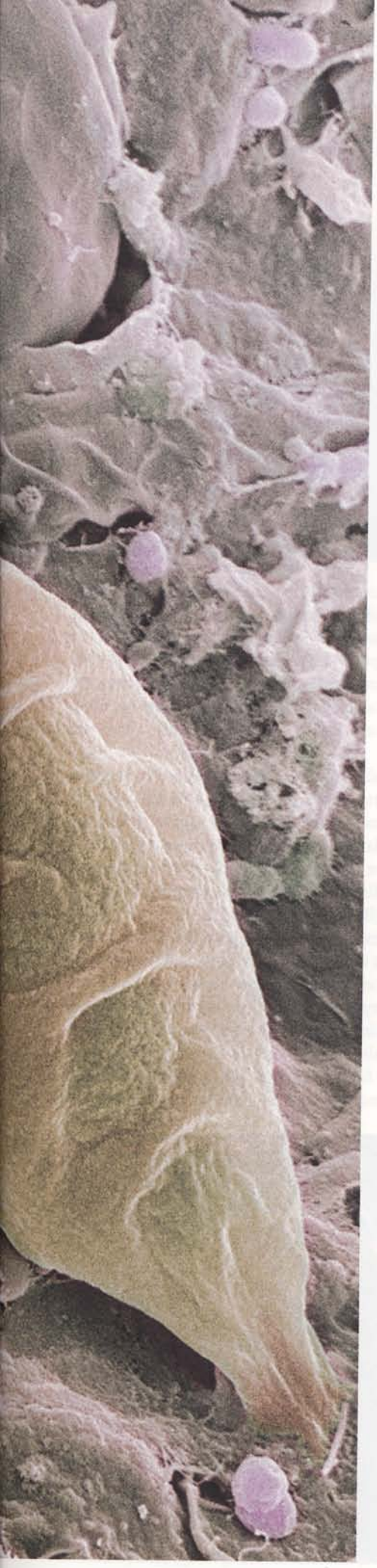


▼ Fig. 2. Epifluorescence image of *Ulva* zoospores (red) attaching to bacteria stained with SYBR Green. *Karen Tait*

Sugar-coated bacteria: wolves in sheeps' clothing?



Many protozoa can get through the sugary matrix of a biofilm and feed on the bacteria inside. What seems like a sticky end may in fact be a means for some bacteria to exploit the grazing organisms for their own benefit, as **Jackie Parry** explains.



Food, food everywhere but not a morsel to eat. This is the common perception of protozoan participation, or the lack of it, in biofilm dynamics. The reason for this is that when bacteria attach to surfaces they notoriously coat themselves in a sugary gelatinous matrix, which has always been considered impenetrable to grazers. But many protozoa can break through the matrix and consume the prey within. But is the grazing of these sticky, sweet bacteria always good for protozoa or does the sugar-coating conceal hidden dangers?

What are protozoa?

Protozoa are the most beautiful microbial creatures on earth, but I am biased. After spending 3 years of a PhD counting actinomycete colonies and phage plaques on agar plates, I often think I was just easily pleased when I saw my first protozoan under the microscope. But 14 years later I am still as excited to see them as I was on that first day. The literal meaning of protozoa is 'first animals' and although some of these single-celled eukaryotes are totally photosynthetic (algae), most of them are heterotrophic, i.e. they consume particulate prey, especially bacteria. Their role in water (plankton) as the major predators of bacteria is undisputed and their ability to excrete waste products such as ammonium and orthophosphate is known to feed bacteria in nutrient-poor environments. Whether protozoa perform a similar role in surface-associated communities (biofilms) remains to be seen.

Protozoa are normally between 5 and 200 µm in size and exhibit a variety of mechanisms to capture their prey. This has led to a considerable diversification of protozoan morphologies, but for simplicity's sake, they can be divided into three groups; flagellates, ciliates and amoebae.

Flagellates and ciliates

The flagellates possess one or more flagella which are used for swimming and the creation of feeding currents. Water, containing prey, is drawn towards the base of the flagellum where they are ingested via cytoplasmic extensions known as pseudopodia. The prey is then deposited into food vacuoles. Some flagellates have a collar of tentacles at the base of the flagellum which only allows the smallest of prey particles to pass through. Many flagellates attach to surfaces by means of a stalk, allowing more effective use of their flagellum/flagella to produce larger feeding currents than if swimming. Flagellates, due to their small size (5–20 µm), are considered the dominant predators of

◀ Coloured SEM of a freshwater ciliate protozoan (*Stentor*). The trumpet-like opening is surrounded by a rim of cilia (not seen). The cilia beat to create a current of water that draws food in, e.g. bacteria (purple). *WG / Science Photo Library*

bacteria in aquatic systems with ingestion rates of 2–300 bacteria per flagellate per hour.

The ciliates are another protozoan group that feeds effectively on bacteria in suspension, but their larger cell size (15–200 µm) allows them to exploit more prey types such as algae, flagellates and other ciliates. Ciliates are morphologically diverse and possess cilia for swimming and creating water currents, which draw prey to the oral area (cytostome) where they are then deposited into food vacuoles. Once again, ciliates can attach to surfaces and produce extensive feeding currents, sometimes yielding ingestion rates in excess of 1,000 bacteria per ciliate per hour.

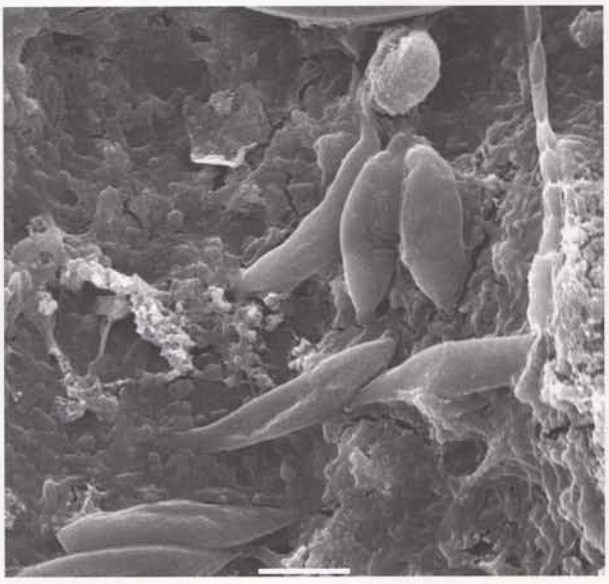
The impact of flagellates and ciliates on biofilm-associated bacteria, in the main, appears to be one of disruption. Their active movement and large feeding currents generate a significant disturbance that dislodges bacteria from the biofilm, releasing them into suspension where they are eaten, i.e. a 'slough and feed' response. They are therefore thought to be important in defining the topography, and subsequent stability, of natural biofilms.

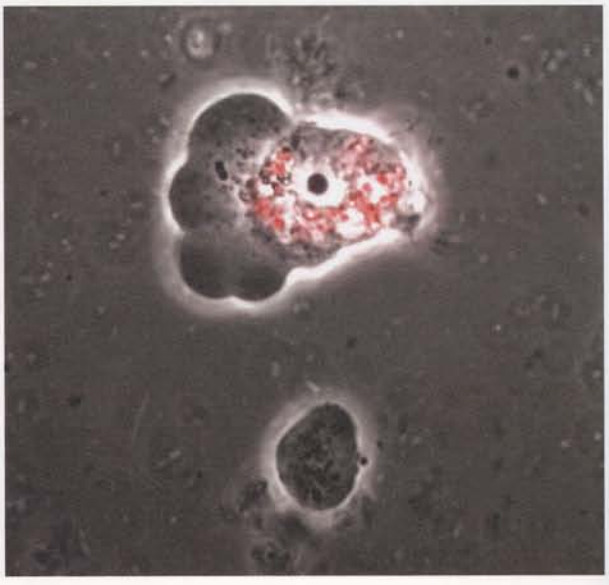
If a bacterium can develop a mechanism to avoid digestion in the wild, it should also be able to avoid digestion in the immune system

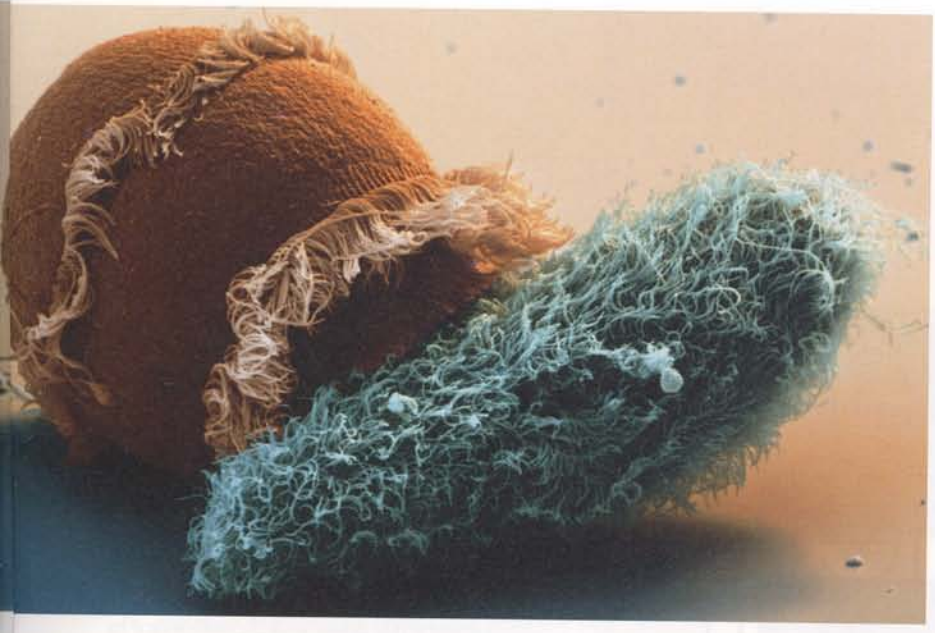
Amoebae

Amoebae cannot feed effectively on suspended prey, but are known to feed on attached bacteria. The naked amoebae move by projecting pseudopodia and crawling, and are more associated with surfaces than free-floating in the plankton. They envelop their prey within pseudopodia before enclosing them in a food vacuole. Some amoebae are housed in a shell (testate amoebae) and protrude sticky extrashellular cytoplasm which trap prey before bringing them to the main body of the cell where they are ingested. Amoebae are not fast-moving and do not disrupt the biofilm to anything like the extent of flagellates and ciliates. Many workers have confused amoebae with multicellular rotifers, which possess trophy and can nibble/grasp biofilm stacks. But the amoebae cannot do this and appear to just sit on top of the biofilm as it grows and patiently ingest the sugar-coated prey beneath. Amoebic grazing rates on attached bacteria, either as naked or alginate (sugar)-coated cells, range from 0·2 to 1465 bacteria per amoeba per hour, which are very significant.

So, in essence, all protozoa have the ability to consume sugar-coated bacteria – either directly from the surface or via a 'slough and feed' mechanism. The question now arises as to whether these predators are influenced by the composition of the sugar-coating or can they detect the prey inside, possibly via cell–cell signalling mechanisms? Studies regarding the effect of prey 'smell', and subsequent 'taste', on protozoan ingestion are non-existent but exciting work at the Plymouth Marine Laboratory shows that different bacterial strains can signal ('talk') to the eukaryotic zoospores of *Ulva*, as described on p. 14; either saying 'come and settle next to me' or 'go away and don't settle next to me'. It would be interesting to see if bacteria could also 'talk' to the eukaryotic protozoa in a similar way. Your first thought might be that they should all say 'go away and don't eat me', but some might want a







protozoan close by, particularly if they have an ulterior motive.

The potential wolves

Certain pathogenic bacteria are known to avoid the digestive mechanisms of protozoa either by escaping from the food vacuole and replicating in the protozoan cytostol, e.g. *Listeria*, or by blocking the digestive processes and multiplying within the food vacuole itself, e.g. *Legionella*. Other bacteria appear to resist the complete digestive cycle and are excreted as viable, 'super-tough' forms, e.g. some coliforms. Either way, being inside a protozoan cell provides a positive aspect to the life of these bacteria by protecting them from the harsh environment outside. In addition, protozoa might have played an important role in the evolution of these pathogens in that, if a bacterium can develop a mechanism to avoid protozoan digestion in the wild, it should also be able to avoid digestion in the macrophages of the immune system. The protozoan–pathogen relationship does appear to be one-sided though, and the protozoa gain nothing, they just waste energy on processing a non-digestible prey or are lysed when bacterial progeny are released from their cell. If cell–cell signalling does occur between bacteria and protozoa then surely they would be able to detect a non-profitable prey, avoid it and feed only on profitable (digestible) prey – unless the prey was not what it seemed. Pathogen densities are notoriously higher in biofilms than in the surrounding water and maybe by being part of this mixed bacterial community, with all its many signals, the protozoa are tricked into thinking they are palatable. Thus sugar-coating themselves may indeed provide these pathogens with a route to the safe haven they desire.

Acknowledgements

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Further reading

Parry, J.D. (2004). Protozoan grazing of freshwater biofilms. *Adv Appl Microbiol* **54**, 167–196.

Zimmermann-Timm, H. (2002). Characteristics, dynamics and importance of aggregates in rivers. *Int Rev Hydrobiol* **87**, 197–240.

Sources of protozoan cultures

Culture Collection of Algae and Protozoa (ccap@sams.ac.uk)
Sciento (sales@sciento.co.uk)

▲ Coloured SEM showing a ciliate protozoan, *Didinium* (brown), in the process of attacking another ciliate protozoan, *Paramecium* sp. (blue). *Eye of Science / Science Photo Library*

◀ Far left EM of a natural biofilm which developed on sandpaper after 5 days incubation in Lake Carter, Lancaster University. The image shows a cluster of flagellated protozoa (10 µm in length) on the surface of the biofilm matrix. It can be clearly seen that the majority of bacteria are embedded in the extensive matrix, although some are more loosely associated with the surface.

◀ Left A naked amoeba which has ingested a number of photosynthetic bacteria (*Synechococcus*) and deposited them in its food vacuoles. This image shows the amoeba under visible light and the prey under green light, which causes the prey's inherent chlorophyll *a* to fluoresce red.

Urinary catheters: ideal sites for the development of biofilm communities

Biofilms containing pathogenic bacteria soon develop in urinary catheters, leading to bladder and kidney infections. **David Stickler** describes how studies on the unique nature of these microbial communities should help to provide solutions to a very distressing problem.

Bladder catheters are the most common prosthetic medical devices, with around 100 million in use each year. Apart from important roles in monitoring urine production from unconscious patients and facilitating repair of the urethra after surgical procedures such as prostatectomy, they are also used to manage urinary retention and incontinence in the elderly and in patients disabled by strokes, spinal injury or multiple sclerosis. They offer a convenient way to drain urine from the bladder, but unfortunately they also provide a passageway for bacteria from a heavily contaminated external skin site to a vulnerable body cavity. The risk of infection is related to the length of time the catheter is in place. Most patients catheterized for less than a week should escape infection, but for those catheterized for periods longer than 4 weeks, it is inevitable. The initial infections are usually by single bacterial species such as *Staphylococcus epidermidis*, *Enterococcus faecalis* or *Escherichia coli*. As time goes by however, a variety of organisms colonize the bladder urine and polymicrobial communities develop which might contain up to six or seven species. This urinary flora is dynamic with some species being transient

residents, whereas others such as *E. coli*, *Providencia stuartii*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Morganella morganii* and *Klebsiella pneumoniae* generally are stable residents.

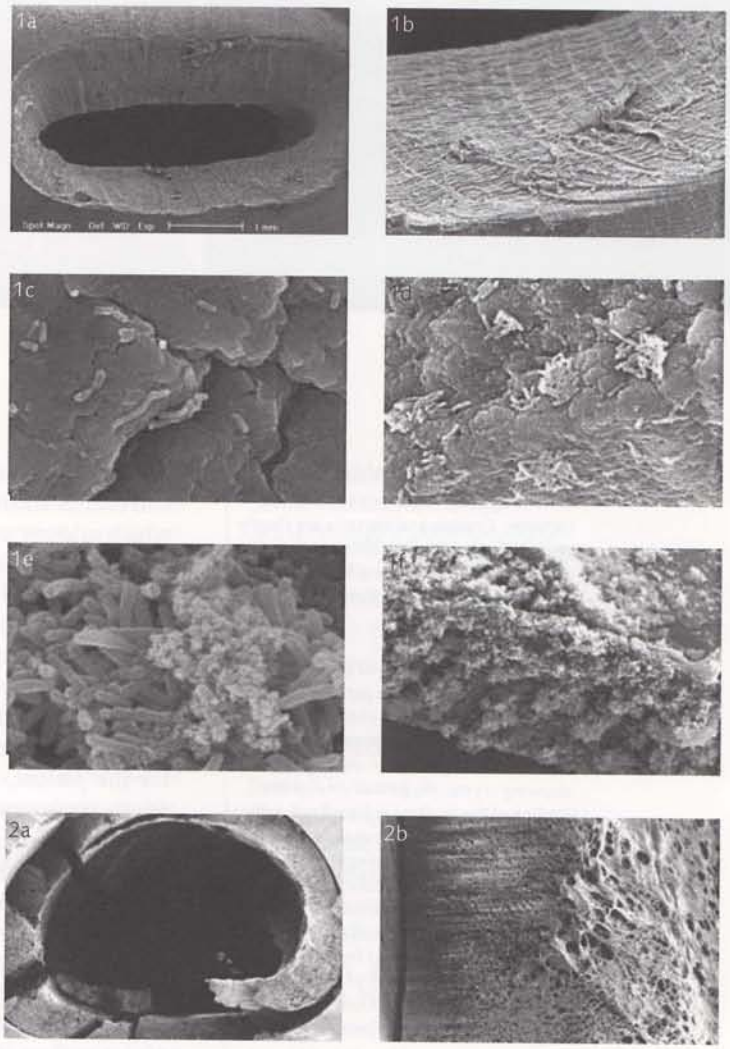
How do the biofilms form?

While the catheter is in place the infections are notoriously difficult to eradicate with antibiotics. It is normal practice to resort to treatment only when there is evidence that the infection has reached the kidneys or the bloodstream. In patients undergoing long-term bladder management, a common regime is to change catheters at 10- to 12-week intervals. So we have a situation in which infected urine flows through catheters for periods of up to 3 months at a time.

Catheters are manufactured from silicone or from latex coated in either silicone or hydrogel. These materials provide attractive, unprotected sites for bacterial attachment. In addition the irregular surfaces left by the manufacturing process, particularly around the eye-holes, can trap cells as the infected urine flows through the device (Fig. 1). Attached to a surface bathed in a constant gentle flow of a nutrient-rich medium, the bacterial populations thrive. Within days extensive biofilms develop, particularly on the lumenal surfaces of the catheter. Towards the end of the device's lifetime, the biofilms can comprise thick layers up to 500 cells deep, embedded in their exopolymer matrix (Fig. 2). These bacterial communities have all the properties conferred by the biofilm mode of growth and the resistance of the cells frustrates attempts to treat infections.

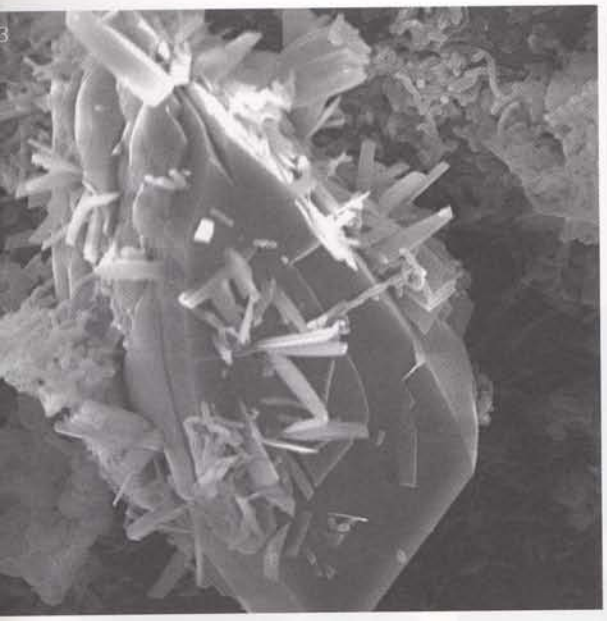
Crystalline biofilms

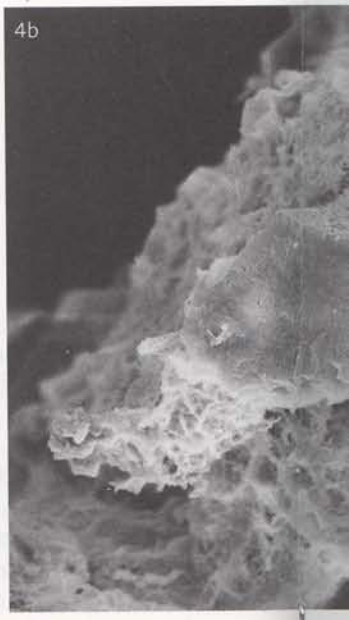
The biofilms produced by *Pr. mirabilis* pose particular threats to the health of catheterized patients. The urease activity



▲ Fig. 1. The early stages in the formation of a *Proteus mirabilis* biofilm on a catheter. Catheters were removed for examination after incubation for various times in an infected laboratory model of the catheterized bladder. The rough irregular surface of the eye-hole is shown in (a) and (b). After 2 h in the model, cells can be seen adhering to crevices in the surface (c). At 4 h, microcolonies have developed in depressions in the surface (d). After 6 h incubation, amorphous crystalline material (e) can be seen associated with the cells. At 20 h, extensive crystalline biofilm has formed around the eye-hole (f). *Reproduced from Urol Res (2003) 31, 306–311, with permission*

▲ Fig. 2. Cross-sections of an all-silicone catheter that had been removed from a patient after 6 weeks. The biofilm was composed of *Pseudomonas aeruginosa* cells at a density of 5×10^9 viable cells (cm catheter surface)$^{-2}$. (a) A freeze-dried preparation of a freeze-fractured catheter section. (b) The biofilm on the catheter surface in more detail revealing the sponge-like structure. *Reproduced from Urol Update (1996) 5, 1–8, with permission from the European Board of Urology*





▲ Fig. 3. Scanning electron micrograph of the biofilm on a patient's encrusted catheter. Crystals and bacteria are clearly visible. *Escherichia coli*, *Enteroccus faecalis* and *Proteus mirabilis* were found in this biofilm community. *Dr Steve Jones, Cardiff School of Biosciences*

▲ Fig. 4. These images are of a worm-like structure that blocked a patient's catheter at four day intervals; (a) shows the 'worm' emerging from a cut section of catheter; (b) is a scanning electron micrograph showing crystalline formations on the surface of the 'worm'; (c) is a fixed, critical-point dried specimen revealing masses of cocci, short rods and a tangle of elongated bacilli beneath the crystalline coat. *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus mirabilis* were recovered from the biofilm. The patient produced 15 of these 'worms' over a 10 week period. *From J Infect (1993) 27, 133–135, used with permission*

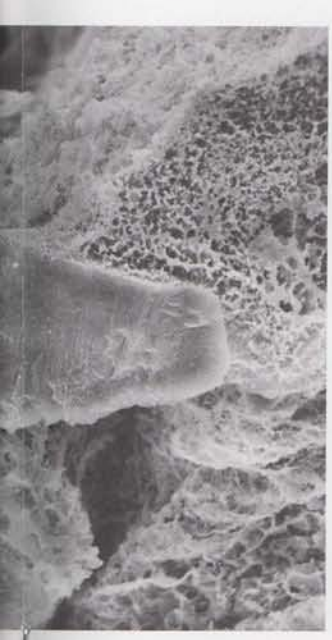
▲ Fig. 5. A scanning electron micrograph of *Proteus mirabilis* biofilm that had developed on a hydrogel-coated latex catheter after just 20 h incubation in a model of a catheterized bladder. Calcification is in progress and pores can be seen in the 'plaster cast' of the biofilm. *Used with permission from the American Society for Microbiology*

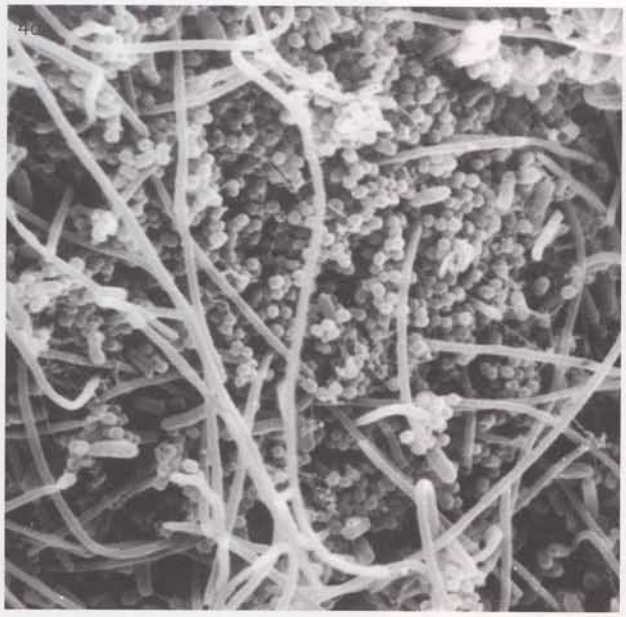
of this organism generates ammonia and creates alkaline conditions under which calcium and magnesium phosphates crystallize in the urine and the biofilm. These unique crystalline biofilms develop rapidly and can completely block the flow of urine from the bladder (Figs 3 & 4). If this situation is not spotted and the catheter changed, it can have disastrous consequences for the patient, leading for example to urinary retention, painful distension of the bladder and reflux of infected urine to the kidneys, which triggers pyelonephritis and septicaemia. Many long-term patients suffer this complication. All currently available types of catheter are vulnerable to encrustation and there is no effective way of controlling the problem – hence our interest in trying to devise novel strategies to deal with it. A clinical colleague tells a story of a patient who was so frustrated with his catheter blocking recurrently that he tried (while the catheter was still in place) blowing through it to clear the obstruction. When this failed he attached a bicycle pump to the end to get enough pressure to do the job!

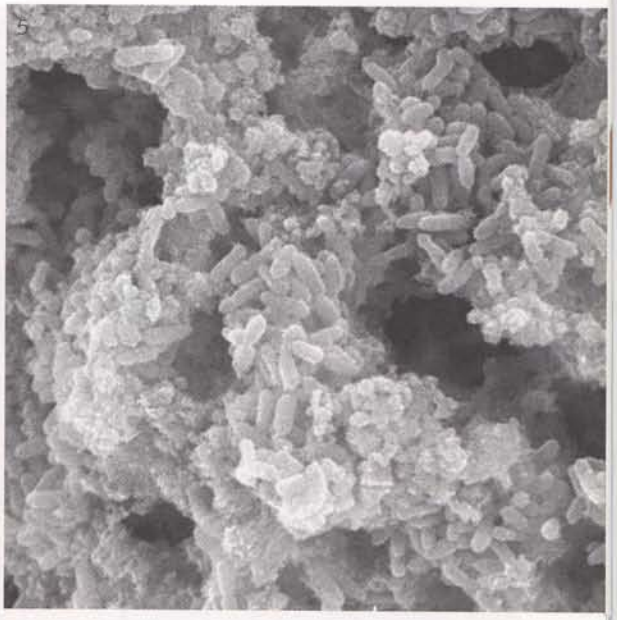
Catheter biofilms and the universal model of biofilm structure

A great deal of work has been done on *Ps. aeruginosa* biofilms growing on surfaces under oligotrophic conditions. Optical sectioning of these biofilms by scanning confocal laser microscopy has revealed the complex nature of the biofilm communities. They are not composed of homogeneous layers of cells, but have a sophisticated structure which develops from a basal layer of cells. These studies have led to a consensus view of mature, natural biofilms as highly organized, structured communities. The bacteria embedded in their gel-like matrix form microcolonies which grow up from the surface into tower or mushroom-shaped structures. Open water-filled channels permeate the structure, presumably acting as primitive circulatory systems, transporting nutrients to and waste products from the bacterial communities. These concepts have led many researchers to investigate the regulatory mechanisms that control the development of these complex structures.

Electron micrograph images of mature biofilms on long-term urinary catheters are hard to reconcile with the consensus model of biofilm architecture







The electron micrograph images we have seen of mature biofilms on long-term urinary catheters are hard to reconcile with this consensus model of biofilm architecture. While the preparation of samples for scanning electron microscopy is notorious for distorting structures, it is hard to imagine how sample preparation could transform mushroom-shaped structures surrounded by open channels into the blocks of biomass visible in Fig. 2. The catheter biofilms are clearly permeated by channels, but they have a sponge-like structure. The crystalline biofilms generated by *Pr. mirabilis* in urine afford a special opportunity to study biofilm architecture by scanning electron microscopy. As the pH of the biofilm becomes alkaline, calcium phosphates are laid down around the cells and create what we have termed a 'plaster-cast' of the biofilm. These solid structures are not so vulnerable to distortion by sample preparation. The electron micrographs suggest a layer reminiscent of a coral reef, with the calcified structure permeated with urine-filled pores (Fig. 5).

Ecology of the biofilms

Apart from the pressing clinical need to solve the problem of catheter encrustation, these crystalline biofilms pose some challenging issues in community microbiology. Most are mixed communities, commonly of three or four species. The close proximity and high population density of several species within a gel-matrix provides ideal conditions for processes such as physiological cooperation, exchange of genetic material and cell-to-cell communication. We have wondered whether the microbial composition of these biofilms comes about by chance or is predetermined by interactions between species? Is coaggregation between species involved? Are there complementary associations of species which produce particularly stable, well-developed biofilm? In the case of biofilms containing *Pr. mirabilis* does the presence of other species modulate the extent and rate of crystal formation?

Currently we are attempting an ecological approach to the study of the mixed community biofilms containing *Pr. mirabilis*. The biofilm flora is being examined to look for significant associations between *Pr. mirabilis* and other species. It would be intriguing if some species were typically not found in the mixed *Pr. mirabilis* biofilm or only found in biofilms where crystal formation was minimal. Any apparent antagonisms between *Pr. mirabilis* and these other organisms, or modulation of the encrustation process, could then be examined experimentally in laboratory models. If bacterial factors could be identified which inhibit crystalline biofilm formation, a possible biological interference strategy might be feasible to control a complication which undermines the health, confidence and quality of life for so many elderly and disabled people.

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Further reading

Jones, B.V., Young, R., Mahenthiraligam, E. & others (2004). Ultrastructure of *Proteus mirabilis* swarmer cell rafts and role of swarming in catheter-associated urinary tract infection. *Infect Immun* **72**, 3941–3950.

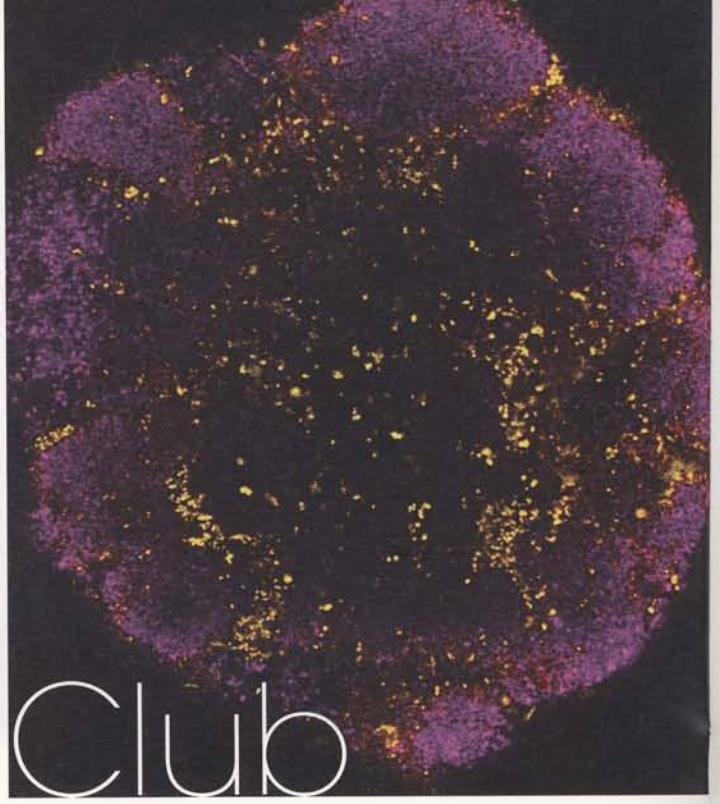
Morris, N.S., Stickler, D.J. & McLean, R.J. (1999). The development of bacterial biofilms on indwelling catheters. *World J Urol* **17**, 345–350.

Sabubba, N.A., Mahenthiraligam, E. & Stickler, D.J. (2003). Molecular epidemiology of *Proteus mirabilis* infections of the catheterized urinary tract. *J Clin Microbiol* **41**, 4961–4965.

Stickler, D.J., Young, R., Jones, G., Sabubba, N.A. & Morris, N.S. (2003). Why are Foley catheters so vulnerable to encrustation and blockage by crystalline bacterial biofilm? *Urol Res* **31**, 306–311.

Sabubba, N.A., Stickler, D.J., Mahenthiraligam, E. & others (2004). Genotyping demonstrates that the strains of *Proteus mirabilis* from bladder stones and catheter encrustations of patients undergoing long-term bladder catheterization are identical. *J Urol* **171**, 1925–1928.

Apart from SGM meetings, where do the researchers that study microbial communities on surfaces network? As **Mat Upton** describes, the Biofilm Club provides this particular microbiological community with an ideal forum.



The Biofilm Club

Over the past 13 years, the Biofilm Club has been a focal point for academic and industrial scientists, both in the UK and overseas, with research interests related to the study of micro-organisms (bacteria, fungi and even protozoa) living in the communities we know as biofilms. The Club was formed in 1992 as the British Biofilm Club when it was realized that there was no common forum in the UK for discussing biofilm research. This was extremely timely as the subject was, and indeed still is, moving very rapidly and impinging upon many fields of fundamental and applied microbiology.

The inaugural 3-day residential meeting was so successful that it was later decided to expand and include biofilm news and interests from an international perspective, and re-convene more appropriately as the Biofilm Club, thereby reflecting our international status. To this day the Biofilm Club continues to go from strength to strength.

There is an elected committee who work to promote the Club and activities of its members. The primary aim of the Club is to further the communication and discussion of research findings and ideas relating to the attachment of micro-organisms to surfaces, their subsequent development into biofilms and the science relating to the biology, control and exploitation of biofilms. Membership applications are open to anyone with relevant research interests and are encouraged from group leaders as well as their postgraduate students.

A regular newsletter publicizes news and upcoming events. To enable members to meet on a regular basis, the Club holds two workshops a year, usually in association with industrial partners or related societies. Topics are chosen to reflect some of the more specialized interests within the membership and recent subjects have included 'Biofilm modelling' and 'Aspects of infection control'. Abstracts are now published in *Biofilms*, the official journal of the Club.

Every other year sees a pilgrimage to Gregynog in mid-Wales for a larger, more general residential meeting. The Gregynog conferences are well known for having a relaxed, sociable atmosphere and are an ideal forum for postgraduate students to present findings of current research. In September 2003, approximately 200 members were registered, representing some 20 different countries. Proceedings from the Gregynog meetings are published in dedicated editions of the BBC series of books, a renowned and much acclaimed publication in the field. Sessions during the 2-day meetings include plenary lectures and offered papers. As a result, the BBC books reflect the current 'hot topics' in biofilm research.

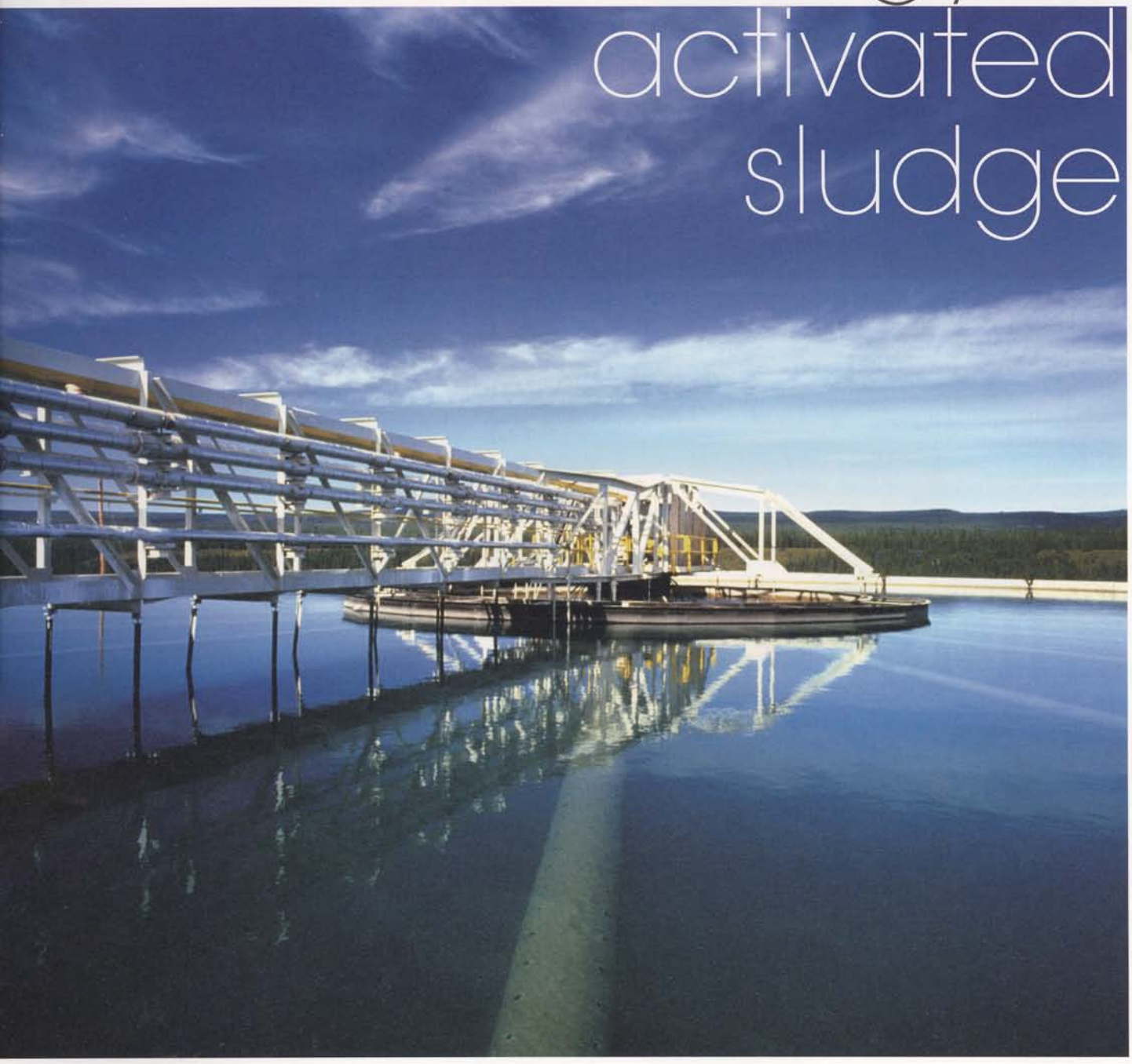
The website (www.biofilmclub.co.uk) acts as a portal for communication between members, with news of events and forthcoming meetings, and details of active research groups. There have been recent efforts to promote the Club outside the UK and Europe by inviting overseas members to contribute to the information held on the site. That said, we always welcome interest and interaction from researchers closer to home! For more information, please contact the Biofilm Club via Mathew Upton or visit the website.

Mathew Upton
Lecturer in medical microbiology and member of the Biofilm Club Committee, School of Medicine, Clinical Sciences Building, University of Manchester, Oxford Road, Manchester M13 9WL, UK (**t** 0161 276 8828, **e** m.upton@manchester.ac.uk)

thebiofilmclub

▲ A confocal section through a subgingival oral biofilm stack. Live organisms (purple) can be seen on the outside of the stack and dead ones (yellow) inside. *Jon Pratten and Chris Hope, Eastman Dental Institute*

Microbial ecology of activated sludge



The activated sludge process relies on a complex microbial community to clean up wastewaters and thus protect our environment. **Michael Wagner** explains how new molecular tools are shedding light on this ecosystem and how the information can be used to improve the treatment process.

The activated sludge process was invented about a century ago and has found worldwide application in the treatment of wastewater before it is discharged into the environment. Modern wastewater treatment plants use this process not only to remove organic matter from the sewage, but also to minimize the concentrations of nitrogen and phosphorus compounds which stimulate eutrophication in lakes, rivers and the ocean. It has long been known that activated sludge consists mainly of bacteria (in concentrations of up to 10^{11} cells ml^{-1}) which catalyse all the important steps of nutrient removal, but are also responsible for the major problems in these plants. Nevertheless, activated sludge research has been dominated for decades by engineers and until quite recently little was known about which micro-organisms were important in these man-made systems and how changes in environmental parameters or process design affect the structure and function of the microbial community. This situation has changed dramatically during the last few years thanks to exciting technical advances in microbial ecology. Using cutting-edge molecular tools, many key players in microbial activated sludge have been identified and partially characterized, and we have begun to understand the laws of biology which underpin this process. In parallel, microbial ecologists have discovered that activated sludge is an ideal model system for developing new methodology and for evaluating hypotheses relating to the ecology of micro-organisms.

Discovery of microbial key players

In developing a detailed theoretical framework for activated sludge microbiology, it is important to find out about the identity and ecophysiology of the micro-organisms responsible for key processes or major problems in wastewater treatment plants. Only if we can measure the abundance of desirable or undesirable populations will we be able to decipher (and later exploit) conditions that might selectively promote their success or failure, and thus rationally influence community assembly. Traditional cultivation methods are inadequate for identifying the key players, since only 10–15 % of the activated sludge micro-organisms form colonies on standard nutrient agar plates. This unintentional selectivity has led to an incomplete and sometimes even erroneous perception of which bacterial species catalyse such important steps as nitrification, denitrification and enhanced biological phosphorus removal, or what the causative agents for activated sludge bulking and foaming are. For example, *Nitrobacter* spp. and *Acinetobacter* spp. were considered for decades to be responsible for nitrite oxidation and enhanced biological phosphorus removal in activated sludge, respectively, and are still listed as model organisms for these processes in recent issues of several standard textbooks on wastewater treatment.

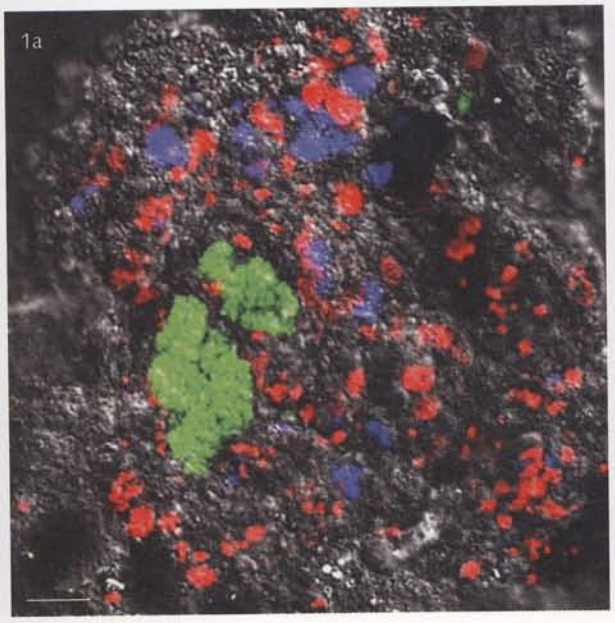
◀ Pool for treating wastewater in British Columbia, Canada. *Kaj R. Svensson / Science Photo Library*

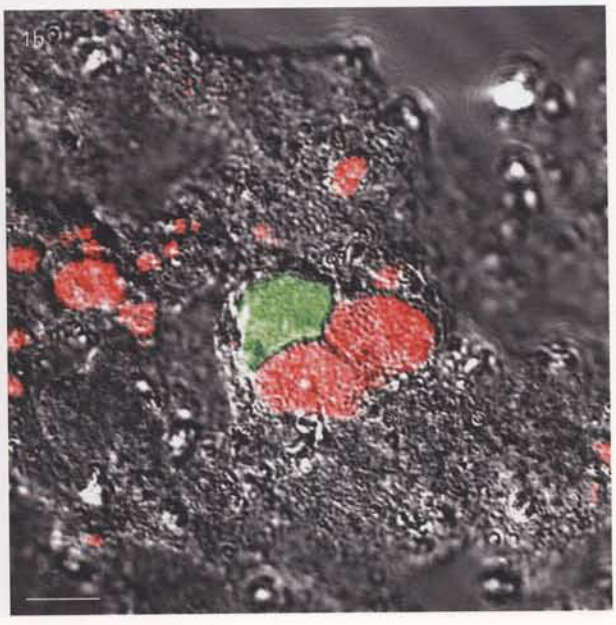
Using cultivation-independent, 16S rRNA-based censuses of microbial activated sludge communities we have learnt that a single reactor may harbour several hundred different bacterial species. This diversity can be easily traced by DNA microarrays which could, for example, be used in the future to monitor community dynamics in activated sludge tanks or the biogeography of activated sludge bacteria. The abundance of selected populations in activated sludge can be precisely measured by quantitative fluorescence *in situ* hybridization (FISH) using rRNA-targeted oligonucleotide probes. In 1994 and 1995, by using this approach, we demonstrated that neither *Nitrobacter* nor *Acinetobacter* were of major importance for N or P removal in most wastewater treatment plants. While perfectly suited to the analysis of the microbial community structure, FISH results alone are insufficient for understanding the ecophysiology of activated sludge bacteria. This limitation can be over-come in microcosm experiments which offer the activated sludge community isotope-labelled substrates under defined conditions. Today, a whole battery of molecular tools is available which allows microbial ecologists to identify which bacteria metabolize the added substrates and so assign specific functions to populations.

This toolbox was used to identify the actual key players in nitrification, denitrification and enhanced biological phosphorus removal, most of which are still uncultured. For example, Stefan Juretschko, a former PhD student in my group, discovered in 1998 that *Nitrospira*-related nitrite-oxidizers and not *Nitrobacter* were the numerically dominant nitrite oxidizers in most nitrifying activated sludge systems. Andreas Schramm and colleagues (now at the University of Aarhus, Denmark) later raised the hypothesis that these nitrospiras are K-strategists for nitrite and oxygen and thus outcompete the putative r-strategist *Nitrobacter* in reactors with low nitrite availability. Recent data from my and other labs support this theory. Furthermore, it is now apparent that these novel nitrite oxidizers can grow mixotrophically in activated sludge, that different *Nitrospira* populations co-occur in nitrifying sludges (Fig. 1) and that these peculiar nitrite oxidizers are also of importance in many other systems ranging from agricultural soil to marine sponges.

Almost at the same time, Alexander Zehnder's group at the EWAG (Dübendorf, Switzerland) reported on the enrichment of uncultured betaproteobacteria tentatively named *Candidatus* 'Accumulibacter phosphatis' which are responsible for enhanced biological phosphorus removal in wastewater treatment plants. In addition, several novel denitrifiers have also been identified in activated sludge. It has become clear that the morphology-based identification key in widespread use for filamentous activated sludge bacteria dramatically underestimates their actual diversity by lumping together genetically very different organisms which share the same morphology.

Recent technological advances have encouraged several research groups to attempt to determine the whole-genome sequences of important but uncultured activated sludge bacteria. Together with the French national sequencing institute (Genoscope) and collaborators from the Netherlands and Germany we are currently analysing the sequences of anaerobic ammonium oxidizers and *Nitrospira*-like nitrite oxidizers. Furthermore, the Joint Genome Institute has launched a





project to decipher the genome of *Candidatus* 'Accumulibacter phosphatis'. These genome sequences will significantly speed up the process of understanding these recently discovered micro-organisms, which serve us in ameliorating anthropogenic damage to the environment.

Ecology and engineers

Some engineers tend to undervalue progress in activated sludge microbial ecology by stating that these new findings simply lead to a change in the names of bacteria responsible for certain processes in wastewater treatment plants, with no relevance to their functions. My response is that these different names represent genetically very different organisms which have diverged early in evolutionary history and thus are most likely to have very different physiological properties. For example, in an rRNA tree *Nitrospira* is as 'closely' related to *Nitrobacter* as humans are to sunflowers (Fig. 2). Now that we have identified the microbial key players in many important processes and problems in activated sludge, and have tools at hand to measure their abundance and activities, we can begin to design experiments to show how operating conditions in a treatment plant affect these parameters. A central follow-up question is how the diversity of functionally important micro-organisms affects the stability of the respective processes (Fig. 1). For example, it is tempting to speculate that a greater diversity of nitrifying bacteria increases the resistance of the nitrification process in a wastewater treatment plant against certain perturbations. Thus, engineers should be interested in monitoring the diversity of important bacterial groups in their treatment plants and ultimately learn how they can influence diversity by changing the operating conditions in order to maximize process stability and performance. Such insights cannot be obtained by traditional bulk parameter measurements, but require high resolution analyses of the structure and function of activated sludge microbial communities, as well as a commitment of researchers and funding agencies to this interdisciplinary research.

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Further reading

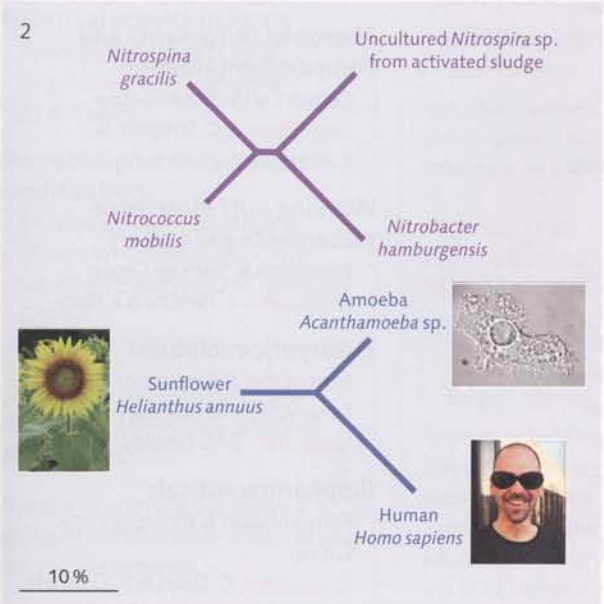
Curtis, T.P., Head, I.M. & Graham, D.W. (2003). Theoretical ecology for engineering biology. *Environ Sci Technol* **37**, 64A–70A.

Daims, H., Nielsen, J.L., Nielsen, P.H., Schleifer, K.H. & Wagner, M. (2001). *In situ* characterization of *Nitrospira*-like nitrite-oxidizing bacteria active in wastewater treatment plants. *Appl Environ Microbiol* **67**, 5273–5284.

Hesselmann, R.P., Werlen, C., Hahn, D., van der Meer, J.R. & Zehnder, A.J. (1999). Enrichment, phylogenetic analysis and detection of a bacterium that performs enhanced biological phosphate removal in activated sludge. *Syst Appl Microbiol* **22**, 454–465.

Wagner, M. (2004). Deciphering the function of uncultured micro-organisms. *ASM News* **70**, 63–70.

Wagner, M. & Loy, A. (2002). Bacterial community composition and function in sewage treatment systems. *Curr Opin Biotechnol* **13**, 218–227.



▲ An outlet pipe discharges sewage onto a North Sea beach. *Simon Fraser / Science Photo Library*

◀ Fig. 1. FISH of nitrifying bacteria in activated sludges from two different wastewater treatment plants. (a) Two different populations of *Nitrospira*-like nitrite oxidizers are stained red and green, respectively. Ammonia oxidizers of the genus *Nitrosomonas* are labelled in blue. (b) *Nitrospira*-like nitrite oxidizers are stained red and ammonia oxidizers of the genus *Nitrosomonas* are labelled in green. Bars, 10 µm. *Courtesy Kilian Stoecker*

◀ Fig. 2. A comparison of phylogenetic distances between recognized nitrite-oxidizing bacteria and between selected eukaryotes. Phylogenetic 16S rRNA and 18S rRNA trees were calculated using neighbour joining with the Jukes–Cantor correction and alignment masks which excluded highly variable positions in the respective data sets. Bar represents 10 % estimated sequence divergence.

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Quorum sensing

This issue of *Microbiology Today* focuses on microbial communities. Microbiologists now know that the organisms in these amazing ecosystems communicate with each other with chemical signals. This system is dependent on population density and is called quorum sensing. **Faye Jones**, who carried out her PhD research project in this subject, explains how it all works.

Bacteria were long thought of as organisms working alone, but over the past 20 years, it has become apparent that they communicate with each other. Microbes naturally live in large communities, often containing hundreds of different species, so it should not be surprising that some co-ordinated teamwork is required for them to co-exist.

Metabolic activities in the cell are initiated by the expression of proteins under the control of specific genes, but how are the genes regulated?

The bacteria secrete small signalling molecules, called microbial pheromones or autoinducers. Signalling occurs through the build-up of the autoinducer in an environment. This is linked to the population density of the bacteria. Once a critical concentration is reached, the inducer is detected by a specific receptor protein in the cells (Protein R), which becomes activated and switches on or off a particular gene or set of genes. This phenomenon is used to regulate a diverse range of phenotypes.

When first discovered, quorum sensing was believed to be a unique signalling system for activating bioluminescence in the Gram-negative, marine bacterium *Vibrio fischeri*. In the past 10 years the number of bacteria found to be using quorum sensing to signal changes has escalated. Some characteristics now known to be regulated through quorum sensing include swimming motility in the human pathogen *Yersinia pseudotuberculosis*, biofilm formation in the fish pathogen *Vibrio anguillarum*, antibiotic production in the plant pathogen *Erwinia carotovora*, formation of nitrogen-fixing nodules on legumes by symbiotic *Rhizobium leguminosarum*, and many factors involved in virulence (lytic enzymes, toxins, etc.) from a whole range of plant, animal and human pathogens. A high percentage of the bacteria known to use quorum sensing are opportunistic pathogens. Virulence factors are not always regulated through the quorum sensing systems in these bacteria, although in many cases they are.

Vibrio fischeri

V. fischeri normally live in symbiosis with certain marine fish and squid, where the bacteria are confined within a special light organ that protects them from the environment. In return, the bacteria produce light that is used by their symbiont partner to attract prey or scare away predators. Within light organs, the bacterial population grows to a high density (ca 10^{10}–10^{11} cells ml^{-1}) and luminescence is induced through accumulation of the autoinducer. When enough autoinducer is present, it can activate a response protein which in turn begins to regulate its designated gene (or set of genes).

V. fischeri can also exist free-living in the sea. Under these circumstances, they don't waste energy on producing light. This lack of light production in free-living cells is due to the autoinducer being diluted in the water as it diffuses away from single cells.

Quorum sensing is a cell-to-cell communication system that allows a single bacterium to work with its neighbours.

◀ Fruiting body of a myxobacterium, *Chondromyces crocatus*. Prof. Hans Reichenbach, GBF, Braunschweig, Germany

The autoinducer that regulates luminescence in *V. fischeri* is *N*-(3-oxo)-hexanoyl-homoserine lactone (OHHL). It comes from the family of autoinducers called *N*-acyl-homoserine lactones (AHLs). The type of side chain on these molecules gives them specificity for different quorum sensing systems. OHHL has also been discovered in cell-free supernatants of the plant pathogen *Erwinia carotovora* and was found to be responsible for regulation of the production of a β-lactam antibiotic. The discovery that AHLs were not restricted to the regulation of luminescence in marine bacteria has led to the production of numerous biosensors for the detection of AHLs in cell-free supernatants of a wide range of bacterial species.

Other signals

Although AHLs are by far the most studied signalling molecules to date, other signals in Gram-negative bacteria include cyclic dipeptides and quinolones, which are found in the opportunistic human pathogen *Pseudomonas aeruginosa*, and 3-hydroxypalmitic acid methyl ester in *Ralstonia solanacearum*, which causes bacterial wilts in a wide variety of plants ranging from potatoes to banana trees.

Other microbes

Myxobacteria

Myxobacteria are interesting gliding bacteria that produce fruiting bodies under starvation conditions. They are commonly found in animal dung and organic-rich soils of neutral or alkaline pH. Some of them grow by utilizing cellulose, but many of them feed by secreting antibiotics to kill other bacteria and then produce enzymes to lyse the cells of their prey. Fruiting body formation is regulated by a diffusible signal, which accumulates to a critical concentration in the environment. When nutrients become limited, some cells commit suicide, while the remaining cells move from various parts of the community to congregate at a focal point, making a mound in which spores can begin to form. This behaviour gives them a chance to survive and germinate when the conditions improve.

Gram-positive bacteria

Many Gram-positive bacteria use small, modified oligopeptides as signalling molecules. These include the human pathogens *Staphylococcus aureus* (used to regulate virulence factors), *Bacillus subtilis* and *Streptococcus pneumoniae* (to regulate genetic competence) and a number of lactic acid bacteria (to regulate production of bacteriocin).

Streptomycetes

Streptomycetes live in the soil and they make many important antibiotics used to treat infections. Several species utilize γ-butyrolactones to regulate the production of antibiotics and other secondary metabolites during the stationary growth phase. Recently, γ-butyrolactones were isolated from another bacterium, *Pseudomonas aureofaciens*, where they were reported to be involved in the production of compounds with antifungal properties.

Useful websites

www.nottingham.ac.uk/quorum/

www.bio.cam.ac.uk/~salmond/research-interests/quorum-research.html

www.medicine.uiowa.edu/greenberglab/

www.med.upenn.edu/micro/faculty/zhu.html

http://helios.bto.ed.ac.uk/bto/microbes/myxococc.htm

Glossary

Gram-negative bacteria	defined by thin cell wall, made up of a small layer of peptidoglycan surrounded by a thicker outer membrane
Gram-positive bacteria	defined by thick cell wall, mostly composed of peptidoglycan
Peptidoglycan	lattice structure formed from linear chains of two alternating sugar derivatives
Phenotype	observable characteristics of an organism/manifestation of gene expression in a particular organism

New resources!

Bioluminescence

Light can be produced by a chemical reaction inside the cells of a whole range of living organisms. This two-page resource explains the basic facts about bioluminescence, with particular reference to the role of microbes, and provides examples of the amazing practical applications of this phenomenon. The leaflet is illustrated in colour. Contact education@sgm.ac.uk

Your Career in Microbiology

This 16-page, full-colour booklet has been completely revised and contains new profiles of the very different career paths of six young microbiologists. Contact careers@sgm.ac.uk

E-source: Microbes & Food

Based around a 'microbial menu', this website covers various aspects of food microbiology – www.schoolscience.co.uk

gradline

Gradline aims to inform and entertain members in the early stages of their career in microbiology. If you have any news or stories, or would like to see any topics featured, contact **Jane Westwell** (e j.westwell@sgm.ac.uk)

All in a day's work

Are you coming to the end of your degree and thinking about your next steps? Have you considered registering with a recruitment agency but were unsure whether it would be a good move? Gradline Editor, **Jane Westwell**, contacted a couple of the major UK recruitment agencies to get a picture of the range of job opportunities for microbiologists.

SRG and Lab Support UK are two well-known agencies that specialize in supplying contract staff to industry and also find permanent staff (usually in senior roles) for employers. Both agencies recruit microbiologists. Over the last 4 years, SRG has placed 235 microbiologists in 115 companies across the UK. In 2004, Lab Support found work for more than 50 microbiologists. Employers tend to be from the pharmaceutical, food and biotechnology sectors, including contract research and water testing organizations. Most biotechnology companies are SMEs (small to medium sized enterprises) although a few spin-outs also recruit through agencies. Despite the relatively high number of microbiologists that are recruited, it is fair to say that there are fewer opportunities for PhDs than for graduate scientists. Those that do exist are predominantly in the pharmaceutical sector.

Opportunities are mainly temporary, but permanent recruiting is on the increase. Also, 30–40 % of temporary posts convert to permanent positions. Whilst working on contract, scientists are employees of the agency and are entitled to the usual employment benefits.

It is rare to place a newly qualified PhD in industry unless they have the specific skills in microbiology or molecular biology that are used in this sector. Possession of 'rare' skills can be an advantage (agencies may list these). Apart from the obvious technical abilities, recruitment agencies are also looking for flexibility, adaptability, and good communication and team working skills. Rather helpfully, all agency websites include a few pages on CV writing and interviews to help potential candidates.

If you are thinking about a career in industry, working through an agency can be good way to gain experience and 'try out' different employers.

Further information

Most recruitment agencies have good websites and it is usually possible to register on-line.

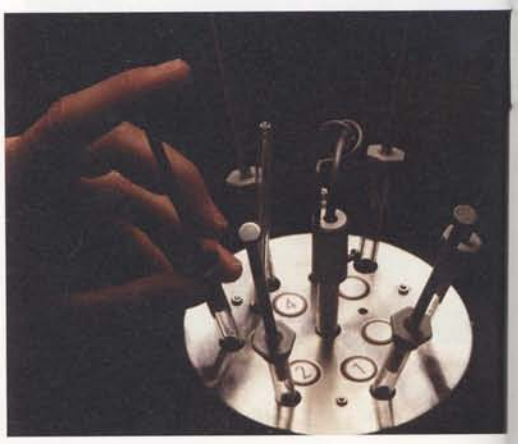
Jobs in Science www.scitemps.co.uk

Lab Support UK www.labsupport.co.uk

SCI www.sci-search.com

SRG www.srg.co.uk

Yoh Scientific www.yohscientific.co.uk



The 'write' stuff

In December 2004, 18 postgraduate students from Irish institutions took part in a course in scientific writing organized by **Catherine O'Reilly**, Convener of the SGM Irish group. The course comprised two half-day sessions and was run by journalist and ex-geneticist **Mary Mulvihill**. I attended as Convener of the Education & Training Group.

The first session began with a reminder of basic principles of grammar: language and punctuation. Mary provided examples of problems arising due to incorrect grammar (such as the importance of the hyphen in the meaning of 'extra marital sex' as opposed to extra-marital sex!). We then wrote an appropriate sentence or phrase for each item. Following this, we tackled the wordiness common to scientific writing by editing out extraneous phrases (e.g. 'was seen to', 'it would appear that') and repetitions (e.g. 'separate entities', 'new beginnings'). We rearranged massive strings of nouns ('blue absorbing

Postgraduate skills workshop

Surviving your PhD

1800–2000, 6 April 2005, Heriot-Watt University

The workshop will consider three key issues that face PhD students at different stages of their training: handling the supervisor/student relationship, effective writing and defending research in the viva. We have brought together three supervisors to share their experience gleaned from guiding many postgrads to PhD success. The workshop will be chaired by SGM Council member **Dr Pauline Handley** from the University of Manchester.

Dr Liz Sockett, University of Nottingham, will start the session with a talk on *Managing your supervisor*. Liz will draw on her years of experience as student, post-doc and then supervisor to take a look at the student/supervisor relationship. She will shed light on that sometimes inexplicable supervisor behaviour and will provide some hints to get over the sticky moments that many research students experience.

Professor Ian Poxton, University of Edinburgh, will follow with a talk on *Writing skills*. Ian has successfully supervised more than 35 students through the thesis-writing process and read many more theses in his capacity as PhD examiner. He will share his knowledge and experience to help those of you tackling transfer reports and theses. Ian is also Editor-in-Chief of *JMM* and will have some words of advice for first time authors of research papers.

Dr Bob Rastall, University of Reading, will end the evening with a presentation on *Strategies for a successful viva*. Bob has more than a decade's experience of helping his own research students successfully prepare for the viva and acts as external examiner to a number of universities. Bob will identify potential viva pitfalls and round up with some sound advice to help you on the way to a positive viva experience.

After a question and answer session chaired by Pauline, we hope you will join us for a glass of wine or two and a bite to eat. Tickets for the buffet and reception will be available at the end of the workshop.

Please note. Wednesday night accommodation will be covered by PG Student Conference Grants for those who wish to attend this session (www.sgm.ac.uk/grants).

pigment spectral curve', 'the negative penicillin skin test result group') and eliminated 'hedging' – excessive use of over-cautious conditional words.

Having tackled the dry and dusty, Mary tried to persuade us to bring some life to our scientific writing but encountered some initial problems. We were all dutiful, passive and unemotional scientific writers who showed great resistance to the use of 'we' and huge reluctance to start sentences with 'and'. However, a bit of expressive/creative writing unleashed a flow of emotions and active writing. We gave our pet micro-organisms a character (precocious, fussy, smelly, tough and resistant, slimy and bitchy) then began writing in a particular genre, such as horror, love, detective story, or diary.

'The smooth, silky colonies glistened from the crimson plate they lay scattered upon. One look at them and I knew I was lost in their magnificence. I set about counting them. Their bodies lay crushed against one another; it was difficult to see where one settlement started and another ended. Where once the undisturbed plate lay naked, exposed to the world, now stood thousands of multiplying factories, expanding before my eyes.' (Matthew Lambert, Trinity College Dublin)

'Once upon a time, there lived13 little Pseudomonas aeruginosas. They loved to hang out in the lungs of cystic fibrosis patients. They were all anti-drug activists, and refused to allow any drugs into their neighbourhood. One, PA13, was the leader of the group. He had absolutely no time for drugs, especially gentamycin.' (Anon)

Nerves now lost, we were given 25 minutes to write a *New Scientist* style article based on some facts provided by Mary. We then compared our efforts and the actual article – a useful exercise in the use of concise, precise but accessible English.

The second day felt more like a 'typical' scientific writing session. Mary and a young lecturer from Waterford Institute of Technology shared tips and experiences of writing for journals. As a final exercise, participants were asked to write an abstract for a paper recently submitted by Catherine (provided prior to the workshop) in only 40 minutes. The abstracts were judged and a prize awarded to the winner. The session ended with some useful discussion on my own and Catherine's experiences as writers and reviewers.

This intensive course was very useful and productive. Mary was careful to involve everyone in all activities. Catherine arranged a popular evening social event for anyone who wished to attend and the group had certainly bonded by the end of the two sessions.

Oral, visual and written communication skills are essential to all postgraduates and are usually addressed via university-organized events. This course covered issues of scientific written communication in an active and interesting manner – and I doubt if I can ever look at colonies on blood agar in the same way again!

Professor Joanna Verran (e j.verran@mmu.ac.uk)

hotoffthepress

Science writer **Meriel Jones** takes a look at some papers in current issues of the Society's journals which highlight new and exciting developments in microbiological research.

A sticky problem

Kusumoto, A., Seto, S., Jaffe, J.D. & Miyata, M. (2004). Cell surface differentiation of *Mycoplasma mobile* visualized by surface protein localization. *Microbiology* **150**, 4001–4008.

Mycoplasmas are strange bacteria. They do not have a cell wall, frequently live as parasites or commensals and glide sedately over surfaces. Typical bacteria propel themselves with flagella, but there is no sign of anything like this in mycoplasmas, neither on the bacteria nor among their genes, and microbiologists have long puzzled about the mechanism.

The authors of this paper have been working on this problem for years. They study *Mycoplasma mobile*, which was isolated from fish gills. The cells are flask-shaped and glide around in the direction of the tapered end, which is therefore called a 'head-like' structure. The authors have identified minute spikes on the head-like structure that stick to glass surfaces and might be involved in motility. To investigate this, they used antibodies to block the interaction between the spikes and the glass. Their reasoning was as follows. Antibodies are made by the immune system to stick to and neutralize pathogenic bacteria. If they could find an antibody that stuck to *M. mobile*, and also stopped the bacterial cells sticking to glass, they could use it to identify exactly what part of the cells adheres to glass.

After testing hundreds of antibodies, they found one, mAB7, with exactly this property. It stuck to the head-like structure only, especially the middle and basal part of it that the researchers dubbed the 'neck'. Other antibodies stuck to other regions on the surface, but the cells could still stick to glass. When the authors discovered that mAB7 interacted with the *M. mobile* protein Gli349, it fitted with information that mutant cells lacking this protein could not glide. Other antibodies reacted with four proteins on the cell surface from the Mvsp family, proving that they were part of the mycoplasma cell surface. *M. mobile* has genes for a further 12 closely related Mvsp proteins.

The authors have put all the information together to give a picture of the *M. mobile* surface. There are around 450 spikes made of Gli349 clustered on the neck of the cell, while proteins MvspN, MvspI and MvspO are present on the head. MvspI and one further protein, MvspK, are found on the body of the cell. The remaining 12 Mvsp proteins could either be on the surface, but were not detected, or may only be made by the cell in a different environment. Since the cell surface proteins, and adhesion, are essential to the parasitic life-style of mycoplasmas, this knowledge is important for our understanding of these strange bacteria.

A complementary approach to systematics

Dawyndt, P., Thompson, F.L., Austin, B., Swings, J., Koski, T. & Gyllenberg, M. (2005). Application of sliding-window discretization and minimization of stochastic complexity for the analysis of fAFLP genotyping fingerprint patterns of *Vibrionaceae*. *Int J Syst Evol Microbiol* **55**, 57–66.

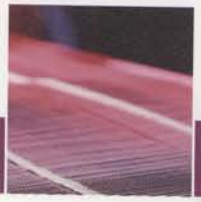
An amazing amount of diversity lurks beneath the anonymous pale, slimy appearance of many bacteria. Species that are more different than a carrot and an elephant can look the same to the human eye. Bacteriologists have put a lot of effort into detecting these differences, and objective ways to assess them. There is a long tradition of using methods that rely on hierarchical clustering to create taxonomies based on both genetic and biochemical characteristics. However, researchers have been aware for over 30 years that the order in which characteristic are grouped together can affect the outcome. Useful relationships may be distorted or missed.

These authors have been trying out alternative, complementary ways to classify bacteria from the *Vibrionaceae*, some of which are important pathogens of fish. A few years ago they recorded genetic fingerprints from 507 strains, and classified them using a conventional method, Ward's hierarchical clustering algorithm. Now, they have used a very different method on the same data, and found some interesting different results.

The method optimizes a given expression in information theory. The authors chose to minimize stochastic complexity (SC), using the BinClass software package, written by Mats Gyllenberg and his colleagues. To do this, they had to convert the data into a vectorized data representation. They already knew that the way they carried out this conversion could affect the final result, but that the sliding-window discretization procedure conserved more of the original information content than other methods, so they used it.

Many of the groupings produced by the two methods were exactly the same, but the SC method also disclosed new clusters that agree with recent information about *Vibrionaceae*. For example, SC brought together strains that had been split into two clusters by the traditional hierarchical method. Recent information about these strains has shown that they are definitely all the same species. One further SC cluster brought together *Vibrio rotiferianus* and four strains of *V. harveyi*. These species have turned out to be highly related, so it is possible that these four strains of *V. harveyi* have been misidentified.

The researchers feel that their two analyses of the *Vibrionaceae* genetic fingerprints bring out the value of using two complementary approaches to reveal the most information about bacterial relationships.



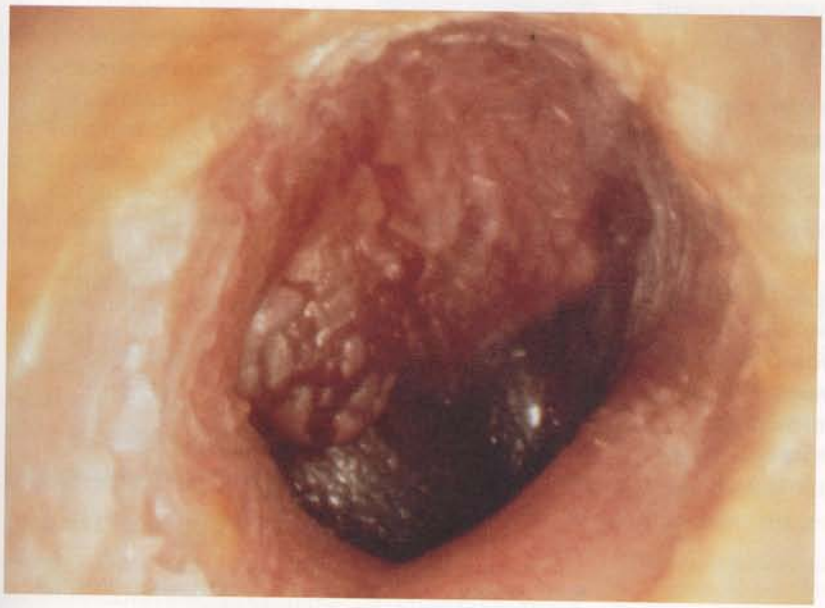
Antimicrobial resistance in otitis media patients

Brook, I. & Gober, A.E. (2005). Antimicrobial resistance in the nasopharyngeal flora of children with acute otitis media and otitis media recurring after amoxicillin therapy. *J Med Microbiol* **54**, 83–85.

Otitis media, or an infection of the ear where fluid and mucus are trapped inside the eardrum, is very common in babies and young children. In developed countries, three out of four children have suffered from this painful condition before they are 3 years old. One complication is that fluid can remain within the ear after the infection is over and may affect the child's hearing. Several types of bacteria and viruses can cause the infection. The bacterial infections can be treated with antibiotics, but the infection sometimes recurs.

Itzhak Brook and Alan Gober from the Georgetown University School of Medicine in the USA have been investigating whether the bacteria that cause recurrent infections are more resistant to antibiotics. They analysed nasopharyngeal cultures of 72 children who had appeared at a middle-class suburban clinic, suffering from uncomplicated otitis media between September 1999 and August 2001. Forty children presented with acute otitis media and 32 with recurrent otitis media that had been treated with the antibiotic amoxicillin. The researchers defined a recurrent infection as one that followed a previous ear infection with an infection-free interval of 4–6 weeks. The clinical microbiology showed that pathogenic bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae* or *Moraxella catarrhalis* had been identified in swabs taken from almost all of the children. Tests of the resistance of the bacteria to antibiotics indicated that many more of those isolated from the recurrent infections were resistant to a broad range of antimicrobials. The amoxicillin therapy might have selected for these resistant strains.

The researchers suggest that for effective treatment of recurrent ear infections, clinicians need to be aware of the resistance patterns of organisms like *S. pneumoniae* and *H. influenzae* within their patient community, as well as any previous treatment the patient has received. In addition, antibiotic sensitivity testing of samples from the patient may be required to prescribe suitable therapy.



▲ Otitis media viewed from the external ear canal. *Prof Tony Wright, Institute of Laryngology and Otology / Science Photo Library*

Filtering prions out of blood

Kobayashi, A., Satoh, A., Ironside, J.W., Mohri, S. & Kitamoto, T. (2005). Type 1 and type 2 human PrP^{Sc} have different aggregation sizes in methionine homozygotes with sporadic, iatrogenic and variant Creutzfeld–Jakob disease. *J Gen Virol* **86**, 237–240.

The protein PrP^{Sc} that causes the neurodegenerative Creutzfeld–Jakob disease (CJD) continues to surprise. For unknown reasons, it can change shape and form aggregates within the brain that result in the lethal neurodegenerative disease. Clinicians identify distinct types of disease (MM1 or MM2) depending on the size and location of the aggregates, the effects of proteinase K digestion and the numbers of sugar molecules attached to the protein. This group of researchers has been investigating how one protein can produce so many pathologies.

They focused on the size of the aggregates made by PrP^{Sc}, as this is also important in measures to prevent transmission. The researchers obtained permission to test brain samples from people who had died from CJD. They filtered extracts from the samples through filters that had pores of around 72 millionths of a millimetre. They already knew that some PrP^{Sc} could pass through these holes and the idea was to use this to check the size of the aggregates, identifying them based on proteinase K digestion patterns. The filters removed particles from patients with MM2 efficiently, while MM1 passed through. As a control, they tested an extract from a person who had died with signs of both pathologies in the brain. Again, most the particles typical of MM2 pathology were caught on the filter, while those characteristic of MM1 passed through.

The results suggest that there is a link between the type of PrP^{Sc} and the efficiency of removing it by filtration. The PrP^{Sc} aggregates in MM1 are generally small, consisting of fewer than 20 protein molecules, while those in MM2 are larger with up to 1,000 molecules. The authors concluded that any filtration methods to remove PrP^{Sc} from, for example, blood donations, should ensure that the smaller type of particles does not pass through.

Science and the Parliament 2004

Faye Jones, Public Affairs Administrator, describes some SGM Microbiology Awareness Campaign activities which aim to raise the profile of microbiology to politicians and opinion-formers.

On Wednesday 10 November 2004, the Royal Society of Chemistry held its *Science and the Parliament* event at 'Our Dynamic Earth', a science centre opposite the new Scottish Parliament building in Edinburgh. The event aims to raise awareness of science issues to MPs and civil servants working in the Scottish Parliament and the focus this year was the Scottish Executive's Science Strategy.

It attracted 340 participants from across the science and political communities, and was complemented by a parliamentary debate on science, which had probably the biggest audience ever for a members' debate. It touched on the burning issue of MRSA and hospital superbugs and its link to the lack of clinical microbiologists.

The host of first class speakers included **Deputy First Minister Jim Wallace**, who addressed the evening exhibition and reception and spoke of the importance of science education and the need to develop and promote the research base that underpins scientific innovation. He also commended the Scottish Parliamentary Science Information Service, which makes use of the expertise of several SGM members, for a successful first year.

Professor Sir Alfred Cuschieri, pioneer of keyhole surgery, emphasized the necessity to be aware of the changing requirements for effective science and technology research and development, as well as the training of the next generation of scientists.

SGM was among the 33 exhibitors, with an interactive display of interesting and relevant microbiological material which attracted a great deal of attention. **Dr David Gally**, University of Edinburgh, provided a full-length animation depicting the key stages of EHEC O157 : H7 colonization of the gut of its cattle host. The SGM exhibit also included an exciting display of bioluminescent bacteria – *Photobacterium phosphoreum* – provided by **Dr Graeme Paton** of the University of Aberdeen and prepared by **Professor Brian Austin** of Heriot-Watt University.

As a fun reminder to the attendees of the many uses of bioluminescence, from basic laboratory research to contaminant testing in the food industry and for bioremediation, SGM gave away hundreds of glow-in-the-dark badges, 'Glo-Bugs', that were worn by exhibitors, scientists and MSPs alike.

▲ Left SGM Executive Secretary Ron Fraser greets a very important guest at the *Science and the Parliament* event.

▲ Right Professor Wilson Sibbett and Dr Elaine Murray MSP, sporting an SGM 'Glo-Bug' (see inset), at the SGM stand in Edinburgh.

SGM at Westminster

On 1 March next year, SGM will be holding an event in the House of Lords to raise awareness of the importance of microbiology to Parliamentarians. The theme will be *Fighting Infection*, following on from the House of Lords Science and Technology Committee report of the same name. Experts will address MPs, ministers and civil servants on issues important to politicians in their constituencies, including tuberculosis, malaria, HIV and other sexually transmitted infections. There will be a small exhibition. The event will be hosted by **Lord Soulsby of Swaffham Prior** and **Dr Ian Gibson MP**, both members of Parliamentary Science and Technology Committees.

What's on in science communication?

Royal Society – www.royalsoc.ac.uk

23–24 May 2005
Science Communication Conference

Hosted jointly by the BA and the Royal Society, the conference seeks to address the key issues facing UK science communicators and to play an important role in helping to develop a national strategy in this arena..

4–7 July 2005
Summer Science Exhibition

This prestigious event showcases some of the UK's best science research. It features 25 exhibits and the visiting public can talk to the researchers about their work.

Competitions and awards

Famelab – www.famelab.org

Are you the new face of science? If so, you could win broadcasting time on Channel 4, a masterclass in science communication, a schedule of speaking events and £2,000 cash. The competition, sponsored by the Cheltenham Science Festival in partnership with NESTA, seeks to find new communicators of science. To enter you need to be over 18, working in science and able to present a scientific topic at one of a series of regional auditions in March and April.

Wellcome Trust Engaging Science Awards – www.wellcome.trust.ac.uk/engagingscience

People Awards of up to £30,000 can be used to support activities such as dramas, exhibitions, art projects etc. with a biomedical focus. Society Awards of £50,000+ are available to support larger activities or academic research.

Visions of Science **05**

a call for images that **inspire** and **intrigue**

www.visions-of-science.co.uk

It's now time to start planning your inspiring and intriguing images for the *2005 Novartis and The Daily Telegraph Visions of Science Photographic Awards*, supported by the Science Photo Library. Winning images should open our eyes to new and interesting areas of science, perhaps show something never seen before or show science in creative and unusual ways. The image may simply show a well known subject with a new twist.

Inspiration for entries may come from the natural world, from research in laboratories or in the field, from art, technology, people, medicine or conceptual science. Judges look for entries with high impact that can make viewers look again at science, nature and the world around them and consider the impact of science on their everyday lives.

The five popular categories are:

Action	Images should capture a scientific process or event as it happens in the natural world.
Close-up	Images that are beyond the naked eye.
People	Images should communicate the impact of science, medicine and technology on people's lives.
Concepts	Images should demonstrate or explain a scientific concept.
Art	Images should illustrate the beauty of science.

New Special Awards for 2005 are *Art meets Science*, *Einstein Year* and *Medicine and Science*.

Information on how to enter and entry forms are available on the *Visions of Science* website. The closing date for entries is **6 May 2005**. Photographs taken on or after 1 January 2000 are eligible for entry and up to six images may be entered in each Category or Special Award.

Winners of the main categories will be awarded first prizes of £1,000 and second prizes of £400, sponsored by the Science Photo Library. Each of the Special Award winners will receive £500. Winning images form a touring exhibition visiting science and arts venues across the UK.

► Upper Coloured SEM of the fungus *Aspergillus fumigatus*. *Eye of Science / Science Photo Library*

► Lower Computer artwork of AIDS virus particles (virions, green) erupting from the surface of T-4 lymphocyte white blood cells. *Hybrid Medical Animation / Science Photo Library*

reviews

These and other reviews are posted on the SGM website, where a classified compendium of reviews from 1996 to the present is also available.

Iron Transport in Bacteria

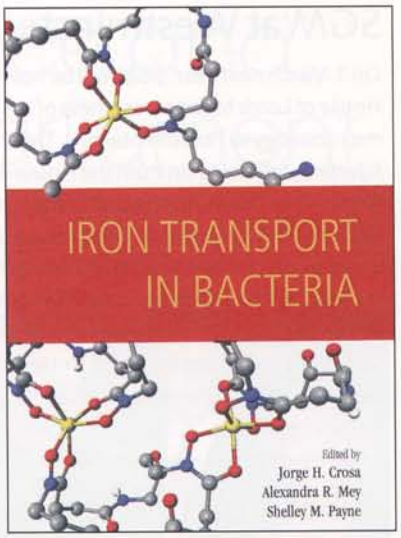
Edited by J.H. Crosa, A.R. Mey & S.M. Payne
Published by American Society for Microbiology (2004)
List Price US$119.95
Member Price US$109.95 pp. 532
ISBN 1-55581-292-9

Iron plays an important role in bacterial physiology: too little and the cell cannot synthesize essential iron-proteins for growth, but too much promotes reactions that produce hydroxyl radicals that damage the cell. As with many important molecules in the cell, the most rational method to control the intracellular concentration is at the level of transport across the cytoplasmic membrane, and this book is an attempt to present the findings on microbial iron transport from the last 50 years.

The Editors, Jorge Crosa, Alexandra Mey and Shelley Payne have assembled the book from 30 small chapters, allowing them to cover many subjects in a reasonably detailed manner. The early chapters focus on siderophores and haemophores, followed by general chapters on the structures of cytoplasmic and outer-membrane transport systems that take up the iron bound by these chelating agents. The details of model iron transporters from *Escherichia coli* make up the next four chapters, with a general chapter on the role of Fur as a repressor of gene expression. Unfortunately, the book was published just before the interesting publication by Vincenzo Scarlato's group that the Fur protein in *Neisseria meningitidis* can act as both an activator and a repressor. The next 14 chapters describe the details of iron transport in a wide range of pathogens, although a chapter on *Helicobacter pylori* seems notably absent. The last few chapters contain a description of iron uptake and regulation in rhizobia, but apart from this and the earlier chapters on *E. coli* K-12, the book has a very strong leaning to pathogens.

The method of presentation generally works well, so that a reader can quickly find specialist information about iron transport in a particular organism, but the Editors chose a method where research papers are not cited in the text and only suggested reading is provided at the end of each article. This does make the text easier to read and is suitable for the introductory chapters, but is infuriating in the specialized chapters as it is not possible to identify quickly the primary source for a particular interesting fact. The Editors have also done a pretty good job of removing large overlap between the chapters, but there is inevitably repetition – the function of a siderophore is described in almost every chapter! The book has also omitted a few topics that it would have been useful to include. Iron storage is an additional method to control the intracellular concentration of iron that is intimately linked to transport and yet there is no coverage of this. Also, many chapters mention iron transport in yeast for a comparison and a chapter on this and other non-bacterial iron transporters would have been helpful. Recent microarray data from Jay Hinton's lab has suggested that the intracellular environment chosen by certain pathogens is not iron-limited and the book would have benefitted from a general chapter relating the *in vivo* environments of these pathogens to the ranges and concentrations of iron sources available, given its strong bias for iron transport in pathogens.

Who will buy this book? It will obviously appeal to all interested in iron transport and can potentially attract more general readers working on the organisms



covered in the specialized chapters, although these might be a little short to justify buying the whole book. Also, as some of the most well characterized solute transport systems from bacteria are involved in movement of iron, the early chapters have a more general value to scientists interested in microbial transport per se and the presentation of the material in relatively short chapters means that it is easy to dip into the book and find accurate information quickly. It will certainly be on my shelf for the immediate future as a general reference text for membrane transport structure and function, and would be an invaluable reference for anyone with an interest in iron transport.

Gold is for the mistress – silver for the maid –
Copper for the craftsman cunning at his trade.
'Good!' said the Baron, sitting in his hall,
'But Iron – Cold Iron – is master of them all.'
[Rudyard Kipling (1865–1936) in *Cold Iron*]

***Gavin Thomas**, University of York*

Microbial Biofilms

Edited by M. Ghannoum & G.A. O'Toole
Published by American Society for Microbiology (2004)
US$115.95 pp. 440
ISBN 1-55581-294-5

As any potential reader may expect from a book with such an encompassing

title, the subject of biofilm research is viewed from a variety of perspectives. Amid chapters on the history of the biofilm concept and future directions are those related to human, plant and environmental biofilm systems. The book's content is a credit to a series of contributors, each of whom has put together a concise and informative chapter. As well as evaluating their complexity, diversity and structure, also discussed is the behaviour of biofilms in terms of antimicrobial resistance and genetic exchange. Of interest is the implication of biofilms in disease. Indeed, this area comprises several sections with one dedicated to the current key issue of hospital-acquired infections.

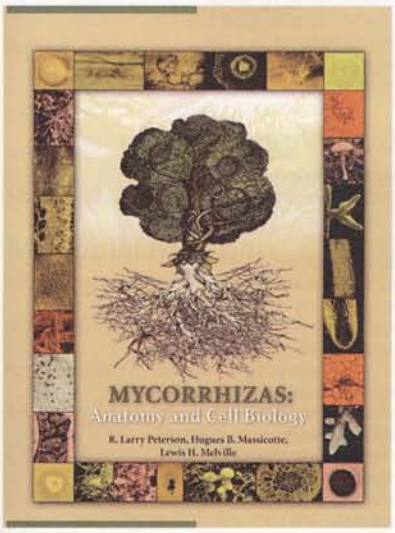
I believe this book would be useful to both new biofilm researchers and those wanting a current reference book on the numerous topics in this interesting area.

***Jonathan Pratten**, Eastman Dental Institute, UCL*

Mycorrhizas: Anatomy and Cell Biology

By R.L. Peterson, H.B. Massicotte & L.H. Melville
Published by CABI Publishing (2004)
£40.00/US$70.00 pp. 196
ISBN 0-85199-901-8

Apart from some mushrooms and toadstools, most mycorrhizal fungi



have few if any above-ground structures visible to the naked eye. I spend most of my time looking at sequences and other molecular data derived from such fungi, so this book came as a refreshing reminder that these fungi have a complex anatomy and some very intricate structures. These are beautifully illustrated in this book, using both micrographs and diagrams and it is an appealing book for these alone. The material is up-to-date, with current techniques and debates presented in an accessible 'box' format. Undergraduate and postgraduate researchers alike embarking on mycorrhizal projects and who will be interpreting unfamiliar microscopic material, should find this a valuable resource. The accompanying text is approachable, and will appeal to field mycologists who would like to explore the world beneath their feet. This is a timely publication in a fascinating field.

***Thorunn Helgason**, University of York*

Molecular Microbial Ecology Manual, Second Edition, Vols 1 and 2

Edited by G.A. Kowalchuk, F.J. de Bruijn, I.M. Head, A.D.L. Akkermans & J. Dirk van Elsas
Published by Kluwer Academic (2004)
€395.00/US$435.00/£273.00
Vol. 1 pp. 849; Vol. 2 pp. 1,774
ISBN 1-4020-2176-3

This multi-author, well referenced, two-volume book describes techniques for molecular microbial ecology. Vol. 1 has three sections on isolation, detection and applications of nucleic acid sequences whilst vol. 2 has five sections dealing with detecting gene transfer, reporter systems, statistical and computer analyses and methods to assess microbial activities. Each chapter has an introduction and detailed protocols with varying amounts of additional information such as useful notes to guide the non-expert. Strengths include protocols for just about any technique a microbial ecologist might wish to use. However, the emerging high-throughput techniques (described in a handful of chapters) may eventually render some of the described methods obsolete. There is no indexing; contents of the volumes are described in a loose booklet, easily lost in a busy laboratory! Overall, an excellent all round reference source of techniques probably aimed at institutional purchase.

***Clive Edwards**, University of Liverpool*

Reviews on the web

Reviews of the following books are available on the website at www.sgm.ac.uk/pubs/micro_today/reviews.cfm

Infectious Disease and Host–Pathogen Evolution

Foot-and-Mouth Disease: Current Perspectives

Lentivirus Gene Engineering Protocols. Methods in Molecular Biology, Vol. 229

Public Health Microbiology. Methods in Molecular Biology, Vol. 268

Pathogenic Fungi (1) Structural Biology and Taxonomy; (2) Host Interactions and Emerging Strategies for Control

Molecular Epidemiology of Infectious Diseases: Principles and Practices.

Concise Encyclopedia of Bioresource Technology

Vaccinia Virus and Poxvirology: Methods and Protocols. Methods in Molecular Biology, Vol. 269

Microbial Functional Genomics

The Pneumococcus

Pocket Guide to Clinical Microbiology 3rd Edition

The Metabolism and Molecular Physiology of Saccharomyces cerevisiae, 2nd Edition

The Innate Immune Response to Infection

The Internet for Cell and Molecular Biologists, Second Edition

Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Mammals, Birds and Bees), Fifth Edition, Vols. I & II

Bacteria for Breakfast: Probiotics for Good Health

Genomics: Applications In Human Biology

Plasmid Biology

obituary

June Lascelles

23.01.**1924**–01.07.**2004**

SGM Member (1947–1978/83)
Council (1954–1958)
Associate Editor (1955–1961)

▲ June Lascelles. *Courtesy Alick Lascelles*

June Lascelles, Professor Emerita of Microbiology and Molecular Genetics at the University of California, Los Angeles, died at her home in Los Angeles, aged 80. June devoted her life to microbial biochemistry and to those she taught and supervised. She had a tremendous and infectious enthusiasm for research on a wide variety of micro-organisms, and she is particularly well known for her work on the purple non-sulfur photosynthetic bacteria. Her pioneering physiological and biochemical studies, which frequently exploited mutants, provided fundamental insights into tetrapyrrole biosynthesis and other metabolic processes, laying down foundations and concepts that are still achieving new relevance in the post-genomic era.

June Lascelles was born and raised in Sydney, Australia. She graduated from the University of Sydney with a First Class BSc degree in Biochemistry in 1944, followed by an MSc in 1947. She was awarded an 1851 Exhibition Fellowship and elected to work in the Microbiology Unit of the Department of Biochemistry in Oxford. Her supervisor was Donald Woods and with him she established that pAB is converted to folic acid, that the conversion rate is tenfold higher in sulfonamide-resistant organisms and that folates are involved in amino acid biosynthesis. After receiving a DPhil in 1952, June stayed in Oxford and was appointed Lecturer in Microbial Biochemistry in 1960.

At this stage she developed new projects in microbial biochemistry, making very significant contributions to our understanding of the general and respiratory metabolism of *Staphylococcus aureus*, the C- and inorganic S-metabolism of photosynthetic bacteria, and she achieved early distinction for her contribution to microbial porphyrin synthesis. An important motivating factor for these studies was the value then attached to the use of bacteria to inform on the basic metabolites and metabolic reactions of higher organisms. Her tremendous enthusiasm for a wide diversity of micro-organisms was confirmed during an exciting year (1956/7) spent with C.B. van Niel at the Hopkins Marine Station (California) isolating and studying various 'exotics' like *Thiovulum*, *Thiothrix* and *Cytophaga* as well many species of anoxygenic photosynthetics. Her broad approach is reflected in publications addressing fundamental metabolic problems in at least 20 species ranging from *Staphylococcus* to *Tetrahymena*, *Campylobacter* to *Physarum*, and *Paracoccus* to purple non-sulfur photosynthetics.

In 1965 June was invited to a Chair in Bacteriology at the University of California, Los Angeles. A major theme of June's research from 1955 to 1980 was the synthesis and regulation of haem and bacteriochlorophyll (Bchl) in *Rhododobacter sphaeroides*. This classic study established that light and oxygen affect the synthesis of several enzymes used in the biosynthesis of both haem and Bchl, and provided the basis of our current understanding of tetrapyrrole synthesis in photosynthetic bacteria. She was one of the first to select mutants having defects in tetrapyrrole synthesis, and exploit them in identifying individual enzymic steps in the biosynthetic pathways. June's influential scientific reviews and exceptional editorial and reviewing skills were widely valued by scientists, publishers and grant-awarding bodies, worldwide. June retired in 1989 and was made a Fellow of the American Association for the Advancement of Science in 1990. She worked every day until 2 years before her death.

June was modest and self-effacing but forthright, plain-speaking, proud of her Australian heritage, and seriously professional. Her industry and thoroughness are legendary. She was an extremely acute and reliable critic and reporter of both scientific matters and human affairs. She had a very positive attitude to life, a great sense of humour, and a highly developed social conscience. She was no respecter of class, royalty or the Church, and impatient of some of the ancient practices of Oxford college life. Most of all she will be remembered for her great kindness and generosity to young scientists. She was always approachable, prepared to listen, took a genuine interest in their work, and gave wise counsel and encouragement. Her death was announced to Departmental colleagues in words that included, '*She will be missed as an accomplished scholar, dedicated learner, highly respected experimentalist, unique role model and rare friend*'.

John R. Guest
University of Sheffield, UK

Howard Gest
Indiana University, Bloomington, USA

comment

MRSA – national disgrace?

MRSA is constantly in the headlines and is even the focus of 'campaigns' in some UK tabloids. It is often suggested that improved hospital hygiene will solve the problem, but as **Mark Enright** describes, the issue is far more complex than the public realize.

Stories in the UK media about the 'scandal' of dirty NHS hospitals have made hygiene a major topic for public debate. The press often links dramatic increases in the prevalence of the 'superbug' – methicillin-resistant *Staphylococcus aureus* (MRSA) – with decreasing standards in hospital hygiene. Such reports often bundle together the cleanliness of the hospital environment and that of staff. There ought to be fewer MRSA infections in clean hospitals yet surprisingly there is little scientific evidence for this.

MRSA causes ~50 % of *S. aureus* bacteraemia in the UK, the second highest rate in Europe according to a recent study, yet much higher rates are reported in the USA and Japan, countries not generally perceived as having failing hospital hygiene regimens. The impact of environmental cleanliness measures is likely to be small compared to proven measures that interrupt transmission such as frequent handwashing by healthcare workers and the isolation of patients carrying particularly transmissible clones.

One puzzling aspect of the MRSA epidemic is its apparent global nature. Isolation rates have been increasing in the UK and elsewhere since the early 1990s. In the UK the emergence and domination of two clones, UK Epidemic MRSA clone-15 and -16, coincided with increases in MRSA bacteraemia, but these genotypes are still uncommon in most countries. MRSA mainly causes invasive disease following infection of the tissues around indwelling devices such as venous catheters. These are increasingly used, as are immunosuppressive drugs.

▲ *Kevin Beebe / Science Photo Library*

The UK MRSA epidemic may largely be explained by the emergence of particularly transmissible genotypes meeting an increasing number of vulnerable hosts. In other countries changes in the population structure of hospital *S. aureus* were not so dramatic although MRSA has become increasingly multidrug resistant.

Although MRSA is a major problem in most industrialized societies, some parts of Europe have very low rates. In Scandinavia and the Netherlands MRSA isolation rates are only 1–2 %, presumably due to radical 'search and destroy' policies which keep affected patients out of shared wards until carriage can be eliminated. UK government policy is to import infection control experts from such countries to help combat endemic MRSA. This may seem laudable but it is not at all clear how personnel who are good at keeping MRSA out of hospitals will respond to the challenge of reducing infection rates – surely quite different strategies are required.

The Department of Health has set a target to reduce the UK MRSA bacteraemia rate by 50 % by 2008, apparently by raising hygiene standards and improving infection control practices. Better hand hygiene will have some effect, but only a radical measure such as isolating all patients with highly transmissible MRSA would really solve the problem. However this would require a politically unacceptable level of resource and lengthen hospital waiting lists.

Analysis of the results of successful local interventions to reduce MRSA rates is needed, but comprehensive data on MRSA from all samples should also be collected nationally. Presently only the number of MRSA blood infections in each NHS Trust is published yet MRSA colonizes and invades many body tissues, including the lung, skin and bone. A true model of how MRSA comes into hospital, colonizes staff and patients and then causes serious disease is lacking and full information on all *S. aureus* in a unit and their transmission routes is required.

There is little large-scale government spending on microbiology research in this area through the NHS or MRC. This is unacceptable. Such studies would give a deeper understanding of how the MRSA pandemic started, which may allow us to begin to tackle the problem and prevent future epidemics. MRSA is changing rapidly and beside hospital strains with resistance to all antibiotic classes, some types are finding niches in the community, causing disease in healthy young people with no prior hospital exposure. Hospital hygiene initiatives are all very well but clean hospitals and motivated staff should be the norm and we should be finding out how to eradicate this international problem.

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Anonymous (1997). *Commun Dis Rep Wkly* **7**, 1.

Anonymous (2003). *EARSS Annual Report.*

Diekema, D.J. & others (2000). *Microb Drug Resist* **6**, 213–221.

Enright, M.C. & others (2002). *Proc Natl Acad Sci USA* **99**, 7687–7692.

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