Microbiologist

The magazine of the Society for Applied Microbiology ■ March 2013 ■ Vol 14 No 1 ISSN 1479-2699

BENEFICIAL BUGS

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INSIDE

- Actinobacterial diversity as a source of new drugs
- Lactic acid bacteria in cheese and salt reduction
- Q&A: Probiotics, prebiotics and bifidobacteria

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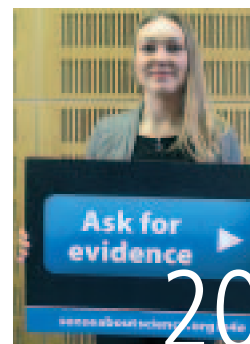

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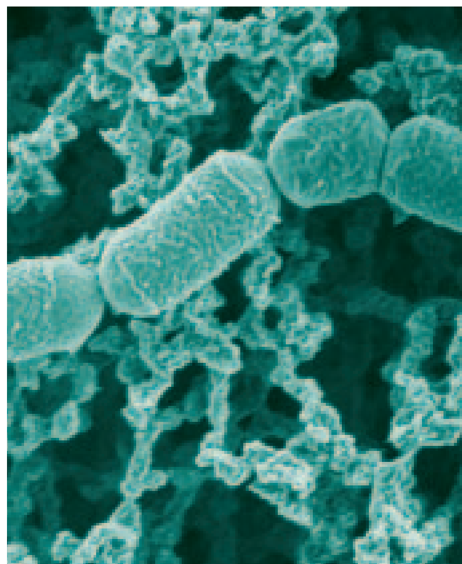
editorial

Lucy Harper discusses the vital role of beneficial microbes



We are all aware of the enormous benefits provided to us by our microbial friends, and in this issue we look at the vital role that microbes play in three of those benefits: food, drugs and probiotics.

This issue of *Microbiologist* follows the theme of our forthcoming Summer Conference: lactic acid bacteria, bifidobacteria and actinobacteria.

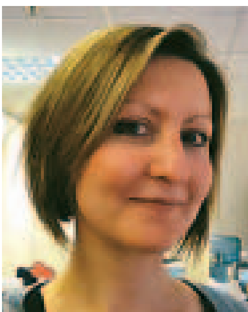
Mike Goodfellow writes about the potential to produce drugs from actinobacteria. He writes: "*The challenges posed by drug-resistant pathogenic bacteria are especially acute, as exemplified by rising levels of infection caused by vancomycin-resistant Enterococcus faecium, fluoroquinoline-resistant Pseudomonas aeruginosa and meticillin-resistant Staphylococcus aureus, as well as the recent reports of panantibiotic-resistant infections attributed to Acinetobacter species and carbopenem-resistant species of Klebsiella (Donadio et al., 2010). However, it is somewhat paradoxical that as the incidence of drug resistance grows the number of newly approved drugs is in sharp decline.*"

Actinobacteria can synthesize novel natural products with a wide variety of activities which can be exploited in drug production. The bioactive compounds formed from these natural products have unique modes of action against panels of drug-resistant pathogenic microorganisms. You can read more on page 8.

The role of lactic acid bacteria in the production of cheese, is the subject of the second feature. Kostas Gkatzionis and Christine Dodd write: "*Cheese production involves ripening and maturation stages that are characterized by the growth of complex bacterial and fungal communities. These microorganisms are either added by the producer as starter cultures or they naturally contaminate the cheese, and comprise its secondary microflora.*" He continues: "*Although the secondary microflora is not controlled by the producer, the physico-chemical environment in the cheese and the external environment favour the development of particular microbial species in each cheese variety and/or dairy.*" Kostas gives us the low-down on lactic acid bacteria and cheese production on page 13.

Finally, we explore the world of probiotics with a Q&A with Professor Glenn Gibson on page 16. He tells us about his work on probiotics and prebiotics and how the future of work in this area will look at the metabolome. He says: "*There's a lot of research going on at the moment on sequencing and understanding the diversity and systematics of the gut microbiome. But, I think the next big challenges are to understand functionally what the gut microbiome offers and how this metabolism can be altered, to improve health.*"

As well as some of the benefits microbes provide to humans, we also look at the benefits provided by SfAM which are highlighted on page 36. Here we describe two new online tools which are free to SfAM Members: Virtual Microbiology Lab and Tropical Microbiology Network.



Lucy Harper

contribute

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

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

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president's column

SfAM President, **Professor Martin Adams** muses on how history can usefully inform the present

The famous crime writer Agatha Christie, speaking of her husband the archaeologist Max Mallowan, said that the good thing about being married to an archaeologist was that the older you got, the more interesting he found you. This sentiment is true in a much broader sense; the history of anything has an intrinsic interest and the longer the history the more interesting it often is. Nor is it purely a matter of academic interest, knowledge of the past is important in shaping our current views and future strategy in a host of different areas. At its simplest, when embarking on a new area of work, the starting point is invariably a trawl of the literature to see what has been done before and this will influence what we then do.

In the case of institutions such as SfAM, history can usefully inform the present so it is important that some memory of our origins and development are retained. This was the motivation of the SfAM Executive Committee when several years ago it commissioned Max Sussman, very ably assisted by Grahame Gould, David Post and Fred Skinner, to write a short history of the Society to mark its 75th anniversary. In doing this they spent many hours working through the archive of documents held by the Society bringing more order to them and ferreting out interesting details for the history volume published in 2006.

During a recent visit to the SfAM office, I took the opportunity to investigate some of the archives. Much of the material, related to Society business, might be considered a little dry, but I did notice a copy of a seminal letter from Robert Stenhouse Williams, dated August 1929, which reported on some preliminary soundings of interest in a meeting of dairy bacteriologists:

So many have expressed the desire for a meeting of dairy microbiologists next September that we have decided to call that meeting and perhaps we may then consider what further steps shall be taken to organize such conferences at regular intervals.

Thus great oaks from little acorns grow.

What particularly attracted my attention though were the laboratory notebooks of the late W. H. (Bill) Pierce. Bill Pierce was Chief Bacteriologist of Oxo Ltd. and a long-time member of the Society. The books cover a period from 1943 to 1962 and illustrate the wide range of activities he was engaged in over that period. The folders with their neat typewritten entries and hand-drawn graphs help dispel the common misconception, held among students particularly, that working in an industrial setting can be rather dull and repetitive when compared with the apparently adrenalin-pumping world of research. Certainly sometimes the materials worked on may seem a little prosaic, corned beef for example, but the records of work investigating microbiological methods, customer complaints, problems with products and processes, development of new products, assessment of new analytical equipment, indicate wide and varied activity demanding a rigorous scientific approach throughout. What struck me was how much Bill Pierce must have known and how much he must have learnt during his career.

W. H. (Bill) Pierce

In 1984 the directors of Oxoid decided to commemorate his life and works and instituted SfAM's W H Pierce Prize. This prestigious prize is awarded each year at the annual Summer Conference to the young microbiologist (in these days of improved healthcare and cosmetic surgery, young is taken to be under 40) who has made a substantial contribution to the science of applied microbiology. If you look back at the list of previous winners, there are numerous examples of scientists who have continued to excel and are now at the very top of our profession. If you know of any likely candidate or if, in less humble moments, you consider yourself a potential candidate then please think about nominating them or persuading someone to nominate you. Full details are available on the SfAM website and on page 37.

In the archive I also found a copy of a songbook that had circulated at conferences in the 1970s. Younger members of the Society may be surprised at the enthusiasm of their forbears for a good sing-song, but this cannot be doubted when you leaf through the typewritten lyrics of no less than 86 songs! Each President hopes to create some kind of legacy for the Society but rest assured reintroduction of the sing-a-long will not be mine. I speak as someone who was once persuaded, very reluctantly, to sing a "British song" at the end of a soiree in Finland during which some alcohol had been taken. For reasons that will be apparent to those that know me, I sang that beautiful air "I'm forever blowing bubbles" after which my host complimented me, but went on to observe that this was quite an easy song to sing since it appeared to consist of only one note.

Martin Adams
President of the Society

ceo's column

Philip Wheat reports on the latest developments within the Society

Thank you to all the members who took the time to complete the recent membership questionnaire. In total we had over 600 responses and there is much useful information which will be very helpful when considering future initiatives. There were a number of non-UK members who commented that they would very much like to attend the Society's meetings but they could not because of lack of funding. I would like to remind all members that we offer grants to enable members to attend scientific conferences.

If a member (UK or non-UK) would like to attend the Summer Conference this year for instance (1–4 July), they could apply for a **President's Fund** to enable them to attend. The total monetary value is up to a maximum of £1200 (approximately US$1900). After the registration costs (which include most meals and three nights' accommodation) are deducted this would still leave over £900 for travel and other subsistence expenses. Full details of all grants (including The President's Fund) can be found by visiting http://www.sfam.org.uk/en/grants--awards/index.cfm. One of the main criteria for a President's Fund application being successful is that you must be contributing to the meeting in some way (e.g. speaking, as chairperson or presenting a poster). Although Student Members are also entitled to apply for the President's Fund, in the case of the Society's Summer Conference a **Conference Studentship** is more appropriate (full details can be found here: http://www.sfam.org.uk/en/grants--awards/conference-studentship.cfm). There is an upper limit to travel costs but all other appropriate expenses will be covered.

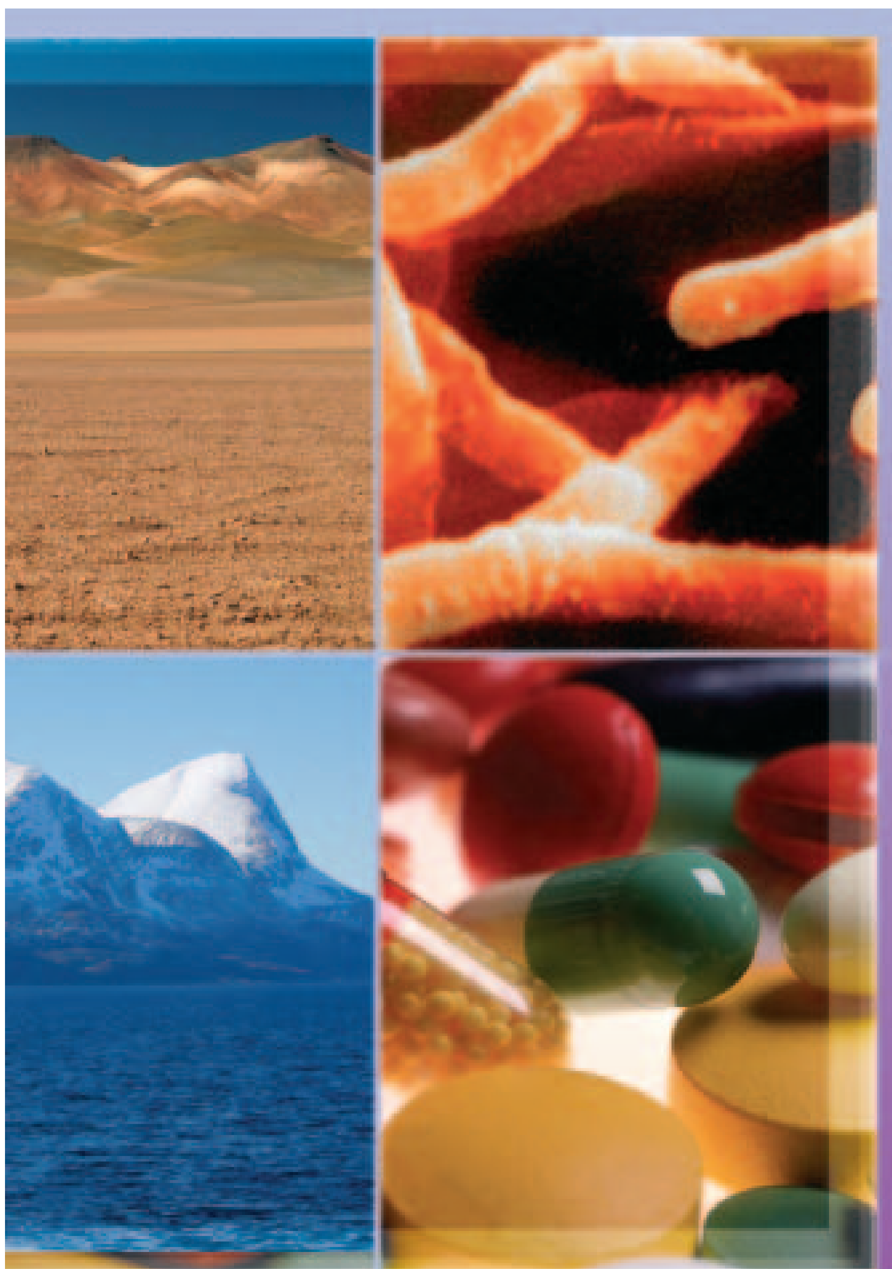
In this issue you will find full details of the forthcoming S*f*AM Spring Meeting (page 31). The title of this year's meeting is "***Sexually Transmitted Infections in the 21st Century***". The meeting is primarily aimed at Biomedical Scientists (BMS) who are working in clinical microbiology laboratories. The Society has a burgeoning number of BMS members who have decided that we offer significant benefits. One such benefit is eligibility to apply for a **Scientific Meeting Grant**. This grant is different from a President's Fund (discussed above) in that the applicant does not have to be contributing to a scientific meeting, attending as a delegate is enough. Whilst the maximum amount (£300) available is much less than a President's Fund it should be enough to enable BMS members (and any other members) to attend the Spring Meeting without incurring any personal expense. Provided

you have paid two subscription fees and there is a relevant applied microbiology meeting you wish to attend, why not apply online for a **Scientific Meeting Grant** (http://www.sfam.org.uk/en/grants--awards/scientific-meeting-attend-grant.cfm)?

Finally, whilst still on the subject of grants, I am pleased to announce that by the time you read this, we will have launched a new grant. It is entitled the **Student Professional Placement Grant**. The purpose of the grant is to allow joint applications from at least two Full Members of the Society, working in the same institution, for funding to help undergraduate students receive relevant practical work experience in applied microbiology. Members who work in commercial institutions are not eligible and neither are applications from the same institution as the students' place of study. The placement period should be for no less than four months and no longer than 12 months. The maximum funding is £2000 per student and the total award is for up to £20000 per institution. There is a deadline for applications which must be received by the 30 June 2013. Further details can be obtained from julie@sfam.org.uk or by visiting http://www.sfam.org.uk/en/grants--awards/index.cfm.

In your December 2012 issue of the *Microbiologist* we enclosed a S*f*AM branded spectacle/computer screen/tablet/smartphone cloth, which hopefully you have found useful. In addition, we also enclosed a copy of the new promotional/marketing booklet. If any member would like to receive further copies for advertising in their own institution or distribution at any meetings they may be attending please contact julieb@sfam.org.uk who will arrange for further copies to be sent to you.

Philip Wheat
Chief Executive Officer



Actinobacterial diversity as a source of new drugs

New drugs, especially antibiotics, are needed to control the spread of antibiotic-resistant pathogens and to treat life-threatening diseases such as cancer (Goodfellow & Fiedler, 2010). The challenges posed by drug-resistant pathogenic bacteria are especially acute, as exemplified by rising levels of infection caused by vancomycin-resistant *Enterococcus faecium*, fluoroquinoline-resistant *Pseudomonas aeruginosa* and meticillin-resistant *Staphylococcus aureus*, as well as the recent reports of panantibiotic-resistant infections attributed to *Acinetobacter* species and carbopenem-resistant species of *Klebsiella* (Donadio *et al.*, 2010). However, it is somewhat paradoxical that as the incidence of drug resistance grows the number of newly approved drugs is in sharp decline.

The most promising source of new drugs remains natural products, partly because alternative strategies such as combinatorial chemistry and fragment-based drug design have yet to make an impact. The selection of prokaryotes for search and discovery programmes can be seen as formidable given the diversity of culturable prokaryotes (Goodfellow *et al.*, 2012a), which in turn represent but a tiny fraction of uncultivated prokaryotes in natural habitats (Stach & Bull, 2005). However, amongst prokaryotes, members of the phylum Actinobacteria, notably the genus *Streptomyces*, have an unrivalled capacity to synthesize novel natural products with a wide range of activities (Bérdy, 2005). Actinobacteria account for nearly half of all microbial bioactive chemicals with nearly 80% of them produced by *Streptomyces* strains; despite this amazing creativity it is estimated that only about 10% of the total number of natural products that can be synthesized by streptomycetes have been discovered (Watve *et al.*, 2001).

The current resurgence of interest in Actinobacteria as a potential source of new chemical entities can be attributed to the discovery that their whole-genome sequences, unlike those of nearly all other prokaryotes, contain 20 or more natural product gene clusters for the synthesis of known or predicted secondary metabolites (Goodfellow & Fiedler, 2010). It has become difficult to find new bioactive compounds from common Actinobacteria as examining

"old friends" leads to the costly rediscovery of known compounds. However, it is becoming increasingly apparent that the discovery of new natural products is enhanced when novel and rare Actinobacteria from new or neglected habitats are examined in existing and new screening assays (Figure 1). It is essential in search and discovery programmes to foster these two aspects of novelty.

The essence of culture-dependent bioprospecting strategies is outlined in Figure 2. The microbiological steps rest upon a judicious choice of selective isolation procedures, the recognition of unusual Actinobacteria and subsequent screening of representative novel isolates. The next steps include the detection of bioactive compounds in dereplicated strain libraries using appropriate growth conditions, primary screening of fermentation broths and mycelial extracts using chemical procedures, such as HPLC-diode array screening, followed by detection of putative novel metabolites and structural chemical elucidation of active principles.

Figure 1. *Twin-tracked approach to drug discovery*

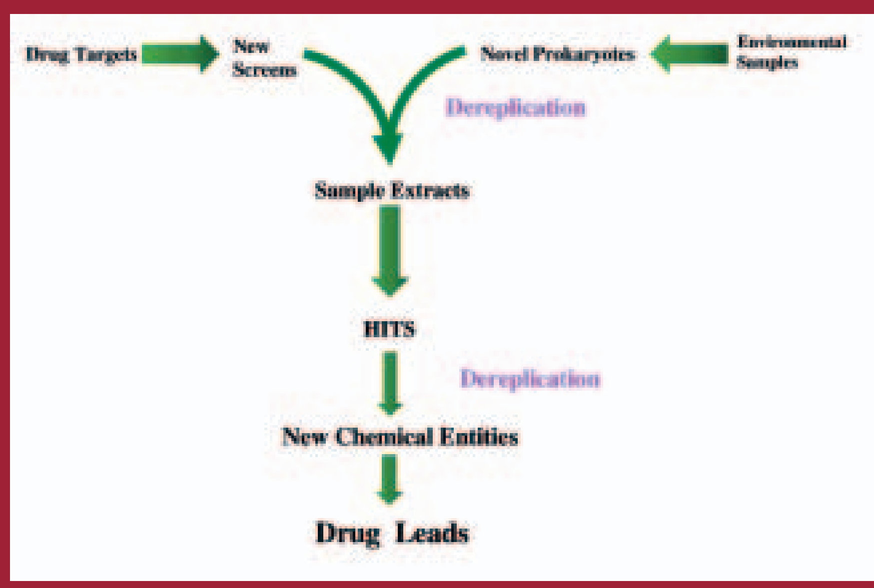
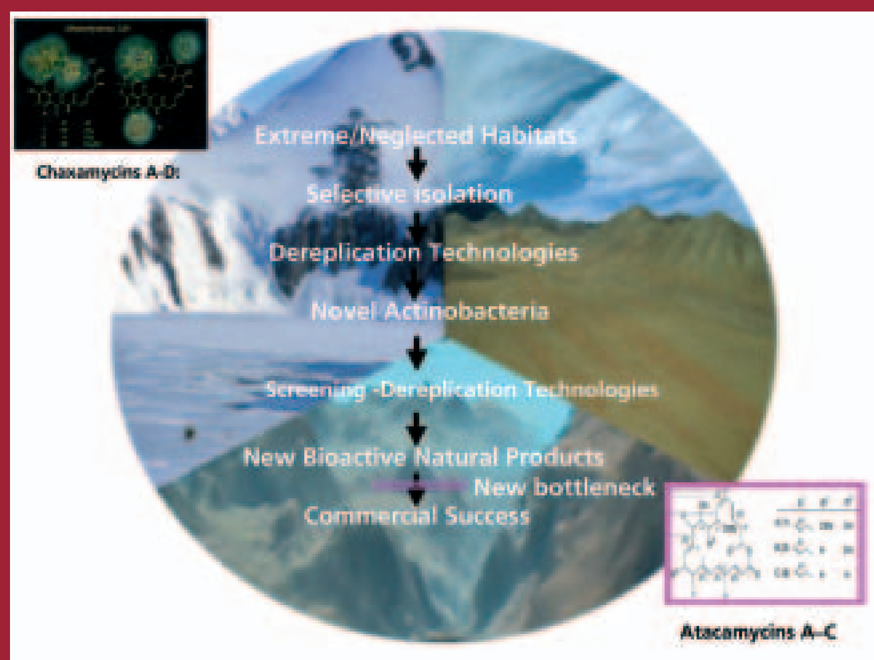


Figure 2. *Culture-dependent bioprospecting strategy*



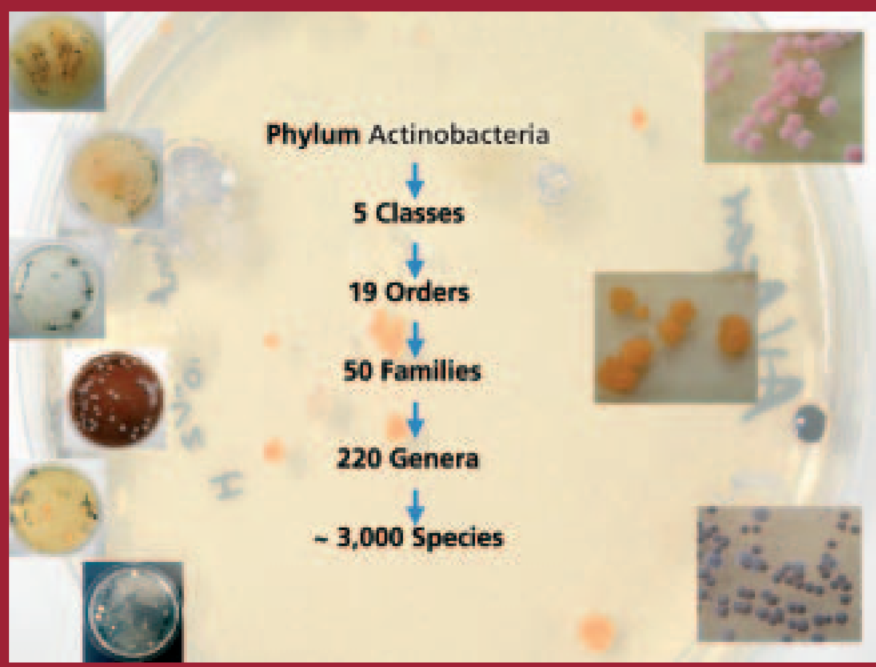
The initial steps in small-scale culture-dependent procedures rest heavily on actinobacterial systematics given the importance of selecting rare and novel isolates for screening programmes. Strain selection has been facilitated by marked improvements in the classification of prokaryotes due to the application of polyphasic taxonomy based on chemotaxonomic, molecular genetics and numerical taxonomic procedures (Goodfellow *et al.*, 2012a). It is apparent from such studies that the taxonomic status of the phylum Actinobacteria is underpinned by analysis of 16S and 23S rRNA genes, the presence of conserved insertion and deletions (indels) in certain proteins, and characteristic gene arrangements.

The current hierarchical classification of the phylum Actinobacteria is outlined in Figure 3. The phylogenetic relationships of taxa above the genus level are based solely on 16S rRNA signatures (Goodfellow *et al.*, 2012a). In contrast, classification at generic and species ranks also takes into account the discontinuous distribution of chemotaxonomic, morphological and physiological properties, together with complementary molecular genetic data. The present classification of Actinobacteria is a marked improvement on earlier taxonomies and provides a sound framework for the choice of high-quality isolates for pharmacological screens. However, the main source of novel bioactive compounds are filamentous Actinobacteria from the class Actinobacteria, a taxon which accommodates 15 orders, 43 families and 203 genera (Goodfellow *et al.*, 2012a). It should be noted that the term Actinobacteria embraces all members of the phylum.

The traditional view that Actinobacteria are soil and freshwater organisms has been overturned by evidence which shows that they are present in all habitats which support life forms. The chances of finding novel bioactive compounds, notably antibiotics, from well-known soil Actinobacteria is low, hence the upsurge of interest in isolating and recognizing rare and novel Actinobacteria from diverse habitats (Tiwari & Gupta, 2012a). The numbers and kinds of Actinobacteria in natural ecosystems is influenced by key ecological factors, such as aeration, pH, temperature, salinity and availability of organic matter

and moisture. Consequently, success in isolating interesting new Actinobacteria is dependent on the nature of environmental samples, as illustrated by the presence of high numbers of novel acidophilic and acidotolerant Actinobacteria in acidic soil and litter. The principles and practices used to isolate uncommon and previously uncultured Actinobacteria have been the subject of recent comprehensive reviews (Goodfellow, 2010; Tiwari & Gupta, 2012b). Most selective isolation regimes involve the extraction of actinobacterial spores and mycelial fragments from environmental samples, pretreatment of samples, choice of selective media and incubation conditions, and colony selection. It is important to use several selective isolation procedures when drawing up inventories of actinobacterial taxa in neglected habitats as different methods favour the isolation of specific fractions of actinobacterial communities.

Figure 3. *Hierarchic classification of the phylum Actinobacteria*



New drugs from Actinobacteria isolated from extreme habitats

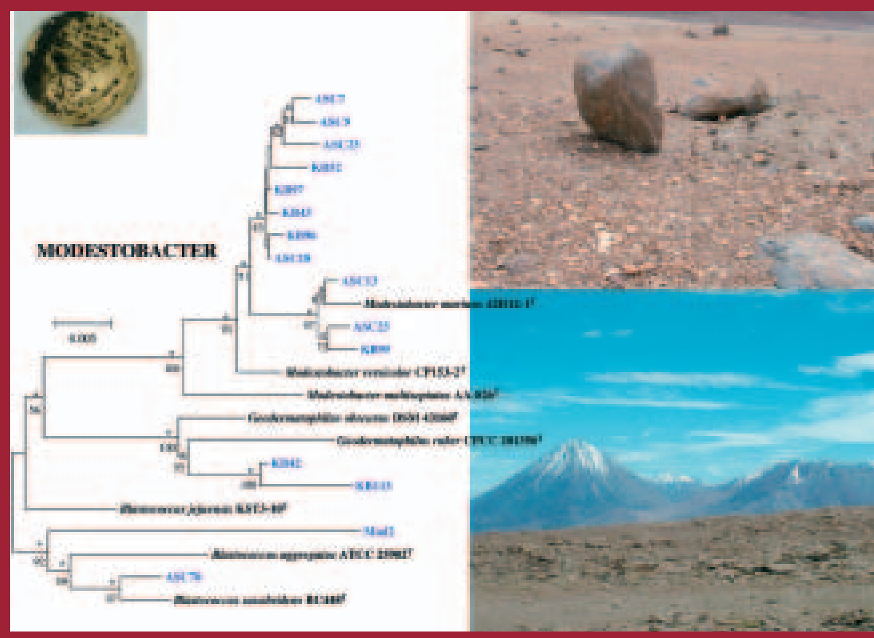
Over the past two to three decades the search for new natural products that can be developed as resources for healthcare has steadily shifted towards the isolation of Actinobacteria from extreme ecosystems, notably marine habitats, on the premise that extreme conditions give rise to unique biodiversity which is the basis of novel chemistry (Bull & Stach, 2007; Bull, 2011). The prevailing view that Actinobacteria from marine sources were restricted to the genera *Micromonospora*, *Rhodococcus* and *Streptomyces* was soon dispelled, as culture-independent surveys revealed the presence of previously unimaginable actinobacterial diversity in marine habitats, as illustrated by a report of over 1,000 novel actinobacterial taxa based on the application of species richness estimators to microbial diversity data (Stach & Bull, 2005).

It also soon became apparent that effective isolation procedures were needed to ensure that representatives of the small actinobacterial populations present in marine habitats were recovered on isolation plates. A multiplicity of selective isolation procedures have been used to good effect, these include treating environmental samples with UV light and extra-high frequency radiation, application of the dispersion and differential centrifugation technique, the employment of highly diluted isolation media and polymeric growth substances, and selection based on the use of actinophages; other worthwhile approaches include dilution to extinction, microencapsulation and virus-dependent cultivation (for further information see Goodfellow, 2010; Tiwari & Gupta, 2012b; Zotchev, 2012). To date, members of over 50 actinobacterial genera have been isolated from marine sources (Goodfellow & Fiedler, 2010) though few of them are indigenous to the marine ecosystem (Zotchev, 2012).

As anticipated, novel Actinobacteria from marine habitats are a rewarding source of biologically active molecules, as exemplified in Table 1. Some of the marine actinobacterially-derived chemical entities have unique structural features that are rarely, if ever, found amongst compounds synthesized by Actinobacteria from terrestrial sources (Fiedler *et al.*, 2005; Zotchev, 2012). It is interesting, but not surprising, that most of the natural products from marine Actinobacteria are synthesized by streptomycetes as these organisms are easier to culture and to induce synthesis of secondary metabolites compared with other Actinobacteria isolated from marine habitats. This certainly proved to be the case with *Streptomyces* sp. NTK 937 which was recovered from an Atlantic Ocean sediment core at the southern edge of the Canary Basin and found to produce a new benzoxazole antibiotic, carboxamycin, which showed inhibitory activity against Gram-positive bacteria, several tumour cell lines and the enzyme phosphodiesterase (Goodfellow & Fiedler, 2010).

Marine Actinobacteria belonging to the genera *Micromonospora*, *Salinispora* and *Verrucosispora* of the family *Micromonosporaceae* are prolific sources of new bioactive agents (Jensen, 2010; Goodfellow *et al.*, 2012b; Tiwari & Gupta, 2012a). Some, like diazepinomicin, an anticancer agent produced by a *Micromonospora* strain, have unique modes of action (see Zotcher, 2012). *Verrucosispora* strains are the focus of considerable interest following the discovery that *Verrucosispora maris* synthesizes a unique family of polycyclic polyketide antibiotics, one of which, abyssomicin C is the first known inhibitor of *para*-aminobenzoic acid synthesis, a primary broad spectrum antibiotic target that is not present in humans (Jensen, 2010; Freel *et al.*, 2012; Goodfellow *et al.*, 2012b). *Salinispora* is an obligate marine genus which shows a pan-tropical distribution in near-shore marine sediments and is an interesting model to study bacterial biogeography

Figure 4. *Neighbour-joining 16S rRNA gene tree highlighting presumptively novel Modestobacter species isolated from Atacama Desert soils*



and species-concepts (Jensen, 2010; Freel *et al.*, 2012). *Salinispora arenicola* and *Salinispora tropica*, the founder members of the genus, have a large fraction of their genomes devoted to the biosynthesis of structurally unique secondary metabolites, which are produced in species-specific patterns. *Salinispora arenicola* synthesizes compounds in the rifampicin and staurosporine classes and *S. tropica* compounds in the salinosporamide and sporalide classes, including salinosporamide A which is in clinical trials for the treatment of cancer. The proposed type strain of *Salinispora pacifica* (Ahmed, pers. com.) produces the secondary metabolite cyanosporoside A which, to date, has not been detected in the other two *Salinispora* species.

Another fascinating, but underexplored, biome that has only recently attracted the attention of microbe hunters searching for potentially useful Actinobacteria is the temperate Atacama Desert in northern Chile. This is the world's most continuously arid desert and is also of interest to astrobiologists as the prevailing environmental conditions are considered to provide an accurate analogue of those prevailing in Martian soils. Most Atacama Desert soils are hyper-arid, that is, the ratio of mean annual rainfall to mean annual evaporation is <0.05, the corresponding ratio for the extreme hyper-arid soils of the Yungay region is <0.002. The harshness of these conditions is compounded by high UV radiation, very low concentrations of organic carbon and, in areas such as the Salor de Atacama, by high salinity.

The most comprehensive investigation of culturable Actinobacteria from Atacama Desert soils is that of Okoro *et al.* (2009) who isolated significant numbers of Actinobacteria from arid, hyper-arid and extreme hyper-arid soils using several selective isolation procedures. Genotypic and phenotypic characterization studies showed that most of the isolates were putatively novel *Streptomyces* species, the remaining isolates formed new centres of taxonomic variation within the rare genera *Amycolatopis* and *Lechevalieria*. To date, additional studies have shown that three of the putatively novel streptomycetes do

Table 1. Selected biologically active products of potential value produced by novel actinobacteria isolated from extreme habitats

Isolate	Source	Compound	Activity
Dermacoccus abyssi	Sediment, Mariana Trench	Dermacozines	Cytotoxic, radical scavenging
Micromonospora sp.	Marine ascidian	Diazepinomicin	Anticancer
Micromonospora sp.	Sediment (off the Mozambique coast)	Thiocoraline	Anticancer
Nocardiopsis sp.	Fjord sediment, Norway	Thiazolyl peptide	Antibacterial (inhibition of protein synthesis)
Salinispora arenicola	Sediment (off coast of Guam)	Saliniketals	Anticancer
Salinispora arenicola	Sediment (off coast of Palau)	Salinipyrones	Moderate inhibition of interleukin-5 production
Salinispora arenicola	Deep-sea sediment	Salinosporamide A	Anticancer (proteome inhibitor)
Streptomyces sp.	Arid Atacama Desert soil, Chile	Atacamycins	Antitumour
Streptomyces sp.	Hyper-arid Atacama Desert soil, Chile	Chaxamycins	Antibacterial, antitumour
Streptomyces sp.	Sediment, Canary Basin	Carboxamycin	Antibacterial, cytotoxic
Streptomyces sp.	Sediment (off Californian coast)	Marinopyrrols	Antibacterial, antitoxic
Streptomyces sp.	Surface of jellyfish, Florida Keys	Salinamides	Anti-inflammatory
Verrucosispora fiedleri	Sediment, Raune fjord, Norway	Proximicins	Cytotoxic
Verrucosispora maris	Sediment, Sea of Japan	*atrop*-Abyssomicin C	Antibacterial (inhibition of *para*-aminobenzoic acid pathway)

Data drawn from Abdel-Mageeb *et al.*, (2010), Goodfellow & Fiedler (2010), Goodfellow *et al.*, (2012b), Santhanam *et al.*, (2012) and Zotchev (2012)

belong to new species, namely *Streptomyces atacamensis*, *Streptomyces bullii* and *Streptomyces deserti*, while two other Streptomyces strains were found to synthesize new ansamycin and 22 membered macrolactone antibiotics (Santhanam *et al.*, 2012). Another putatively novel *Streptomyces* strain, isolated from the Chilean highlands of the Atacama Desert, produces novel aminobenzoquinones, the abenquines, which inhibit bacteria and dermatophilic fungi (Schulze *et al.*, 2011). Extensions of the pioneering work of Okoro and her colleagues have revealed further extensive actinobacterial diversity in Atacama Desert soils, notably within the genus *Modestobacter*, a member of the family *Geodermatophilaceae* (Figure 4).

Conclusions

Diverse Actinobacteria, notably streptomycetes, can be isolated from extreme ecosystems using carefully chosen combinations of selective isolation methods. Preliminary analyses of resultant dereplicated groups of isolate show that many of them form new centres of taxonomic variation. Strains taken to represent such taxa are proving to be a rich source of potentially useful bioactive compounds, notably ones with unique modes of action against panels of drug-resistant pathogenic microorganisms. Strong support for such culture-dependent bioprospecting strategies comes from studies, notably on the genus *Salinispora*, which provide strong evidence of a coupling between taxonomic and chemical diversity. This taxonomic approach to drug discovery should be especially effective with respect to novel Actinobacteria derived from poorly studied extreme environments where habitat-specific challenges promote the synthesis of new secondary metabolites.

However, innovative selective isolation methods and rapid, but effective, taxonomic procedures are needed to isolate, recognize and cultivate the plethora of diverse novel Actinobacteria detected in desert and marine ecosystems by the application of culture-independent surveys. In short, novel Actinobacteria are still the prokaryotes of choice when panning for chemical gold!

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Lactic acid bacteria in cheese and salt reduction

Cheese production involves ripening and maturation stages that are characterized by the growth of complex bacterial and fungal communities. These microorganisms are either added by the producer as starter cultures or they naturally contaminate the cheese, and comprise its secondary microflora. This secondary microflora originates in the production environment, equipment and raw materials, and is made up of a diversity of bacterial species and fungi that succeed to become the dominant species and strains during the ripening and maturation of the cheese. These organisms enhance proteolysis, lipolysis and the production of cheese flavour. Although the secondary microflora is not controlled by the producer, the physico-chemical environment in the cheese and the external environment favour the development of particular microbial species in each cheese variety and/or dairy.

Lactic acid bacteria (LAB; mainly lactococci, lactobacilli, streptococci) are by far the most commonly used starter cultures in cheese production and together with non-starter LAB (NSLAB) form a major part of the microflora during ripening and maturation. For example, in blue cheeses starter LAB (mainly *Lactococcus lactis*) are added together with *Penicillium roqueforti* spores in order to promote the acidification of the curd. But the ripening and maturation is promoted by the development of a complex secondary microbial community composed of yeasts and NSLAB, mainly *Lactobacillus* species (*Lact. plantarum*, *Lact. brevis* and *Lact. curvatus*) (Figure 1). This change in flora is in part driven by the stage at which salt is added, as *Lactobacillus* is more salt tolerant than *Lactococcus*. Although *Lactobacillus* eventually dominate the LAB communities, the profiles and diversity of *Lactobacillus* species seems to be related to the cheese quality as well as differentiating between the profiles associated with individual dairies. In Stilton, the homofermentative *Lact. plantarum* has been associated with good quality blue cheeses where it was present alone or at least dominated the *Lactobacillus* species. In contrast, the heterofermentative *Lact. brevis* was associated with cheeses of poor quality. Whenever *Lact. brevis* was co-present

Figure 1. *Blue cheeses at (i) 24 hours (development of starter LAB) and (ii) 6 weeks — (development of NSLAB and secondary microflora dominated by fungi and lactobacillus spp.)*

with *Lact. plantarum* in good quality cheeses, they were accompanied by other homofermentative lactobacilli (*Lact. curvatus*, *Lact. casei*) demonstrating the fine equilibrium of NSLAB that develops during ripening and maturation (Whitley, 2002).

Modern trends in food production demand the reduction of salt in food because of the association of high sodium intake with negative health effects, including hypertension. Salt reduction is not a new issue to the food industry. The World Health Organization called for lowering sodium levels in foods worldwide in 2007. This was adopted quickly in the production of ready meals, bread and meat. Interestingly, in the last decade its application was expanded to fermented products, and the cheese industry has been researching ways to reduce sodium. There is increasing pressure for Cheddar cheese manufacturers to reduce salt as the typical level of NaCl in Cheddar cheese is around 2.0% (w/w) (Rulikowska *et al.*, 2013); some blue cheese varieties contain up to 3.5% making salt reduction even more challenging.

Salt serves several purposes in cheese: it has a direct impact on the control of the metabolism and survival of the starter bacteria, as well as influencing the development of the secondary microflora during the ripening period. It also inhibits the survival of pathogenic bacteria. Also, salt indirectly influences the development of LAB in the cheese matrix through its impact on syneresis, control of final moisture and texture of the cheeses (Johnson *et al.*, 2009). NaCl controls microbial growth by interfering with the utilization of substrates, ceasing cellular function (Harper & Getty, 2012). Reducing salt in cheese can affect the LAB and NSLAB equilibrium and, as an extension, the quality and safety of the cheese.

During ripening, the populations of NSLAB increase, overtaking the starter LAB that die off. This is important in order for the secondary microflora to grow and promote the intensification and acceleration of flavour development, as well as imparting desirable flavour notes to the cheese. Their interactions and biochemical activity is associated with the products' flavour, quality and sensory properties (Addis *et al.*, 2001). Interestingly, reducing salt in cheeses allows the starter LAB to remain at high populations for longer, competing with the NSLAB. Indeed, Cheddar cheeses with low levels of NaCl develop high initial populations of starter LAB and NSLAB and these remain higher and survive significantly longer than in cheeses with high salt concentrations, resulting in less flavour formation and greater bitterness (Johnson *et al.*, 2009; Rulikowska *et al.*, 2013). Salt levels therefore affect the rate and degree of sugar fermentation by LAB in cheese and consequently the levels of acid present during ripening and maturation, affecting proteolysis and the development of the secondary microflora. Proteolysis is required for flavour development; however, excessive (or insufficient) proteolysis can lead to flavour and texture defects (Johnson *et al.*, 2009). When salt is reduced, bitterness and an unpleasant aftertaste are further promoted non-microbiologically because of the effect of salt on the sensory profiles of the cheeses.

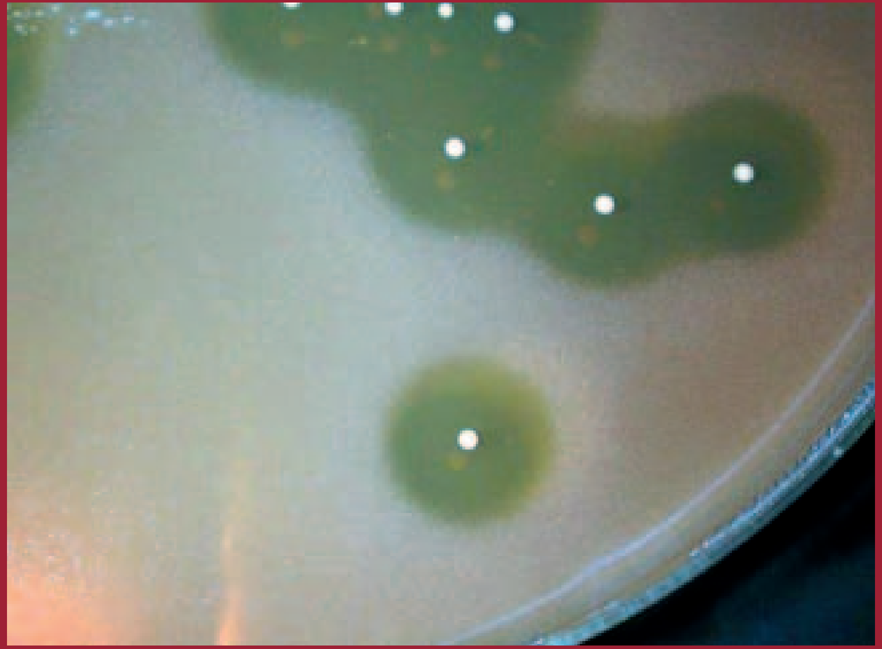
The impact of less salt on the safety of cheese is also important to understand. Sodium reduction in cheese can be achieved by simply lowering the NaCl content. The reduction of NaCl in cheese would not inhibit the growth of

foodborne pathogens such as *Listeria monocytogenes* (Ribeiro *et al.*, 2006). Also, sodium reduction is achieved by substituting NaCl with other salts with similar taste. Potassium chloride (KCl) is the most popular substitute. There are indications that the growth of *Listeria* in food is not affected by the type of salt (Harper & Getty, 2012). However, some LAB inhibit the growth of undesirable bacteria such as *Listeria* due to the production of a combination of organic acids, hydrogen peroxide, carbon dioxide, acetaldehyde, diacetyl or bacteriocins. Therefore, the effect of salt on the starter LAB and NSLAB could result in microbial equilibria that change the balance of these compounds and consequently allow the growth of pathogens in the cheese.

One potential solution is the use of anti-*Listeria* bacteriocin-producing NSLAB strains as adjunct cultures in order to control the growth of *Listeria* spp. in low salt cheeses. In the past their use has been offered as an option for increased product safety. The most popular NSLAB adjuncts are lactobacilli, however, other LAB species present interesting antimicrobial activities. For example, *Enterococcus* spp. are able to produce bacteriocins which inhibit *L. monocytogenes* and are naturally present in milk and cheeses (Chanos & Williams, 2011). Also, pediococci have been widely used as starters in food preservation and include strains presenting strong anti-*Listeria* activity (Olaoye & Dodd, 2010) (Figure 2). Additionally, the use of NSLAB adjunct cultures can promote increased secondary proteolysis. This balances the bitterness (Johnson *et al.*, 2009) that results from extensive primary proteolysis because of bacterial overgrowth due to salt reduction.

The reduction of salt in cheeses, although recognized as having potential health benefits, could affect the quality and safety of cheeses. These effects come about through the effect of salt on starter LAB and NSLAB populations and interactions in the cheese microenvironment influencing the composition and the metabolism of the microbiota. Information is needed to optimize production in a way that inhibits and promotes the growth of undesirable and desirable species respectively. The isolation and use of NSLAB as adjunct cultures could be key as levels of salt in cheese decrease.

Figure 2. *Antagonistic activities of P. acidilactici NCIMB 700993 against L. monocytogenes. Live broth cultures of the former were spread inoculated on agar plates previously seeded with cells of the latter*



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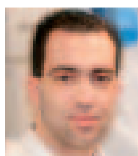

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Professor **Glenn Gibson** is Professor of Food Microbiology and Head of the Food Microbial Sciences Unit at the University of Reading. His research area can be described as gut microbiology, but his more specific interests include pro/prebiotic mechanisms, acute and chronic gut disease, autism, obesity, novel prebiotics and human metabonomics. Specific projects on pro/prebiotics, the molecular genotyping of gut bacteria, H_2S production, gastroenteritis in sportspersons, gut flora development with age and colonic homeostasis are being carried out.

Q&A: Probiotics, prebiotics and bifidobacteria

Q That's quite a list, Professor Gibson, can you tell us about the one thing you're most excited about working on at the moment?

A It's actually not that much — basically I work on human gut bacteriology and the use of diet to intervene as appropriate. That involves testing probiotics and prebiotics using model systems of the human gut and ultimately in volunteer trials. In answer to your question about what's most exciting, I think it's exploring the metabolic capacity of gut bugs. There's a lot of research going on at the moment on sequencing and understanding the diversity and systematics of the gut microbiome. But, I think the next big challenges are to understand functionally what the gut microbiome offers and how this metabolism can be altered, to improve health. Now we have the opportunity to look at how changing the gut microbiome and resulting metabonome can affect clinical states which can't currently be very well managed.

Q Which clinical states are you referring to?

A Well, something like irritable bowel syndrome (IBS) for instance which is said to affect 20% of people living in the West, and if that's anywhere near true it's a massive medical and economic concern. There are very few, if any, decent ways of dealing with this condition which is characterized by unpredictable bouts of diarrhoea, constipation or sometimes alternating between the two.

At the moment we think that there's a link between IBS and an imbalance or inconsistency in the gut flora, and if these imbalances can be reset, we can see some improvement in the condition. Some of our recent research, as well as results from another group in Cork have shown this to be the case. More chronic conditions like inflammatory bowel diseases and obesity are also being looked at. Then there is protection from acute gastroenteritis — something which we are all susceptible to.

Q We've all heard of probiotics and I have anecdotal evidence that they really help to settle the 'upset stomach' side effects of taking antibiotics. What else are probiotics good for?

A Well I think firstly it's fair to say it's not really an upset stomach it's more an upset large intestine. But yes, there are issues of distension or

discomfort often felt when taking antibiotics and this happens further down in the large intestine which as we know, is really where most of the bacteria in the human body reside. So the research into understanding the metabolism of the microbiome is very much directed towards the large intestinal community at the moment.

Probiotics, if they work well, boost the beneficial components of the microbiome and obviously an orally ingested probiotic is a live microbial feed addition which sets out to do so. So, looking at the science, there are many research papers published now, I think you can find something like 9,000 on PubMed, around 1/10th of which are human studies. I guess traditionally probiotics were used to reduce the symptoms of gastroenteritis.

There's also been research showing a reduction in traveller's diarrhoea, as well as potentially more chronic ailments like ulcerative colitis (UC), and even some markers of bowel cancer have been suggested to be reduced using probiotics. And then outside of that, now there's a lot of interest in systemic effects and looking at cognitive problems like autism, and also the issue of the gut microbiome and its role in obesity and metabolic syndrome — that may well have implications for the use of probiotics as intervention tools.

Q You mentioned autism, is that because people with autism have different bowel metabolomes?

A You're right, especially in children: a lot of autistic kids have gut problems — they have a lot of distension difficulties, abdominal pain and discomfort. You may not think that something which is clearly a cognitive issue has an effect further down in the body, but there is a big link between the gut and systemic organs like the brain. For instance, if you feel nervous you have butterflies in your stomach, and similarly if you have something like constipation, you mentally don't feel great. But going back to autism, we became interested because, as I said, a lot of children in particular who have autistic spectrum disorders also suffer gastrointestinal difficulties. When we began to look at the microbiology, we found elevated levels of clostridia. It may well be that the clostridia are manufacturing toxins that at least partly explain the gut problems and also manufacture quite a lot of gas, which explains some of the distension difficulties.

So, as with all these gut issues, if you try to understand the culprit microbes responsible for the aetiology or maintenance of these factors, you can then begin to build interventions against them. We tested a probiotic intervention against clostridial carriage. Unfortunately it was only partly successful because we had a lot of drop-outs during this human study, though it's something we'd very much like to repeat in a slightly different manner.

Q I know there's been some discussion about the ability of probiotic bacteria to survive the acidic conditions of the stomach when taken orally, and that this results in a decrease in their effectiveness. Can you talk a bit about that?

A Inevitably I think when you ingest a probiotic they're in stomach acid for about half an hour if not longer than that. Next, they pass into the small intestine where we have bile salts and pancreatic enzymes to deal with. If they survive that, they've then got to compete with the resident large intestinal microflora, and so there are challenges to the long-term stability of probiotics after ingestion. But some of the best research strains are bred to have some inherent resistance to these challenges. I don't think there is ever 100% recovery — you will get some losses, but if a big enough dose is taken initially, then enough bacteria will get down to the large intestine to make some kind of difference.

Building on that, there are now products which use a protective coating to actually try and deliver the probiotic past the stomach and the small intestine in an intact form. So there are ways that you can protect during this passage, but it is certainly a challenging journey nonetheless.

Q So we've heard about probiotics, now can you tell us: what are prebiotics?

A Prebiotics are kind of a fertilizer for beneficial bacteria in the gut. So a prebiotic would target certain populations which are already there.

These are selectively fermented ingredients, usually carbohydrates, like certain oligosaccharides, and these boost the indigenous populations of bacteria that are said to be beneficial. A probiotic is introducing the bugs themselves, and a prebiotic uses the diet to pick out the positive elements of the gut flora to enhance their numbers and activities.

Q I assume the prebiotics are targeted at specific bacteria in the gut to encourage their growth?

A They are yes — they tend to target growth of bifidobacteria because there are quite a few bifidobacteria in the gut already, so it makes sense to try and elevate these bacteria as a target. Because of the long history of health and safe use of probiotics, it tends to be these strains of bacteria which are targeted by prebiotics. Having said that, one of the interesting avenues of the current diversity driven projects has begun to unravel other bacteria seen to be beneficial but which aren't traditional targets. By that I mean eubacteria, faecalilbacteria and roseburia. These offer certain health benefits or traits which traditional probiotics may not necessarily offer. So, new research in the pro- and prebiotic area may bring in these other genera which are safe and could offer certain additional benefits.

Q Can you give some examples of 'bifidogenic factors'?

A A bifidogenic factor is basically another name for a prebiotic which targets bifidobacteria, and if you look at the literature there are a lot that claim to do so. But for me, the ones which have been most reliably tested, and by that I mean human studies in different labs with reproducible results, are fructoligosaccharides, or FOS and galactoligosaccharides or GOS. These seem to produce significant effects on bifidobacteria within a few days of feeding and are very much the focus of the type of research that we do both in our gut models and in our human studies. To lengthen the list a little bit more there are other candidates like isomaltoligosaccharides, xylooligosaccharides, pectin derivatives, polydextrose, but I don't think the evidence for these oligosaccharides is as advanced as it is for FOS and GOS so far. That will change in the future.

The reason FOS and GOS are selective to bifidobacteria is because of their size, but also their structure. The enzymes which bifidobacteria have, are very adept at breaking open the bonds which link FOS and GOS together. And once that happens, they become very favourable and selective energy sources for the bacteria which respond by growing well.

Q What health benefits would targeting bifidobacteria confer?

A Bifidobacteria are very powerful inhibitors of pathogens, so you could argue that higher levels of bifidobacteria in the gut should reduce the carriage rate of transient or indigenous pathogens and we know the different mechanisms by which this can occur, for instance, acid production, competition for nutrients or excretion of antimicrobials. Importantly, bifidobacteria are seen to be harmless innocuous bacteria so if you boost their numbers, you should be able to tackle pathogenic traits in the gut and it doesn't really matter whether you're trying to ease an acute trait like gastroenteritis, or something more long term like IBS or UC.

You could make an analogy with food poisoning in that if there's an outbreak, some people suffer pretty badly, some people don't suffer at all and some people are moderately ill. I think one of the reasons for that comes down to different levels of bifidobacteria – the people who suffer the least probably have the highest levels and are then better able to deal with the pathogen. Some of our research in traveller's diarrhoea has shown that this is the case when people take a prebiotic and go to high-risk destinations — for gastroentiritis their risk of onset as well as duration of diarrhoea was markedly reduced.

Q What do you see as the most important direction that research into pro-/prebiotics can take?

A I think the whole area of metabonomics where you use NMR or mass spectrometry to analyse all the metabolites present in all biological specimens, such as blood, urine, faeces. This opens up really powerful information on what the gut microbiome is offering the host metabolically. We know we can change the composition of the gut microbiome with prebiotics so the next big challenge is to find out if we can change metabolism (and health status) as a result of that. For example, one of the big 21st century disorders is metabolic syndrome, which is linked to obesity, and research into pro- and prebiotic intervention in this direction should create some fascinating outputs. It's a harmless approach which could offer a lot of benefits to a lot of people. And then obviously there are the more traditional targets like gut difficulties. But, I think again in research we really need to understand a lot more about the mechanisms.

Trials where pro- and prebiotics are fed and some outcome is measured are probably gone. Now I think we need to measure those outcomes but we also need to understand mechanistically what's happening in-between the intervention and the outcome. I think science now offers the opportunity and the technologies to really understand the mechanisms of the effect that pro- and prebiotics have on human and animal health — there is massive potential.

Q Finally, what will you be doing after this interview?

A Well I'm speaking to you just before Christmas and every year the professors in our department at the University of Reading, and the management team, cook Christmas dinner for the whole department. So right after I've finished speaking to you, I'm going to go down to our food processing hall to join my colleagues and work out how we can stop 150 people getting food poisoning. Dinner will happen tomorrow so we'll begin preparations straight after this interview. Some people in the department claim this is the only time of year they see the professors do any real work!

Lucy Harper
*Sf*AM Communications Manager

Clare Doggett
*Sf*AM Communications Officer

bio**Focus**

Mark Downs talks teaching in higher education

www.societyofbiology.org

Teaching is almost always associated with schools and the education of 5–16 year olds. It is a skilled profession with dedicated training lasting up to four years with life-long learning requirements as the norm. Why then do we still fail to fully train, support and recognize more of those who teach beyond the 16 year barrier, especially in higher education (HE)? For example, being an excellent research scientist may well inform university teaching and help create a dynamic environment but it still doesn't guarantee a great learning experience for the student. Teaching well and inspiring students, matters at all levels of education, in all types of institutions, and certainly not just schools.

To be fair, many universities are placing far greater emphasis on teaching skills and training support for lecturers is growing. But there is still a long way to go, especially if research-led university funding continues to be allocated without full recognition of the teaching a researcher can offer. The good news is that there are many ways in which the bioscience sector can help support the university and further education teaching. The Society of Biology has been working hard to provide more support, stimulate debate and inform policy, greatly aided by HUBS (Heads of University Biosciences), which became a special interest group of the Society in 2011.

HUBS is open to heads of departments and subject leads or nominees, and aims to represent the particular challenge of managing biological and life science departments and units in UK HE. The group acts as a forum for discussing national issues on the provision of research and teaching in the biological sciences, and as a source of informed comment on the consultations of the day which affect HE institutions in their delivery of life science teaching and research. The most recent HUBS conference, held in November focused on HE and research policy. The meeting covered current topics in HE including the Research Excellence Framework, research funding, and open access to publicly funded research and it traditionally splits its two annual meetings between teaching and research. If you are involved in HE life science teaching and your university is not involved do think about getting involved and visit: www.societyofbiology.org/aboutus/special-interest-groups/hubs.

To better recognize the value of HE teaching the Society now runs the Higher Education Bioscience Teacher of the Year Award originally established by the HE Academy. By identifying the UK's leading bioscience teachers we provide an annual opportunity for bioscience academics to receive national recognition for their outstanding learning and teaching practices. The criteria look for evidence of the candidates design and development of teaching approaches, undertaking scholarly and professional developmental activities to enhance student learning, and influencing bioscience student learning beyond their own department and institution. It is also an opportunity to highlight and share best practice through the creation of teaching case studies.

The 2012 award winner, Dr Neil Morris presented his case study on '*Using technology to enhance the quality of the student experience*' at the HUBS spring learning and teaching focused meeting, as well as writing a guest blog post on '*Tablets, podcasts and text messaging in education.*' The 2013 shortlist has just been announced.

What HE teachers often lack, however, is not just recognition but resources to support them. The Society is trying to help with this and last summer received funding from the Higher Education Academy[1] and JISC[2] through their Open Education Resources (OER) Programme to identify, collect and promote UK OER to the bioscience community. We worked closely with HUBS throughout this project and recently launched a new HE teaching website at http://heteaching.societyofbiology.org to signpost these resources. We aimed to reduce the time spent by individuals searching the web, ensure better access to quality teaching resources, and help to introduce and encourage those who are new to OER in the bioscience community to use these resources. The website focuses on resources that support practical biology in HE and features lab and field work protocols, videos and animation, multimedia alternatives to wet labs and health and safety information.

Setting up this new website has been the start of this project for us, and we look forward to working with you all on this in the future. We will be adding new resources as they are released to keep the website up to date. If you are creating resources, or know of a great resource that we have missed, then please let us know via the 'submit resources' section of the site.

The Society has also been developing new training courses to support our membership, including a Workshop on Learning and Teaching in Higher Education. The course covers the role of research and experience in learning and teaching in the biosciences, session planning and preparation for effective learning, constructive alignment of outcomes and assessment, and opportunities to develop independent learners. All members of the Society's member organizations receive a 50% discount on these courses which we hope to repeat during 2013 to match demand. HUBS have more planned over the next year including producing a position statement on the value of teaching in HE, launching a platform for universities to advertise their lab space for other organizations to use for practical work, and releasing a database of external examiners.

Hopefully, together with the supportive work of other learned societies, HEIs themselves and many other interested parties, HE teaching will increasingly be better supported and valued.

■ [1]http://www.heacademy.ac.uk ■ [2]http://www.jisc.ac.uk

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

mediaWatch

microbiology and the media

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

Sense about Science

Standing up for Science Media Workshop November 2012

SfAM policy on the media

We will:

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

I was lucky enough to receive funding from SfAM to attend the Standing up for Science Media Workshop in Glasgow in November. The day began with Julia Wilson, the Development Manager of Sense about Science introducing the organization. Sense about Science is a charity that brings together over 500 scientists who want to ensure good science is represented in the media accompanied by supporting evidence. Voice of Young Science is a part of Sense about Science and is a network for early career scientists to provide a platform for young scientists to stand up for science.

The whole day was divided into three panel sessions interspersed with group work. During group work activities we were asked to discuss 'bad and good things' about the media and the contribution of social networks and public engagement.

The first session entitled '*Science and the media*' was a panel session with the panellists: Professor Sergio Della Sala, Dr Eleanor Gilroy from The James Hutton Institute and Professor Miles Padgett, Professor of Optics and Kelvin Chair Natural Philosophy from the University of Glasgow providing an overview of their experiences with the media. The session was chaired by Victoria Murphy, Events and Campaigns Officer for Sense about Science. Miles Padgett, Professor of Optics in the Department of Physics and Astronomy at the University of Glasgow, started the discussion by stating how important it is for scientists to appropriately communicate unbiased science and that opinions need to be separated from personal views bearing in mind the institution being represented. He also touched upon the subject of Scottish independence (as a scientist based at a Scottish University). Giving opinions on political subjects needs to be done carefully as once the information is passed on to the media it remains there forever. Dr Eleanor Gilroy, a geneticist

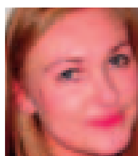
from The James Hutton Institute in Dundee strongly voiced her disapproval on the general negativity in the media regarding GM crops. Eleanor stressed the importance and necessity for changes to be made to the Scottish curriculum to ensure the younger generation feel more positive about GM crops. Sergio Della Sala, Professor of Human Cognitive Neuroscience at the University of Edinburgh, controversially stated that "we", the scientists, blame the "bad guys", journalists, for communicating "science badly" whilst scientists are the ones responsible for how the science is being communicated to the mass media. "*We are responsible to communicate real evidence to avoid misconception and miscommunication*", said Sergio. The panellists' opinions created a lively discussion between panellists and the audience which all came down to one conclusion — scientists are responsible for communicating the science clearly to avoid misconception and as a scientist it is your duty to speak up — if you won't someone else, potentially less qualified, will!

The second session — '*What journalists are looking for*?' — was led by journalists; Peter Ranscombe (*The Scotsman*, speaking in a personal capacity), Julie-Anne Barnes (*Scottish Daily Mail*) and Eleanor Bradford (Health Correspondent for *BBC Scotland*). Peter started the discussion trying to draw the audience's attention to the ordinary journalist's day filled with very stressful deadlines and short timeframes. Once a story is identified, it needs to be out within 24 hours as after that it no longer counts as 'news'. The urgency of gathering information and opinions over such a short period of time sometimes does not leave the journalists with the choice of trying to find the best expert on earth — they may have to settle for any expert. Hence, as a scientist if you refuse to comment on a story acting as an expert in your field and then you find the same story being inappropriately delivered to the public — do not complain, you had your chance. Journalists may end up seeking out 'any expert' which can potentially have serious consequences, so if you're approached do not refuse to give an opinion — as a scientist you have a duty to maintain the public's trust in scientists. Julie-Anne reminded the audience that journalists are the people who deliver the story that is wanted — "*the public loves scientific stories*". Sometimes the editorial restrictions of certain media are a source of misconception. Eleanor explained that a journalist's job is to translate science into an understandable format. Journalists are restricted to very tight deadlines, whereas the research world is used to a slower pace. This is one of the main reasons these two, so different, worlds clash. The following discussion was very lively with the audience

suggesting journalists often presented biased, cynical scientific stories. The journalists reminded us that it is not for journalists to judge what is right or wrong; however, it is their duty to present the story as accurately as possible. The truth is somewhere in the middle — both groups share the responsibility for communicating accurate scientific stories; but for this to happen these two groups need to start working closely together. We, as scientists, were urged to 'make friends' with journalists as it could prove beneficial for our careers.

The third session '*Standing up for science — the nuts and bolts*' provided a summary of earlier sessions and offered practical guidance for early career researchers on how to respond to bad science and how to manage journalists. The session was led by Ross Barker, Media Relations Officer for the University of Glasgow; Jaime Earnest from Voice of Young Science and Victoria Murphy. Panellists reminded us, as young scientists, of the importance of networking and developing communication skills as it is important for recognition and making sure science is accurately reported.

Lastly, Sense about Science invited us to join the Voice of Young Science network, which is made up of many interesting people from different backgrounds. The panellists and workshop sessions made the discussion lively and vibrant and left all of us with lots to think about. As young scientists we were urged to ask for the evidence behind anything that is being communicated to the media and to learn to recognize the value of scientific enquiry.



Agnieszka Piotrowska
PECS Communications Officer

Public engagement by the infectious disease research community — recent activity, barriers and is it worth it?

What is public engagement?

The term public engagement (PE) has been defined as a mutually beneficial interaction between members of the public and higher education and research institutes (National Co-ordinating Centre for Public Engagement, 2012). One common purpose of PE is to engage outside communities with work taking place in universities, NHS Trusts and public health agencies. Anecdotally, the prominence of PE seems to be increasing, with funders including the Biotechnology and Biological Sciences Research Council (BBSRC) placing expectations on universities to carry out an increasing amount of PE. There also seems to be significant enthusiasm amongst researchers to promote their own area of science to the lay public. The areas of infectious disease and microbiology cover a vast spectrum of knowledge which provides scope for a diverse range of exciting and informative PE activities.

Funding for PE is currently available from a wide variety of different sources. Professional bodies including the Wellcome Trust offer PE grants, as do the Societies of Applied Microbiology and General Microbiology. The Nuffield Foundation offer bursaries to encourage students to gain valuable experience working alongside researchers during the summer months. The National Co-ordinating Centre for Public Engagement (NCCPE) was founded in 2008 as part of the Beacons for Public Engagement Initiative, with the primary aim to support researchers develop their skills in engagement. The NCCPE receives its funding from the UK Higher Education Funding Councils, Research Councils UK and the Wellcome Trust, and provides a comprehensive list of funding opportunities for university staff and researchers with an interest in PE. To find out researchers' attitudes to PE in the area of infectious disease, we surveyed members of the Infectious Disease Research Network (IDRN) and members of the wider infectious disease research community.

Our survey

We collected data on researchers' views related to various aspects of PE by sending out two separate surveys to the members of the IDRN mailing list. The surveys were also forwarded through other contacts and colleagues. One survey was tailored to individuals who had previously carried out PE activity in the field of infectious disease or microbiology; the second survey targeted researchers who had never been involved in PE. Responses were anonymized. The surveys were mostly structured as multiple-choice questions to allow responses to be easily quantified; there were also free-text boxes to allow the opportunity for additional comments should individuals wish to expand on their responses. This provided valuable qualitative information.

The results

The survey which was sent to those who had carried out PE generated a total of 59 responses. The survey for those who had not undertaken PE produced 50 responses.

As shown in Figure 1, the most popular style of engagement, with a total of 18 responses, comprised of a talk or lecture at a public event or venue. Visits to schools and classrooms were also favoured, with 16 responses however, laboratory-based experiments were sparsely conducted with only three respondents opting to use this form of activity. This may correlate with our findings on the barriers to PE, as difficulties with health and safety requirements when trying to accommodate school students in working labs were flagged by some respondents as a barrier.

Figure 2 shows greater than half the PE activities conducted received no specific funding. Nevertheless 30% of respondents did manage to secure funding specifically to carry out their PE work. This funding was provided by a variety of sources, including the Department of Health, National Institute of Health Research and the Wellcome Trust. Universities were a key provider of funds, as were specialized societies including the Society of Applied Microbiology.

Figure 3 highlights the main barriers to PE from a researcher's perspective. A lack of time to plan and carry out the necessary work was mentioned as a significant barrier by researchers who had and had not carried out PE, with 47 and 23 responses respectively. Figure 4 illustrates whether researchers would be interested in taking part in PE activities in the future.

Figure 1. *Most popular style of engagement*

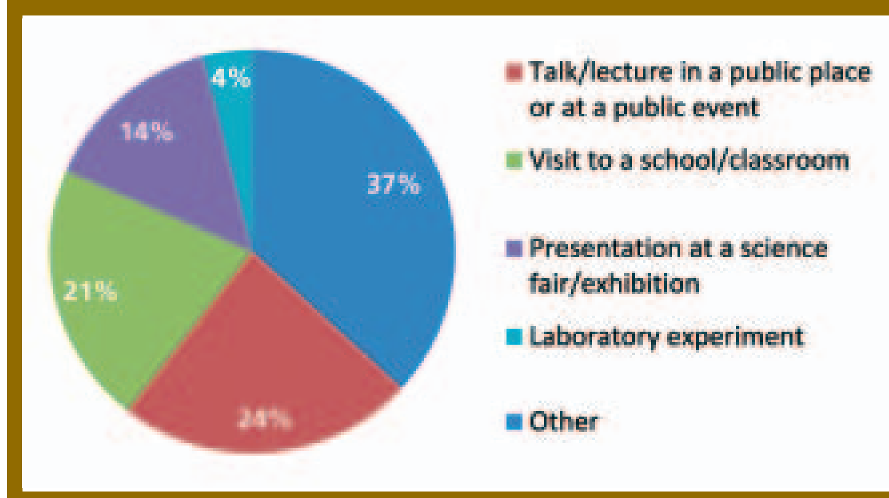
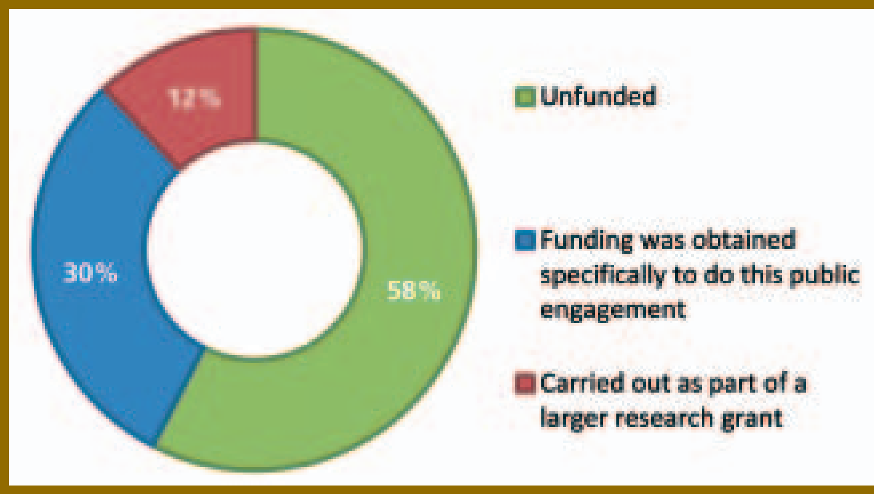


Figure 2. *Funding distribution*



Overall, feedback appeared encouraging with 95% (56) of respondents stating they enjoyed partaking in PE compared with 3% (2) who did not. Many received positive feedback from their audience and 90% suggested that the audience were receptive to their work.

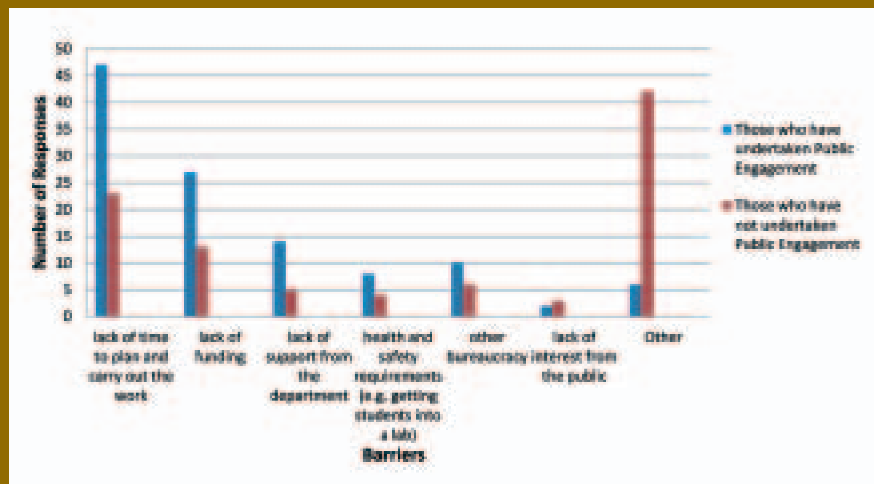
Interestingly, of those who participated in PE, 90%, as depicted in Figure 4, would definitely like to be involved in such activity again. The majority of researchers surveyed admitted to having previously undertaken PE on more than one occasion, thus indicating a continued interest in this area of work. Our survey revealed doubts amongst researchers over the extent to which PE enhances career prospects, as well as the level of recognition received for piloting such events.

Individuals not directly involved with PE did demonstrate awareness of the different funding sources available to them, if they were to engage with the public in the future, as shown in Figure 5, with several respondents demonstrating awareness of multiple sources of funding.

What can we learn?

Our results show common trends in the factors researchers consider barriers to engaging with the public. Thirty two responses, from those who have not undertaken PE stated uncertainty about how to get involved as a reason for lack of participation. Further comments revealed researchers had concerns over the suitability of their work in relation to PE activity. Whether or not they held the appropriate level of expertise to fulfil the role also appeared to be a common barrier.

Figure 3. *Main barriers to PE from a researcher's perspective*



A respondent emphasized this by commenting, "*I am unsure about how useful my expertise is and whether I would be a good ambassador for science*." The practicalities of public involvement, including recruiting staff or volunteers to the activity, gaining permission and finding the correct guidance were listed as additional reasons for not contributing.

Further comments revealed that obtaining appropriate permissions to carry out PE can be difficult. The fact that only a small amount of laboratory work was carried out may be explained through strict health and safety legislation and liability concerns. As a result, students can be limited to observational rather than interactive work.

Both surveys listed an identical hierarchy of barriers to taking part in PE. The greatest barrier was a lack of time to plan and carry out the work. Similarly the next three barriers were lack of funding, lack of support from the department, and health and safety legislation — these were mutual between surveys. The level of recognition received from the relevant departments and research institutions appears to be a key factor in a researcher's decision to carry out PE work. One respondent commented: "*Not sure how useful it is to my career — I should really be writing papers*." At present, there is little acknowledgment for a researcher engaging with the public with another respondent stating: "*Although there is considerable effort by a number of people — we now have our own PE department of four people, the research scientist who volunteers to help out receives little recognition and does not benefit their career prospects*." The large number of researchers who are carrying out PE activity with no financial support does seem to indicate an eagerness and will to do so, rather than any particular personal gain or recognition, which is admirable; however, how much research groups can do without proper support from their institutions, remains unclear.

Within the respondents who have carried out PE, there were several individual comments that showed great enthusiasm for such activity. One response stated, "*I would very much recommend PE activities to other scientists. It's very rewarding and makes you look at your work more*

objectively." Another suggested, "*It is hard work to do, but very satisfying. It is fun, and it has a knock on boost to my research. I have managed to incorporate a new research project within the activities which helps get support from work and colleagues*" and a further commentator said that they would "*Strongly recommend PE to all*". There is a genuine delight from some, and the fact that 90% of respondents would like to get involved in PE once again shows that, despite the barriers, the enthusiasm is there.

Not without its limits

Collecting data by means of a survey however, is not without its limitations. Response bias is introduced, with perhaps those most keen on PE most likely to respond. This was demonstrated by the increased response rate from those who have taken part in PE as compared with that from those who had not (illustrated with 59 responses from those involved in PE compared with 50 from those not involved). The demographics of the respondents were not collected. There has been a suggestion previously that the demographics of respondents to electronic surveys may differ, for example, from respondents to postal or telephone surveys (Mayr *et al.*, 2012). No incentives were offered for completion of the surveys, when widening the sampling frame (Glidewell *et al.*, 2012), though providing an incentive may have increased the number of responses (Olsen *et al.*, 2012). Consequently our results can't be applied to the wider research community.

Conclusion

Public engagement is a fast emerging concept. Our surveys reveal 79% of respondents have taken part in PE and have also been actively engaging with the public on more than one occasion. Recent expectations from funders including the BBSRC may partially account for this increased surge in activity. Nevertheless, PE is not always at the top of the work agenda for all university staff under pressure to meet institutional targets such as those associated with the Research Excellence Framework; a lack of support, encouragement and appropriate guidance have shown to be major barriers for researchers wishing to collaborate with the community outside their respective universities. Our findings are useful for informing organizations and departments involved with PE about both barriers and successes within the area of infectious diseases and microbiology, and also to highlight some of the reasons why such activity may not take place.

Figure 4. *Future participation response*

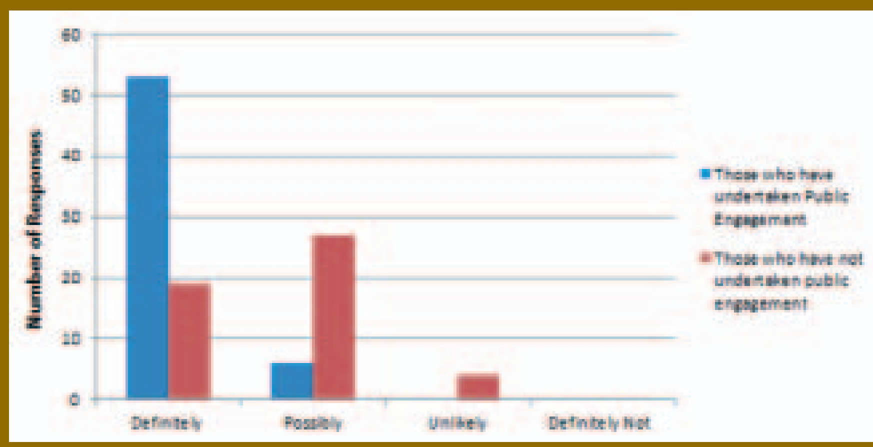
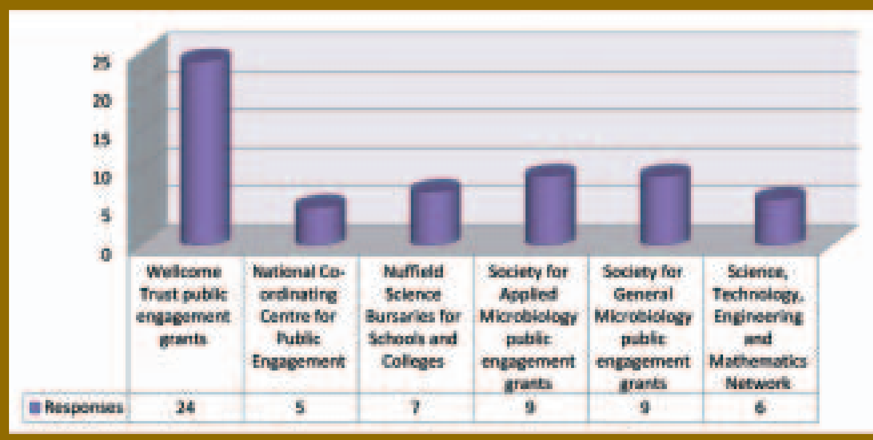


Figure 5. *Public engagement response*



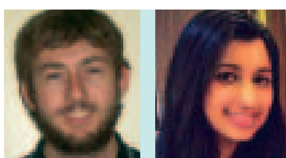
references

■ Glidewell, L., Thomas, R., and Maclennan, G. (2012). Do incentives, reminders or reduced burden improve healthcare professional response rates in postal questionnaires? Two randomized controlled trials. *BMC Health Services Research*, **Vol. 14**, No.12(1), p250.

■ Mayr, A., Gefeller, O., Prokosch, H. U., Pirkl, A., Fröhlich, A., and de Zwaan, M. (2012). Web-based data collection yielded an additional response bias — but had no direct effect on outcome scales. *Journal of Clinical Epidemiology*, **Vol. 65**, No. 9, pp970–977.

■ National Co-ordinating Centre for Public Engagement. (2012). '*What is Public Engagement: NCCPE definition*', [Online]. Available at http://publicengagement.ac.uk [Accessed: 29 August 2012].

■ Olsen, F., Abelsen, B., and Olsen, J. A. (2012). Improving response rate and quality of survey data with a scratch lottery ticket incentive. *BMC Medical Research Methodology*, Apr 19, 12, p52.



Michael Head and Raidah Haider
University College London and Infectious Disease Research Network

appendix 1

Survey questions directed towards those who had previously been involved in public engagement

■ What was the title of your most recent public engagement work?

■ What form did the activity take? (Tick as many as apply)

❑ Laboratory experiment
❑ Talk/lecture in a public place or at a public event
❑ Visit to a school/classroom
❑ Presentation at a science fair/exhibition
❑ Other (please specify)

■ What was the main age group targeted? (Tick as many as apply)

❑ Children under 11
❑ Children aged 11–18
❑ Adults
❑ Mixed age groups / families

If relevant, add further clarification here

■ Funding — was this public engagement work...

❑ Unfunded
❑ Carried out as part of a larger research grant
❑ Funding was obtained specifically to do this public engagement

■ If the activity received funding (either as part of a larger grant, or specifically for this work), who was the funder?

■ To what extent do you agree with the following statement: *"I enjoyed the public engagement activity..."*

❑ 1 (strongly disagree)
❑ 2 (disagree)
❑ 3 (neutral)
❑ 4 (agree)
❑ 5 (strongly agree)

■ To what extent do you agree with the following statement: *"my audience was very receptive to my work..."*

❑ 1 (strongly disagree)
❑ 2 (disagree)
❑ 3 (neutral)
❑ 4 (agree)
❑ 5 (strongly agree)

■ Have you previously taken part in public engagement work before this activity?

❑ Yes, once
❑ Yes, more than once
❑ No, this has been the only time

■ Would you like to take part in public engagement again?

❑ Definitely
❑ Possibly
❑ Probably not
❑ Definitely not

■ If any, what have been the main barriers to taking part in public engagement work?

❑ Lack of time to plan and carry out the work
❑ Lack of funding
❑ Lack of support from the department
❑ Health and safety requirements (e.g. getting students into a lab)
❑ Other bureaucracy
❑ Lack of interest from the public
❑ Just not interested in this kind of work previously
❑ Unsure previously on how to actually go about getting involved
❑ Other (please specify)

■ Any further comments?

appendix 2

Appendix II – Survey questions directed towards those who *had not* previously been involved in public engagement

■ Are you likely to be involved in public engagement in the future?

❑ Yes/probably
❑ Possibly
❑ Unlikely

■ If you would consider taking part in public engagement activity in the future, then are you already aware of any of the following bodies, societies and information/funding sources? (Not an exhaustive list, merely a few high-profile examples. Tick all that apply)

❑ Wellcome Trust public engagement grants (http://www.wellcome.ac.uk/Funding/Public-engagement/index.htm)

❑ National Co-ordinating Centre for Public Engagement (http://www.publicengagement.ac.uk/)

❑ Nuffield Science Bursaries for Schools and Colleges (http://www.nuffieldfoundation.org/science-bursaries-schools-and-colleges)

❑ Society for Applied Microbiology public engagement grants (http://www.sfam.org.uk/en/grants--awards/innovative-projectpublic-engagement-grant.cfm)

❑ Society for General Microbiology public engagement grants (http://www.sgm.ac.uk/grants/pem.cfm)

❑ Science, Technology, Engineering and Mathematics Network (STEMNET) (http://www.stemnet.org.uk/)

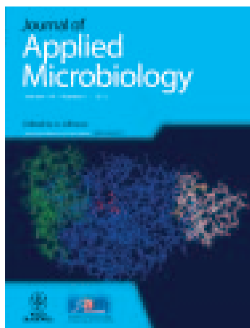
■ What are the main reason(s) why you have never taken part in public engagement? (Tick all that apply)

❑ Lack of time to plan and carry out the activity
❑ Lack of funding
❑ Lack of support from the department
❑ Health and safety requirements (e.g. getting students into a lab)
❑ Other bureaucracy
❑ Lack of interest from the public
❑ Just not interested in this kind of work
❑ Unsure how to actually go about getting involved
❑ Other (please specify)

■ Any further comments?

journal**Watch**

News about the Society's journals



Journal of Applied Microbiology

Antimicrobial activity of essential oils and other plant extracts.
http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2672.1999.00780.x/abstract
K. A. Hammer, C. F. Carson, T. V. Riley.

The antimicrobial activity of plant oils and extracts has been recognized for many years. However, few investigations have compared large numbers of oils and extracts using methods that are directly comparable. In this study, 52 plant oils and extracts were investigated for activity against *Acinetobacter baumanii*, *Aeromonas veronii* biogroup *sobria, Candida albicans*, *Enterococcus faecalis*, *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella enterica* subsp. *enterica* serotype Typhimurium, *Serratia marcescens* and *Staphylococcus aureus*, using an agar dilution method. The results from this study support the notion that plant essential oils and extracts may have a role as pharmaceuticals and preservatives.

Antimicrobial agents from plants: antibacterial activity of plant volatile oils.
http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2672.2000.00969.x/abstract. H. J. D. Dorman, S. G. Deans.

The volatile oils of black pepper [*Piper nigrum* L. (Piperaceae)], clove [*Syzygium aromaticum* (L.) Merr. & Perry (Myrtaceae)], geranium [*Pelargonium graveolens* L'Herit (Geraniaceae)], nutmeg [*Myristica fragrans* Houtt (Myristicaceae)], oregano [*Origanum vulgare* ssp. *hirtum* (Link) Letsw. (Lamiaceae)] and thyme [*Thymus vulgaris* L. (Lamiaceae)] were assessed for antibacterial activity against 25 different genera of bacteria. These included animal and plant pathogens, food poisoning and spoilage bacteria. The volatile oils exhibited considerable inhibitory effects against all the organisms under test while their major components demonstrated various degrees of growth inhibition.

To view these and other articles from *Journal of Applied Microbiology*, as well as our two latest Virtual Issues on **Plant Resistance** and **Probiotics**, please visit: **www.journalappliedmicro.com**.

Letters in Applied Microbiology

Antifungal activity of thyme (*Thymus vulgaris* L.) essential oil and thymol against moulds from damp dwellings.
http://onlinelibrary.wiley.com/doi/10.1111/j.1472-765X.2006.02032.x/abstract. M. Šegvić Klarić, I. Kosalec, J. Mastelić, E. Piecková, S. Pepeljnak.

This study aims to characterize antifungal activities of the essential oil of thyme (*Thymus vulgaris* L.) and pure thymol, as a comparative substance, on different mould species isolated from damp dwellings, with samples from Croatia and Slovakia. The study concluded that thyme essential oil was shown to possess a wide range spectrum of fungicidal activity, and the vaporous phase of the oil exhibited long-lasting suppressive activity on moulds from damp dwellings. This suggests that the essential oil of thyme and thymol could be used for disinfection of mouldy walls in the dwellings in low concentration.

Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity
http://onlinelibrary.wiley.com/doi/10.1046/j.1472-765x.2000.00731.x/abstract. A. Nostro, M. P. Germanò, V. D'Angelo, A. Marino, M. A. Cannatelli.

This article reports on a comparative study on the antimicrobial properties of extracts from medicinal plants. The screening of the antimicrobial activity of extracts from six plants was conducted by a disc diffusion test against Gram-positive, -negative and fungal organisms. The results indicated that the diethyl ether extracts were the most efficient antimicrobial compounds. The activity was more pronounced against Gram-positive and fungal organisms than against Gram-negative bacteria. Bioautography showed that the antimicrobial activity was probably due to flavonoids and terpenes.

To view these and other articles from *Letters in Applied Microbiology*, please visit: **www.lettersappliedmicro.com**.

Environmental Microbiology

Fresh fruit and vegetables as vehicles for the transmission of human pathogens.
http://onlinelibrary.wiley.com/doi/10.1111/j.1462-2920.2010.02297.x/abstract.
C. N. Berger, S. V. Sodha, R. K. Shaw, P. M. Griffin, D. Pink, P. Hand, G. Frankel.

Much research into foodborne human pathogens has focused on transmission from foods of animal origin. However, recent investigations have identified fruits and vegetables are the source of many disease outbreaks. Now believed to be a much larger contributor to produce-associated outbreaks than previously reported, norovirus outbreaks are commonly caused by contamination of foods from hands of infected workers. Similarly, while infections with *Salmonella* have mainly been linked to consumption of foods of animal origin, many outbreaks have been traced to contaminated fresh produce. A better understanding of plant, microbiological, environmental, processing and food handling factors that facilitate contamination will allow development of evidence-based policies, procedures and technologies aimed at reducing the risk of contamination of fresh produce.

Heavy use of prophylactic antibiotics in aquaculture: a

growing problem for human and animal health and for the environment.
http://onlinelibrary.wiley.com/doi/10.1111/j.1462-2920.2006.01054.x/abstract. F. C. Cabello.

The accelerated growth of finfish aquaculture has resulted in a series of developments detrimental to the environment and human health. There is widespread use of prophylactic antibiotics in this industry, and the variety of antibiotics in large amounts, including non-biodegradable antibiotics useful in human medicine, ensures that they remain in the aquatic environment for long periods of time. Accumulating evidence indicates that unrestricted use is detrimental to fish, terrestrial animals and human health and the environment, and global efforts are needed to promote more judicious use of prophylactic antibiotics in aquaculture.

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Environmental Microbiology Reports

Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. http://onlinelibrary.wiley.com/doi/10.1111/j.1758-2229.2008.00004.x/abstract. H. W. Paerl, J. Huisman.

Cyanobacteria are the earth's oldest known oxygen-evolving photosynthetic microorganisms, and they have had a major impact on shaping our current atmosphere and biosphere. Their long evolutionary history has enabled cyanobacteria to develop survival strategies and persist as important primary producers during numerous geochemical and climatic changes that have taken place on earth during the past 3.5 billion years. Here, we review recent studies revealing that regional and global climatic change may benefit various species of harmful cyanobacteria by increasing their growth rates, dominance, persistence, geographic distributions and activity.

Local and regional factors influencing bacterial community assembly. http://onlinelibrary.wiley.com/doi/ 10.1111/j.1758-2229.2011.00257.x/abstract. E. S. Lindström, S. Langenheder.

The classical view states that microbial biogeography is not affected by dispersal barriers or historical events, but only influenced by the local contemporary habitat conditions (species sorting). This has been challenged during recent years by studies suggesting that regional factors such as mass effect, dispersal limitation and neutral assembly are also important for the composition of local bacterial communities. In this paper we summarize results from biogeography studies in different environments, i.e. in marine, freshwater and soil as well in human hosts.

Read these and other online articles including the *Environmental Microbiology Reports* Editor's Choice Virtual Issue Volume 5 at **www.env-micro-reports.com**.

Microbial Biotechnology

Marine genomics: at the interface of marine microbial ecology and biodiscovery. http://onlinelibrary.wiley.com/doi/10.1111/j.1751-7915.2010.00193.x/abstract. K. B. Heidelberg, J. A. Gilbert, I. Joint.

The composition and activities of microbes from diverse habitats have been the focus of intense research during the past decade with this research being spurred on largely by advances in molecular biology and genomic technologies. In recent years environmental microbiology has entered very firmly into the age of the 'omics'— (meta)genomics, proteomics, metabolomics, transcriptomics – with probably others on the rise. This review explores the brief history of genomic and metagenomic approaches to study environmental microbial assemblages and describes some of the future challenges involved in broadening our approaches — leading to new insights for understanding environmental problems and enabling biodiscovery research.

Bacterial persistence increases as environmental fitness decreases.
http://onlinelibrary.wiley.com/doi/10.1111/j.1751-7915.2011.00327.x/abstract. S. H. Hong, X. Wang, H. F. O'Connor, M. J. Benedik, T. K. Wood.

Since persister cells cause chronic infections and since *E. coli* toxin MqsR increases persisters, we used protein engineering to increase the toxicity of MqsR to gain insights into persister cell formation. In this study, we explored how the toxicity of MqsR is related to persistence by using protein engineering (Wood *et al*., 2011) to increase the toxicity of MqsR. We found that MqsR increases persister cell formation by repressing acid resistance, multidrug resistance and osmotic resistance, and that the general stress response master regulator RpoS is important for persister cell formation. Together with the result that wild-type cells that are stressed prior to antibiotic treatment increase 12,000-fold in persistence, we concluded that stressed cells become more persistent than those that are not stressed.

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In the 32nd of a series of articles about statistics for biologists, **Anthony Hilton** & **Richard Armstrong** discuss:

The partial correlation coefficient

StatNote 32

In previous StatNotes (Hilton & Armstrong, 2008a, 2008b, 2009a, 2009b), the application of correlation and regression methods to the analysis of two variables (*X*,*Y*) was described. The most important statistic used to measure the degree of correlation between two variables is Pearson's 'product moment correlation coefficient' ('r') (Hilton & Armstrong, 2008a).

Over the last 50 years, the use of regression methods has increased at the expense of correlation (Snedecor & Cochran, 1980). There are three main reasons for this change. First, 'r' only measures the degree of 'closeness' of the linear relation between two variables whereas a regression analysis can describe the relationship in more detail (Hilton & Armstrong, 2008b). Second, to make valid use of 'r' assumes that the pairs of values are a random sample from a bivariate normal distribution. In reality, however, the values of the X variable are often specifically selected, i.e., the physiological response of a bacterium may be measured at selected values of temperature. Hence, a regression analysis, which only makes the assumption that the Y values are normally distributed, would be more valid. Third, the correlation between two variables may be due to their common relation to other variables. This phenomenon often arises due to the 'size effect', i.e., a larger cell, organism or population may by 'large' in all of its properties, so that any two measurements made on it are likely to be correlated (Snedecor & Cochran, 1980). In addition, many apparently unrelated variables may be significantly correlated. For example, the correlation is -0.98 between the annual birthrate in the UK (1875–1920) and the annual production of pig iron in the USA (Snedecor & Cochran, 1980), and many other similar examples of 'nonsense' correlations could be quoted.

Hence, investigators using correlation studies need to be alert to the possibilities of spurious correlation and the methods of 'partial correlation' are one method of taking this into account. This StatNote applies the methods of partial correlation to three scenarios. First, to a fairly obvious example of a spurious correlation resulting from the 'size effect' involving the relationship between the number of general practitioners (GPs) and the number of deaths of patients in a town; second, to the relationship between the abundance of the nitrogen-fixing bacterium *Azotobacter* in

soil and three soil variables (Martyniuk & Martyniuk, 2003), and finally, to a more complex scenario, first introduced in StatNote 24 (Hilton & Armstrong, 2011a), and involving the relationship between the growth of lichens in the field and climate.

Scenarios

Doctors and deaths

The total number of deaths in 12 towns in the UK in a single year (X_1) was obtained from official statistics as well as the total number of GPs (X_2) in NHS affiliated practices in the town. In addition, an estimate of the population size of the town was obtained from official statistics, (X_3). The question to be investigated is whether there is a relationship between the number of doctors and the number of deaths in the town.

Azotobacter in soil

Azotobacter is a genus of aerobic, nitrogen-fixing bacteria frequently found in soil. The organism is an important component of the nitrogen cycle in ecosystems and is useful to humans in the production of fertilizers, food additives and biopolymers. It can also synthesize biologically important compounds, including plant hormones such as auxin, and can therefore promote plant growth. *Azotobacter* is common in soils which are neutral to weakly basic and is usually absent from acid soils. Hence, an investigator wished to determine the extent to which the abundance of *Azotobacter* in soils was related to three soil variables, *viz*., soil pH, total nitrogen (N) content and percentage of organic carbon (Martyniuk & Martyniuk, 2003). Thirty different soils were studied and 10 samples of each soil were collected and the pH, total N (total Kjedahl nitrogen, nitrite-N and nitrate-N), and per cent carbon measured in each sample.

Growth of lichens and climate

We return to the scenario described in StatNote 24 (Hilton & Armstrong, 2011a). The radial growth rate (RGR) of thalli of the crustose lichen *Rhizocarpon geographicum* (L). DC., was measured in the field in 17, successive three-month periods over 51 months at a site in North Wales (Armstrong and Smith, 1987). Radial growth was measured at between eight and 10 randomly chosen locations around each lichen thallus at three-month intervals from April 1993 to June 1997 (Armstrong, 1973). Radial growth in each period was averaged for each thallus and then over the 20 thalli to examine the pattern of seasonal growth. Climatic data relevant to each three-month period included records of: (1) total rainfall, (2) the total number of rain days, (3) maximum (Tmax) and minimum (Tmin) temperature recorded on each day and averaged for each period, (4) the total number of air and ground frosts, (5) the total number of sunshine hours, and (6) average daily wind speed. Therefore, the data comprise for each three-month period, a single dependent (*Y*) variable, *viz*., radial growth of the lichen and eight possible defining climatic (*X*) variables. The major problem is to determine the variables which are actually related to growth given the degree of inter-correlation often present between climatic variables.

How is the analysis carried out?

Theory

Pearson's 'r' is closely related to the bivariate normal distribution but if more than two variables are studied, then the multivariate normal distribution is a more appropriate description of the data. In the multivariate normal distribution, any variable has a linear regression on any other variable or on any subset of the variables with deviations which are normally distributed (Snedecor & Cochran, 1980). If there are three variables under study, then there are three simple population (p) correlations among them, *viz*., correlation between variable 1 and 2 ($p_{1.2}$), between variables 2 and 3 ($p_{2.3}$), and between variables 1 and 3 ($p_{1.3}$). Hence, the partial correlation $p_{12.3}$ is defined as the correlation between variables 1 and 2 in a cross-section of individuals all having the same value of variable 3. Hence, the third variable is held 'constant' so that only variables 1 and 2 are involved in the correlation, $p_{12.3}$, being the same for every value of variable 3.

Calculation of the partial correlation coefficient

The basic principle of the analysis is to calculate that part of the correlation between variables 1 and 2 which is not simply a reflection of their mutual relationship with variable 3. The sample estimate $r_{12.3}$ of the population value $p_{12.3}$ is obtained by calculating: (1) the deviation of variable 1 from its sample regression on variable 3 ($d_{1.3}$), (2) the deviation of variable 2 from its regression on variable 3 ($d_{2.3}$), and (3) the simple correlation coefficient between $d_{1.2}$ and $d_{2.3}$ ($r_{12.3}$) and is given by:

$$r_{12.3} = r_{12} - r_{13}\, r_{23} / \sqrt{(1-r_{13}^2)\,(1-r_{23}^2)}$$

The partial correlation, which has N - 3 degrees of freedom, where N is the number of experimental units sampled, is referred to the table of Pearson's 'r' to judge statistical significance.

Interpretation

The results of the doctors/death example are given in Table 1. There is a highly significant positive correlation between the number of doctors in a town and the number of deaths in the town ($r = 0.84$, $P < 0.001$) leading to the unlikely conclusion that doctors may have caused the deaths! This scenario, however, is an example of how large entities are large in many of their properties as both the number of doctors ($r = 0.91$, $P < 0.001$) and the number of deaths ($r = 0.78$, $P < 0.01$) in the town are positively correlated with a third variable, *viz*., the size of the town. Hence, does the correlation between doctors and death still hold if the size of the town is held constant? The partial correlation coefficient between doctors and death, size of town being held constant,

Table 1. The correlation (Pearson's 'r') between the number of deaths in a town, the total number of general practitioners (GPs) in the town, and the size of the town for 12 towns in the UK (** P < 0.01; ***P < 0.001)

	Variables		
	Number of deaths	**Number of GPs**	**Size of town**
Number of deaths	—	0.84***	0.78**
Number of GPs		—	0.91***
Size of town			—

Partial correlation r (deaths/GPs.size of town) = 0.49 (N - 3, P > 0.05)

Table 2. The correlation (Pearson's 'r') between the abundance of *Azotobacter* in soils and three soil variables, *viz*., soil pH, total nitrogen (N) content and percentage of organic carbon (C) (*P < 0.05;**P < 0.01; ***P < 0.001)

	Variables			
	% Organic C	**% Total N**	**pH**	**Azotobacter**
% Organic C	—	0.89***	0.04	0.37*
% Total N		—	0.21	0.58**
pH			—	0.62*

Partial correlations: *Azotobacter*/ % Organic C (other variables held constant) = -0.26 (P > 0.05); *Azotobacter*/ % N (other variables held constant) = 0.49 (P < 0.01); *Azotobacter*/ pH (other variables held constant) = 0.56 (P < 0.01).

is not significant (r = 0.49, P > 0.05). Consequently, the correlation between doctors and death is spurious, the result of both variables being correlated with the size of the town. A similar relation can be found between the number of fire engines attending a fire and the damage caused by the fire! These examples are fairly obvious from the context but it may be much more difficult to disentangle 'real' from 'spurious' correlations in scenarios in which there are many inter-correlated variables.

The correlation matrix between the abundance of *Azotobacter* and three soil variables is shown in Table 2. There were significant positive correlations between the abundance of *Azotobacter* and organic carbon (r = 0.37, P < 0.05), total nitrogen (r = 0.58, P < 0.01), and soil pH (r = 0.62, P < 0.05). However, there was also a correlation between total nitrogen and organic carbon (r = 0.89, P < 0.001) and the partial correlations suggest that there is no significant correlation between *Azotobacter* and organic carbon when that part of the correlation attributable to total N is removed. In addition, removing the effect of the variables reduces the size of the remaining correlations but the correlations with pH and N remain significant. It can therefore be concluded with more confidence that the abundance of *Azotobacter* is related to pH and N but not necessarily to organic C.

Table 3. The correlation (Pearson's 'r') between radial growth rate (RGR) of the lichen *Rhizocarpon geographicum* and climatic variables (Tmax = Maximum temperature, SH = Sunshine hours, AF = Air frosts, RD = Rain days, RF = Total rainfall, Tmin = Minimum temperature, GF = Ground frosts, WS = Wind speed). Significant correlations are underlined

	Variables								
	RGR	**Tmax**	**SH**	**AF**	**RD**	**RF**	**Tmin**	**GF**	**WS**
RGR	—	0.76	-0.55	-0.13	-0.07	0.28	0.34	-0.31	-0.21
Tmax		—	-0.22	-0.38	-0.09	0.20	0.56	-0.47	-0.47
SH			—	-0.25	-0.30	-0.49	0.28	-0.29	-0.33
AF				—	0.02	-0.21	-0.82	0.85	0.02
RD					—	0.69	-0.17	-0.05	0.61
RF						—	0.13	-0.23	0.33
Tmin							—	-0.94	-0.29
GF								—	0.16
WS									—

Partial correlation r (RGR/Tmax.SH) = 0.78, (N - 3 DF, P < 0.01)

An example of a more complex scenario involving eight independent variables is shown in Table 3. The correlation matrix suggests that lichen growth is positively correlated with maximum temperature (r = 0.76, P < 0.001) but negatively correlated with total sunshine hours (r = -0.55, P < 0.05). Hence, does the correlation between growth and Tmax still hold if sunshine hours are held constant? This suggestion is plausible as there is very little correlation between sunshine hours and Tmax (r = -0.22, P > 0.05). The partial correlation between growth and Tmax, sunshine hours held constant, confirms this impression since r = 0.78, (N - 3 DF, P < 0.01). These relationships are further complicated because there are mutual correlations between Tmax and Tmin and between sunshine hours and total rainfall. Hence, with several inter-correlated variables, even the techniques of partial regression may not be sufficient to disentangle the relationships among them. In examples like this, we would recommend multiple regression (StatNotes 24, 25, Hilton & Armstrong, 2011a,b) as the most efficient method of analysis.

Conclusion

There are a number of problems that have to be considered in any correlation study. Hence, 'r' only measures the degree of 'closeness' of the linear relation between two variables, makes the assumption that the data are a random sample from a bivariate normal distribution, and suffers from the weakness that correlation between two variables may be due to their common relation to other variables (Snedecor & Cochran, 1980). Partial correlation may help to disentangle real correlations from those which are simply spurious. In addition, investigators should be aware of the 'size effect' in which many attributes are likely to be mutually correlated because of their size.

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Tuesday 23 April 2013

Spring Meeting

STIs in the 21st Century

The Stratford Q Hotel, Stratford-upon-Avon, UK

Programme

09.25 – 10.25	**Tea, coffee, trade exhibition and registration**
10.25 – 10.30	**Chairman's welcome**
Chair:	**Sally Cutler**
10.30 – 11.10	**Changing trends in chlamydia infection** Gwenda Hughes, HPA, London, UK
11.10 – 11.50	**Gonorrhoea — may be untreatable by 2015!** Cathy Ison, HPA, London, UK
11.50 – 12.30	**Epidemiology of STIs in the UK** Ian Simms, Health Protection Agency Communicable Disease Surveillance Centre, London, UK
12.30 – 14.00	**Lunch and trade exhibition**
Chair:	**Steve Davies**
14.00 – 14.30	**The overlooked problem — *Trichomonas vaginalis*** John White, Guy's and St Thomas NHS Foundation Trust, London, UK
14.30 – 15.00	**HPV vaccines, are they doing the job?** Margaret Stanley, University of Cambridge, UK
15.00 – 15.30	**Resurgence of syphilis the "great pretender" in the UK** Andrew Turner, HPA, Manchester, UK
15.30 – 16.00	**BV** Philip Hay, St George's, University of London, UK
16.00	**Close, tea and coffee**

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Programme

Monday 1 July 2013

11.00 – 17.00 **Workshop session**

18.00 – 19.00 ***Journal of Applied Microbiology* Inaugural Annual Lecture**
Peter Setlow, University of Connecticut, USA

19.00 – 20.00 **Drinks reception and buffet**

20.30 – 22.30 **Quiz**

Tuesday 2 July 2013

Session 1: Lactic acid bacteria and bifidobacterium

Chair: **Louise Fielding**

09.00 – 09.35 **Genomics based insight in starter bacteria**
Todd R Klaenhammer, NCSU Raleigh, USA

09.35 – 10.10 **Synthetic approaches in engineering antimicrobial peptides in *Lactococci***
Oscar Kuipers, Groningen University, The Netherlands

10.10 – 11.05 **Tea, coffee, trade show and posters**

11.05 – 11.40 **New insights in viral protection in lactic acid bacteria**
Sylvain Moineau, Laval University, Québec, Canada

11.40 – 12.15 **Pangenomics of paradigm probiotics**
Willem M de Vos, Wageningen University, The Netherlands

12.15 – 13.15 **Lunch, trade show and posters**

Session 2: Lactic acid bacteria and bifidobacterium

Chair: **Willem M de Vos**

13.15 – 13.50 **Growth and physiology of bifidobacteria**
Luc de Vuyst, Brussels University BE, Belgium

13.50 – 14.25 **Functional genomics of bifidobacteria for health**
Douwe van Sinderen, UCC, Cork, Ireland

14.25 – 14.45 **Tea, coffee and trade show**

14.45 – 15.20 **Bifidobacteria in early life**

These preliminary programme times and titles were correct at the time of going to press.

Jan Knol, Laboratory of Microbiology, Wageningen University, The Netherlands

15.20 – 15.55 Targeting bifidobacteria with prebiotic substrates — state of the art
Glenn Gibson, Reading, UK

16.00 – 17.00 Attended poster session

17.00 – 18.00 Student session

17.00 – 19.30 Trade show with wine and a competition

Wednesday 3 July 2013

Session 3: Actinobacteria

Chair: Samantha Law

09.00 – 09.35 Bergey's taxonomic outline of the actinobacteria
William B Whitman, University of Georgia, Athens, USA

09.35 – 10.10 Evolution of sporulating actinomycetes
Gilles van Wezel, Leiden University, The Netherlands

10.10 – 10.45 Ecology and evolution of obligate marine actinobacteria belonging to the genus *Salinispora*
To be confirmed

10.45 – 11.05 Tea and posters

11.05 - 11.40 Actinobacteria from extreme habitats: new opportunities for drug discovery
Michael Goodfellow, Newcastle University, UK

11.40 – 12.15 Central metabolism in the evolution and diversification of natural product biosynthetic pathways
Francisco Barona-Gomez, Laboratorio Nacional de Genómica para le Biodiversidad, Irapuato, Mexico

12.15 – 13.15 Lunch and posters

Session 4: Actinobacteria

Chair: Mike Goodfellow

13.15 – 13.50 Diversity of nitrogen-fixing actinobacteria associated with root nodules of crop plants
Martha Trujillo, Universidad de Salamanca, Spain

13.50 – 14.30 Leaf-cutting ants and their actinomycetes
Matthew Hutchings, University of East Anglia, UK

14.30 – 15.30 Student presentations

15.30 – 16.00 Tea, coffee and posters

S*f*AM Award Lectures

Chair: Martin Adams

16.00 – 16.05 Introduction to the New Lecturer Research Grant
Martin Adams

16.05 – 16.40 S*f*AM New Lecturer Research Grant Lecture
To be confirmed

16.40 – 16.45 Introduction to the W H Pierce Prize
Martin Adams

16.45 – 17.20 W H Pierce Prize Lecture
To be confirmed

17.20 – 18.00 Annual General Meeting

19.00 onwards Drinks reception and conference dinner

Thursday 4 July 2013

Session 5: Actinobacteria (continued)

Chair: Martin Adams

09.00 – 09.35 Genome mining to understand and manipulate antibiotic production in actinomycetes
Mervyn Bibb, John Innes Centre, Norwich, UK

09.35 – 10.10 Applications of phage integrases in streptomycetes and beyond
Maggie Smith, University of York, UK

10.10 – 10.40 Tea and coffee

10.40 – 11.15 Biology of plant pathogenic streptomycetes
Dawn Bignell, Memorial University, St. John's, Newfoundland, Canada

11.15 – 11.50 Secrets from the genomes of human pathogenic streptomycetes
Paul Hoskisson, University of Strathclyde, Glasgow, UK

12.00 – 13.00 Lunch and close

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

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

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

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

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

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

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

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

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

JOIN US!

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

Membership changes

NEW MEMBERS

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

Australia

K. J. Broadby

Belgium

G. Vanantwerpen

Ghana

J. Agbenorku; P. Foriwaa; S. S. Mahamudu

India

I. Deshpande

Ireland

A. Campion; A. McGrath; J. O'Connor; D. Zizyte

Kuwait

A. Yateem

Malaysia

J. X. Hay; W. H. Kam

Mexico

D. Medina

Nigeria

O. E. Abioye; E. Adedigba; A. Adeeko; O. Adesanya; G. Adewale; O. K. Awojobi; M. K. Bakare; M. O. Bello; I. S. Egbewumi; C. D. Fashina; Y. M. Feruke-Bello; J. O. Jagboro; A. S. Kehinde; B. O. Lawal; T. M. Momoh; C. Obi; O. Ojo; O. Oyedara; G. O. Taylor

Russia

B. Lapin; O. Mikhailova

Switzerland

M. Blanc

UK

A. Abrams; T. Adebajo; S. Ahmed; S. Alhagh; R. Allison; M. Alqurashi; F. Alzahrani; E. J. Anah; O. A. Aning; P. Arivaradarajan; K. Baldwin; G. Barnwell; J. Bird; E. Blackburn; L. Blackmore; R. Borne; S. Buchan; G. B. Burnett; F. Burns; K. Busarakam; H. L. Cargill; L. Chaston; A. Chouankam; C. Colenutt; A. Cosgrove; D. Coulbeck; S. Coulson; J. Czyzewska-Khan; B. Davies; A. Day; B. Dickinson; V. Dolan; J. Dormon; G. Duncan; A. Dunn; A. Edwards; M.M. Elshawish; B. Eren; U. A. Eze; M. J. Fleming; D. J. Fletcher; A. Giffen; M. P. Gilks; P. Grayson; E. Green; B. Greenwood; T. Hafiz; J. Hall; E. C. Hambridge; J. Healey; S. Heshmatifar; R. Hills; T. E. Hlangabeza; R. Hodrien; R. L. Hollis; S. Hurley; R. Hussain; H. Idris; C. Ince; S. Iqbal; N. Izuchukwu; R. Jones; W. Kiam-Laine; K. S. Kyi; R. M. La Ragione; G. Laverty; E. J. Lee; L. Lessa Andrade; N. Lley; L. Loughran; S. Love; A. Lyle; B. Manford; K. F. McArthur; M. McCall; S. McGrath; K. McGuirk; O. J. McInnes; A. Milne; M. Mohamed; A. Moorhouse; H. Moule; K. Muddiman; J. J. Mulamootil; G. Mulley; J. Munnoch; M. Muzafari; E. Newire; J. Ngai; A. Ogunleye; S. A. Okafor; R. Okelo; A. T. Oladunjoye; C. Olateju; T. Palmer; M. Pascoe; H. Pickford; G. Quinney; S. N. Raeisi; R. Ragupathy; A. Ridley; J. Roberts; D. P. Roulston; W. L. Scott; S. Sharpe; W. Shaw; D. Sheard; H. S. Shortland; A. Smyth; A. Sorbie; L. J. Stanley; L. Stewart; A. Syanda; L. Sykes; S. Thompson; R. Tirados; C. Umeobi; A. Van Den Bos; P. Varghese; L. Vella; P. Vikhe; G. Wai; A. Wallace; B. Walsh; M. C. Wareing; E. White; Z. Whitehouse; S. Williams; A. Williamson; S. Wilmore; A. Wishart; D. J. Wootten; M. W. Wren; E. Wright; M. Yacoub; D. Yara; D. Young; H. Zahid

USA

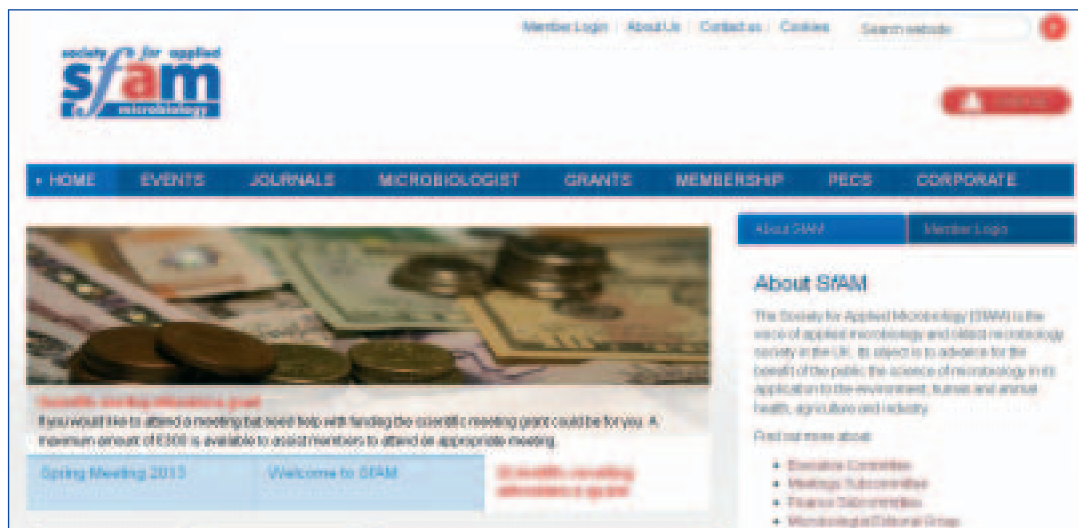
R. Gonzalez; D. J. Grimes; J. Stratton

Call for nominations to Committee

There will be up to four vacancies on the *Sf*AM Executive Committee in **July 2013.** Nominations are invited from all Full Members of the Society for these vacancies. Nominations must be made in writing and received by the Society Office by Friday **3 May 2013**.

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

New online tools for members



Now that we have an all-singing, all-dancing website, we've been thinking about what we can DO with such a flexible and innovative tool and I'm delighted to announce that we have two excellent new facilities which are now available exclusively to S*f*AM members.

Virtual Microbiology Laboratory

Created by Nottingham University, this excellent facility provides all the information you need to know about basic practical microbiology techniques, from culturing and aseptic techniques right through to the accurate identification of pathogens. Log on to the site as a S*f*AM member and visit: http://www.sfam.org.uk/en/members-area/virtual-microbiology-lab.cfm.

Here you can read and listen to a description of the technique, watch an animation, or see a video of somebody carrying it out. As there are so many different ways of learning about the technique, all types of learner will benefit. If you teach basic microbiology, this is an ideal tool to use in your teaching laboratories or lectures. It also provides tests to ensure your students have learnt everything they need to know to carry out the technique successfully. If you're out of practice and need to brush-up your knowledge of a particular method, the Virtual Microbiology Lab (VML) is a great place to start. The VML is available to ALL S*f*AM members, so if you'd like to make it available to your undergraduate class, encourage them to sign up for free eStudent membership, by emailing membership@sfam.org.uk requesting eStudent membership, and they can run through the demonstrations themselves after class. There'll be no excuses for failed experiments ever again!

membership
matters

Tropical Microbiology Network

Another online tool we've just launched is the Tropical Microbiology Network (TMN). Aimed at scientists working in areas where tropical diseases are endemic, this tool provides detailed information about these diseases and how samples containing them should be treated and analysed. Log in to the site as a S*f*AM member and visit: http://www.sfam.org.uk/en/members-area/tropical-microbiology-network/

Here the process of laboratory diagnosis is described clearly in colourful, informative diagrams, as is bacterial classification. Diseases such as HIV, TB, malaria and meningitis are described in detail, including instruction on how to go about their laboratory diagnosis, rapid tests and the basic aetiology of each disease and its causative organism. Again, this is available to S*f*AM members only, so if you are based in a band 1 country and would appreciate access to the TMN, email membership@sfam.org.uk requesting eAffiliate membership, and our Membership Co-ordinators will get in touch with instructions on how to join and access the TMN.

I think you'll agree, both these facilities are excellent new benefits of S*f*AM membership

Lucy Harper
Communications Manager
Society for Applied Microbiology

Obituary

Peter Walker

It is with great sadness that we record the death of Professor Peter D. Walker who passed away at home suddenly on 9 October 2012.

Peter was a long-standing member and enthusiastic supporter of the Society having first joined in 1957. He served as Hon. Treasurer from 1967–1982, almost reaching the record for longest serving officer set by the Society's first Treasurer L. J. Meanwell, and was Hon. President from 1983–1985. He was active in FEMS and in several S*f*AM working parties and was involved with negotiating the transfer of S*f*AM journals to their current publisher, Wiley-Blackwell. He was a regular attendee at our scientific meetings and other functions; most recently attending the Past Presidents' Lunch only a week before his death, when he took an active part in a convivial occasion reviewing recent progress and developments within the Society.

Peter received his undergraduate and postgraduate education at the University of Leeds, obtaining his PhD studying clostridia under the direction of Professor C. L. Oakley FRS. These were remarkable years for the Department at Leeds when almost every postgrad eventually got a chair and became the head of a university department or laboratory.

Peter went on to work with distinction at the Wellcome Research Laboratories in Beckenham for many years. Much of his work centred on spore-forming bacteria and their medical and veterinary importance. Among his noteworthy achievements was his work on the severe foodborne necrotic enteritis that occurred in Papua New Guinea known as pig bel. Among his numerous publications on this subject were the report on the isolation of *Clostridium perfringens* Type C from cases of the infection, identification of how low proteolytic activity in the gut of victims predisposes them to illness since they are unable to degrade the beta toxin of *Cl. perfringens* which is normally very susceptible to proteolysis, and the success of immunization against pig bel with a toxoid produced from the toxin. In 1966 he was a co-author of the much cited paper 'The ecology and epidemiology of the pig-bel syndrome in man in New Guinea (*J. Hyg. Camb*., 1966, 64, pp375–396)'.

As a consequence of his long and special research interest in the structure and resistance mechanisms of bacterial spores, Peter was much involved with like-minded colleagues in the setting up and running of a 'British Spore Group' in the late 1960s. Members met regularly to discuss research findings, invited colleagues from overseas, and published proceedings in a series of books with Academic Press (Spore Research 1971, 1973 & 1976). Scandinavian colleagues were particularly enthusiastic members, such that it became informally known as the 'UK-Scandinavian Spore Group'. Activities were not all strictly work-orientated and Peter took part in all activities to the full! At one time, Scandinavian colleagues called their British friends the 'Whisky-o-philes'. He was Group Treasurer for most if its existence, which ended in the early 1980s as genetics began to replace much of the physiological/biochemical interests of most of its members.

Peter was an outstanding microbiologist who played an important role in his profession and in the building of the Society. He will be sorely missed by family and friends alike.

Call for nominations for W H Pierce Prize

Do you know a young microbiologist (under 40 years of age) who has made a substantial contribution to microbiology? If so, why not nominate them for this prestigious and substantial award which is now worth £3000. The award was instituted in 1984 by Oxoid to commemorate the life and works of the late W H (Bill) Pierce, former Chief Bacteriologist at Oxoid Ltd and a long time member of the Society. The prize is presented annually at the Summer Conference. Full Members wishing to make a nomination for the 2013 prize should write in confidence to the Honorary General Secretary, Professor Mark Fielder, at the Society Office in Bedford, including a full CV of the nominee and a letter of support. Please note that application is through nomination by Full Members of S*f*AM only and that there are no official forms for this award. **Closing date for nominations is Thursday 18 April 2013.**

S*f*AM PECS Research Conference

The inaugural S*f*AM **PECS Research Conference** was held on the 25 October at Charles Darwin House in Central London. Organized by the S*f*AM Postgraduate and Early Career Scientists Committee and attended by students and early career scientists, the topic of the meeting was molecular and microbiological techniques

The focus of the meeting was to provide a supportive environment for postgraduates and early career scientists to disseminate their research findings through poster and oral presentation sessions. In addition, delegates were introduced to the latest techniques in molecular microbiology, from top researchers in the field.

The day started with a warm welcome by Professor Martin Adams, President of the Society. Martin thanked the PECS Committee for their dedication in organizing the meeting and expressed his wish to see an annual PECS Research Conference.

The first session of the morning chaired by Emmanuel Adukwu, Chairman of the PECS Committee, provided an opportunity for two invited speakers to give delegates an introduction to two molecular techniques. The first speaker was Professor Valerie Edwards-Jones of Manchester Metropolitan University. Val began by presenting her predictions for the future applications of mass spectrometry in the identification and characterization of microbes. She continued with a discussion of the parallel development of microbiology and mass spectrometry and how surprisingly, this has only recently begun to combine to develop novel applications. Val focused on the technology behind MALDI-TOF and how rapid identification of clinical specimens has been a driver in the development of the technology. Professor Edwards-Jones concluded with the prediction that within 20 years, we could see a very simple identification system involving a swab, solvent and processing in a mass spectrometer to give immediate results, thereby enabling rapid and effective treatment of infection.

Following Val, Dr Lori Snyder, Reader at Kingston University London, discussed the potential uses of next-generation sequencing in mixed bacterial populations. Lori has worked on *Neisseria* spp. throughout her career and has most recently been using next-generation sequencing to generate and interpret very complex data sets. These have identified a multitude of highly variable gene regions, repeats and rearrangements which have only been hinted at before the application of this technology. Lori underlined the ease, speed and variety of uses of next-generation sequencing by explaining how it had been used for several undergraduate projects to great effect.

After a break and an attended poster session, the first of the student presentation sessions began. David Cleary discussed the use of next-generation sequencing in defence. His PhD had assessed this technology in its ability to provide bio-warfare contaminant identification, source and dissemination patterns in the field. The next speaker, Benjamin Folwell spoke about his research into bacterial-algal co-cultures and their ability to degrade waste products from tar-sands refining processes. He has generated an artificial co-culture which may degrade waste substances which have historically proven impossible to degrade.

Diriisa Mugampoza was up next and presented his research on *Lactobacillus* spp. isolated from Stilton cheese. Diriisa had studied *Lactobacillus plantarum* strains isolated from three precisely sampled sites of a Stilton cheese and had found they were genomically different. Diriisa then grew the strains for seven weeks under conditions designed to simulate cheese ripening. He found that the strains produced different types and levels of volatile compounds which could

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

influence the aroma of the cheese and, in turn, lead to batch variation. The final presenter before lunch, Jonathan Nzakizwanayo, talked through his work on the mechanisms of host-probiotic interactions in *E. coli* Nissle. His research has identified mutants which show altered biofilm and cell adherence, and this has implications for understanding interactions between *E. coli* and intestinal cells.

After a delicious lunch and opportunity to network and visit the trade show, the second set of student presentations began with Tolulope Oyebanji. Tolulope compared a Smartcycler® and commercial assay method with the current standard TaqMan® method for diagnosing chlamydia infections. The CPA recommend using a combination of test methods for reliable identification, the Smartcycler® and assay method was shown to have high specificity, reliability and importantly it reduced turnaround time when used in combination with another current method. The penultimate talk of this session was by Aled Roberts, looking at the effects of manuka honey on *Pseudomonas aeruginosa*. Aled cultured *Ps. aeruginosa* with manuka honey and investigated the effects using a variety of molecular techniques. Interestingly, the results suggested the honey disrupts the cell envelope causing lysis and also reduces the number of cells with flagella in the surviving populations.

The last student talk, by Dewi Yunita, was on the microbial floral dynamics of Stichelton cheese. Using 16S rDNA, she assessed the species found in the cheese at various stages and concluded that surface contamination, piercing practices and competition affect the composition of the microbial community in different portions of the cheese.

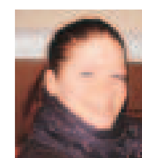
Following the fascinating student presentations we returned to the main topic of the meeting. The first invited speaker of the afternoon was Dr Andrew Sails, Health Protection Agency, Newcastle. The topic of his talk was the applications of real-time PCR in the clinical microbiology lab and how this technology had revolutionized the way infectious agents are identified. He illustrated how completely different infections can cause identical symptoms and how outcomes ranged from minor to fatal. This uncertainty has led to the use of real-time PCR in an attempt to give rapid diagnoses and appropriate treatment. Dr Sails suggested the main problems with older PCR techniques and ELISA assays were the technical skill, reliance on gel electrophoresis and time required to get from sample to diagnosis. These factors meant that PCR was of limited use in diagnostic laboratories. With real-time PCR, a closed tube system limits amplicon carryover and monitoring in real-time gives immediate identification and accurate quantification. Andy finished by stating that the success of this system, and the move toward its use in frontline laboratories, has seen a significant improvement in the diagnosis of infections.

The final speaker of the day was Dr Ali Ryan of Kingston University, London, who presented a talk on the application of microbiology to recombinant protein production. Dr Ryan discussed how most protein production systems utilize *E. coli* due to its high growth rate and high protein yields. It has been found that more complex proteins which require post-translational modification were not suitable for production in *E. coli*. Ali ran through the strengths and weaknesses of the bacterial versus the yeast system and how the protein production system needs to be chosen with the end product in mind. Ali finished with a warning about choosing an appropriate system as the quality and quantity of protein produced may be severely affected by the use of an inappropriate type of system.

The day finished with congratulations from Professor Mark Fielder for a successful first conference and presentation of the awards for undergraduate and postgraduate poster and oral presentations.

The poster awards went to Tosin Onabanjo (postgraduate) and Victoria Bonner (undergraduate).

The oral presentation award went to Benjamin Folwell (postgraduate). Plans are already underway for next year's conference and we hope you can join us for another fun and informative day!



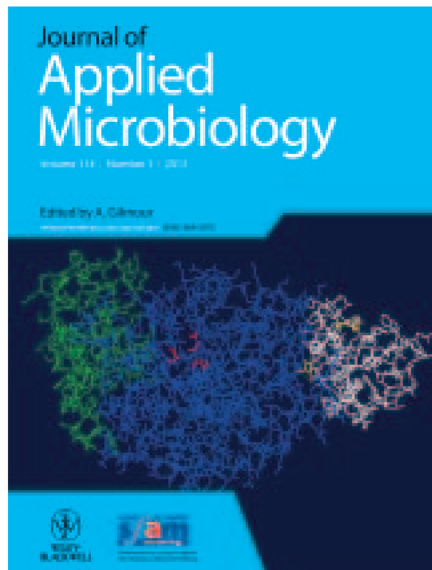
Jo Tarrant
PECS Committee Member

Journals how to get the best out of them

There are certain vital tools needed to carry out scientific research. One of these is the capacity to access up-to-date research in your field. Although textbooks can be a fantastic source of information, the process of editing and publishing a book is long, whereas research papers, periodicals and clinical trial reports etc. are regularly (and more quickly) published in scientific journals. The ability to use journals for a literature search is one that improves with experience, but this article could provide you with a good place to start.

To perform high-quality research whether you are an undergraduate/MSc undertaking your dissertation, or a postgraduate embarking on the daunting task of a doctoral thesis, knowing how to access, use and reference published peer-reviewed articles is imperative. There are a number of difficulties with accessing journal articles. Not only do you have to take into consideration the quantity of articles published regularly (it can become difficult to isolate the information you need) but also the issues surrounding availability of the journals (is it online? Do you have to pay for it? Has the article been published yet?).

For easy access to journal articles, using databases such as Web of Knowledge, MEDLINE, ScienceDirect and PubMed among many others is very handy. Other tools that are used include search engines such as GoogleScholar, which in addition provides detailed information on the number of citations made from each article. University library catalogues can also be a good source, and in cases where journals are unavailable, utilizing the inter-library loan system can be really useful (try contacting your local librarian to find out more about this). Important things to consider at this point include the journal publisher. For example, the Society for Applied Microbiology publishes several journals such as '*Journal of Applied Microbiology*', '*Letters in Applied Microbiology*', '*Environmental Microbiology*', '*Environmental Microbiology Reports*' and '*Microbial Biotechnology*'. Make sure that you look at the impact factor of a journal; this equates to the average number of article citations that have been recently published within it (the higher the better really). This can be used as an indicator of the importance of a journal within its specific field.



Now that you know how to find the journals, the first aspect of research is to know what you are searching for. Here are some helpful searching tips that enable easy identification and access to relevant published materials: **refine your research questions into single concepts**; what are the key words for your search? Only use what is most relevant to your investigation. Are you interested in a specific phrase? Try using quotation marks "...". Are there alternative words for what you are researching? Add all of these in by inserting the word "OR", alternatively try truncating the word by using "*". Try combining concepts by introducing "AND" into the search. Now we are going to run through an example of a research question...

"An investigation into the fortification of wholemeal bread with vitamin C"

Key words: Fortification, vitamin C.

Alternative words: fortification/ fortify/fortified; vitamin C/ascorbic acid.

Truncated words: Fortif*.

Phrase searching: "Wholemeal bread".

Combining search words: Fortif* AND "wholemeal bread" AND (vitamin C OR ascorbic acid).

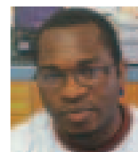
Try to be selective with the articles that you identify from your search. If you are planning on conducting a human trial, then try to focus more on the articles about trials rather than literature reviews. Look at the date of the article; if you are using a new method to analyse your data the chances are that an article from 1972 will not be helpful! Also assess the particulars of the authors, the research methods used in the publication, the data collection, research design and statistical significance of the study.

Journals can be used not only for literature searches, but also to discover new methods, and to verify findings with other researchers in the same field. Don't be scared to expand your search and then narrow it later; try not to feel overwhelmed with all of the information out there — eventually you will find what you are looking for! With all this in mind, you are ready to begin your search for your journal articles. Get searching and have fun!

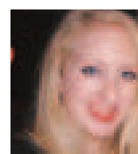
handy tips

Top three handy tips when searching journals:

- COMBINE SEARCH WORDS — use *AND* to narrow and *OR* to enlarge your search.
- Use TRUNCATING SYMBOL (*) to include plurals of words.
- PHRASE SEARCH using ("...") for more specificity.



Emmanuel Adukwu
PECS Chairman



Jenni Drever-Heaps
PECS Publications Officer

Beetles, bees and bugs

Cynthia Sheffield describes the long and convoluted path of her microbiological career

My path to working in the field of microbiology is a long and convoluted one. I was interested in science from a young age, loved to solve puzzles and figure out how things worked. As a kid I was fascinated by insects and loved to walk in the woods and watch the beetles and ants going about their business.

I began my college career with a BS in Entomology from the University of Florida. As an undergraduate, I was fortunate to have the opportunity to become involved in research at the United States Department of Agriculture (USDA) Man and Animals Research Laboratory in Gainesville, Florida. I first worked on plum curculio beetles looking for attractants. Later I worked with fire ants as an undergraduate. These opportunities really sparked my interest in scientific research as a career path. After receiving my BS I worked for a couple of years as a laboratory technician at the University of Florida in the Food Science Department.

I then took a 90 degree turn from the laboratory and pursued a Master's degree in Extension Education at the University of Florida. While extension education was interesting, it seemed that I was destined to return to research because after graduation I moved to Washington and got a job in the Entomology Department at Washington State University. The work involved preventing bacterial and fungal diseases of leafcutter bees through the use of food preservative compounds. This was my introduction to microbiology. I spent many afternoons and evenings in the alfalfa fields of middle Washington State spraying powdered food preservative agents into leafcutter bee nesting boards. During this time I also worked with ground nesting bees and spent time using a backhoe to dig up nests for study in the lab.

After a change of venue from Washington to Texas I again got a job with the USDA working as a technician at the Food Animal Protection Research Laboratory in College Station, Texas. I began working with stable flies, examining their dietary requirements and feeding habits. One of the challenges of this work was to devise a system that would isolate the various components of red blood cells without cross-contamination.

After about two years I was offered a graduate fellowship to pursue a PhD in animal physiology with a subspecialty in immunology. My research focused on designing a means of extracting the various components of red blood cells such as the haemoglobin and cell wall without cross-contamination of the components. I successfully designed a system that extracted the haemoglobin from red blood cells without any contamination from the cell wall components. This pure haemoglobin was used for stable and tsetse fly diet research, as well as the basis for a cross-species blood substitute, while the "ghost" red blood cells were loaded with anticancer drugs and used as a delivery system.

I know what you're thinking about now: where's the microbiology connection? Hold on, it's coming. As a freshly minted PhD I made my way to the USDA Plum Island Animal Disease Research Laboratory in Plum Island, New York. There I worked on the foot-and-mouth disease virus. We worked on possible methods to generate an effective vaccine for use in cattle.

Another opportunity arose and I found myself in Guatemala City, Guatemala, serving as the regional director for a company that was employing the techniques I developed during my PhD to design a human blood substitute based on cattle haemoglobin. After some very successful clinical trials, greed reared its ugly head and the company I worked for became entangled in a protracted legal battle with one of their partners.

So, once again my career changed direction and I found myself back with the USDA at College Station, Texas. As a postdoctoral student, I was given the task of developing an immunoassay against bovine haptoglobin for the detection of preclinical illness in cattle.

This work led to a patent for the immunoassay which was later licensed to a private company.

After my postdoctoral appointment was concluded, I decided to once again venture into the world of private industry. I went to work for a small start-up research company based in College Station, Texas. There I initiated research that led to the development of a first-generation orally administered sustained release vaccine against *Bacillus* species for the US Army at Ft. Detrick, Maryland. I also conducted research into the use of ozone to sterilize poultry layer facilities and transport vehicles.

After a couple of years at this company I decided to move into management, so I became the Assistant Laboratory Director for SpectraCell, Inc. which was a private company that conducted immunological based tests to examine the body's vitamin status. At this job I spent most of my time away from the bench and on the phone with physicians explaining how our assays worked. I also supervised about 30 people, which I quickly learned was not nearly as much fun or rewarding as research. So, after about three years I came back to my true calling, bench science.

I again found myself at the USDA in College Station, Texas. I became involved with pre-harvest poultry food safety. I primarily try to devise ways to assist the broiler producers to alter their management practices to reduce or eliminate the presence of *Salmonella*, *E. coli*, *Campylobacter* and opportunistic bacterial pathogens within their production facility. Here is where the microbiology comes into play: my focus is on manipulating the microbiome within the gut of the birds and the production facility environment. This work is rewarding and constantly challenging as anything that I may find that holds promise, in addition to working in the field must be simple to use and, most of all, must be extremely inexpensive. The requirement that the "solution" be inexpensive is probably one of the most challenging aspects of this work. As scientists we can often find ways to eliminate problems in the lab or even in the field but making them fit the financial needs of our producer partners is not always so easy.

I look forward to continuing this area of work for several more years to come or until one of my fellow researchers finds the magic bullet to make our poultry food supply safe in an economical way.

Cynthia Sheffield
United States Department of Agriculture, Texas, USA

Practical tips for protecting your inventions

When setting up an intellectual property (IP) capture and filing strategy for a company or an academic organization, it may seem that there are an endless number of pitfalls for the unwary. However, a few tips can save you money and stress in the long run

As an initial point, a company or academic organization will want to ensure that all of its potentially valuable IP is captured and assessed so that it can decide what to do with that IP. Options include filing a patent application, keeping that information confidential as know-how, or protecting it using other forms of registerable IP such as trade marks or registered designs. It is good practice to ensure that scientists keep up-to-date lab books, whatever the option ultimately taken, and then the lab books can be reviewed on a regular basis, for example every six months, to ensure that any potentially interesting developments are assessed.

There can be competing pressures on scientists, particularly in academia, to publish their results in scientific journals whilst their employer may want to file patent applications. Here, IP education is essential to ensure that the scientists are aware of the importance of not disclosing their inventions until a patent application is safely on file. Also, be aware that an advance online publication also counts as a publication for patent purposes.

However, care should be taken not to rush into filing a patent application without having the requisite level of data. The main requirements of patentability are:

1. Novelty — the invention must not

have been disclosed in any form, anywhere, before the patent application is filed. This includes written disclosures (such as published patent applications or journal articles), oral disclosures (such as presentations at conferences), and uses of the invention.

2. Inventive step — the invention must not be obvious to a notional "person skilled in the art". This is an ordinary, unimaginative practitioner possessing common general knowledge in the specific technical field to which the invention relates.
3. It must be described in a manner sufficient for the person skilled in the art to carry it out.

This latter requirement of sufficiency is one that bites for many inventions in the biotechnology field, and means that a patent application needs to be supported by some data which shows that the invention actually works. Having said that, you should not hold back from seeking an opinion on patentability from a patent attorney, if you are concerned about the level of data obtained. Your patent attorney will be able to advise whether the level of data is sufficient, and if not, will also be able to guide you on the type of experiments that need to be carried out to achieve the required standard.

Once a potential invention has been identified, you will likely consult a patent attorney to discuss the patent filing procedure. Many will think that the process of drafting and filing a patent application is an expensive business, and there is no denying that this can be the case, but costs can be reduced if you can provide your patent attorney with a little extra information at this time.

Firstly, if you can carry out some internet searching beforehand, this will generally be very useful as it can flush out the most relevant documents. Even something as simple as a Google search using some keywords can therefore help, as can a search using a tool such as PubMed. Using the European Patent Office database, esp@cenet, will help identify relevant patent documents. Providing any search results that you have identified to the patent attorney when giving them the initial information may save you a huge amount of money; if you identify a document that is extremely relevant to your invention, then it may not be worthwhile filing a patent application at all. It is better to know this early on, rather than paying to get a patent application drafted and filed, then have the Patent Office tell you the same!

Next, once a decision has been taken to actually file a patent application, you can help the patent attorney in the drafting process by providing some further information. Some of the questions that your patent attorney might ask include: Which features are essential to the functioning of the invention? Are there any other ways that the invention can be carried out apart from the one you have described/identified? Does the invention solve a particular technical problem? Is there something surprising about the results that you have obtained?

Many companies and academic organizations have invention disclosure forms set up for completion by the inventors when a potential invention has been identified, and these will pose the most relevant questions, the answers to which will help in both deciding whether to pursue patent protection, and in the later preparing of any patent application. Providing your patent attorney with the answers to these questions will enable them to draft the patent application so that it covers the invention in an appropriately broad manner, and also to demonstrate that there is an inventive step involved.

Once the patent application has been drafted, the most common filing strategy for UK applicants is to file a UK patent application. It is also worthwhile asking the UK Intellectual Property Office to carry out a search during the priority year. This is relatively cheap and again should identify the most relevant documents. If you are happy to spend a bit more, you may wish to consider filing a European patent application instead of a UK patent application, as the search carried out by the European Patent Office is generally considered to be more thorough and may identify some documents not identified by the UK Intellectual Property Office. However, the filing fees are more expensive. That said, as noted earlier, it is probably better to know sooner rather than later if there is a particularly relevant document out there which means that your invention is not novel or lacks an inventive step.

A further cost-saving filing strategy used by the majority of applicants is to file an international (Patent Co-operation Treaty — PCT) patent application at the end of the priority year. Although the cost of filing a PCT patent application is relatively high, the so-called "international phase" lasts another 18 months, thus giving the applicant this extra time to consider in which countries they wish to finally file their patent application, taking into account any commercial developments during this time. Also, another search and examination is carried out during the international phase, which allows for a further assessment of the patentability of the invention.

At the end of the international phase, the applicant must select the countries in which they wish to seek protection and file so-called "national phase" patent applications in each of these countries. As many will be aware, the usual procedure for applicants wishing to cover the European (EU and EEA) countries is to file a European patent application, rather than national patent applications in the countries of interest.

Another cost-saving measure which can be implemented at that stage is to use one of the Patent Prosecution Highways. These are a series of agreements between numerous different Patent Offices around the world, aimed at reducing workload between examiners in the different Patent Offices by sharing any search and examination results. In practice, it means that an applicant with a patent allowed before one Patent Office can use that allowed patent to expedite proceedings before a different Patent Office. There are currently a large number of Patent Prosecution Highways in operation and your patent attorney will be able to advise on the applicability of these to your patent application.

As can be seen, there are lots of issues to consider and different options to take. However, your patent attorney will be able to advise on the most cost-effective approach for you, depending on the nature of your business, your commercial goals and the type of technology.

Sarah Lau
Patent Attorney,
Kilburn & Strode LLP

Laboratory Fellowship Grant Report

A summer well spent: diving into the diversity of microbes

The Microbial Diversity Course is an intensive six-and-a-half-week summer course held at the Marine Biological Laboratory in Woods Hole (Massachusetts, USA), which creates an unmatchable atmosphere for 20 scientists from all over the world.

A typical day at Woods Hole started with two morning lectures about the latest discoveries and most up-to-date findings in microbiology. Topics covered included: how do viruses avoid extinction, ecology and physiology of ammonia-oxidizing archaea, nitrogen physiology and niche adaptation in marine cyanobacteria, the phototrophic way of microbial life, competition and cooperation in microbial communities, phylogenetic approaches to microbial diversity, stable isotope probing in microbial ecology, chemotrophic anaerobic respirers: acetogens, methanogens and sulfate reducers, microbial ecology of deep-sea hydrothermal vents, microbial population ecology and evolution, metagenomics and chlorinated solvents, unravelling the pathways and roles of organisms in anaerobic benzene-degrading cultures, SIMS and microbial ecology, the human microbiome and host metabolism.

Following the morning lectures, lunch represented a perfect opportunity to interact with the speakers, ask further questions and discuss the students' projects at their home institutions. Laboratory training sessions were scheduled for afternoons and evenings with some sessions taking place late at night until the early morning. These sessions were under the supervision of the course instructors and were designed to provide the students with intensive training in state-of-the-art microbiological techniques.

Amongst the various techniques that we learnt, a great deal of importance was given to enrichment and isolation techniques for microbes from marine, freshwater and terrestrial environments. Students were divided into groups of five and each group was responsible for enriching and isolating many different microbes and was taught and driven to think thoroughly about the choice of inoculum, media composition and other conditions in order to successfully enrich for the organism of interest. Each group routinely monitored the cultures macroscopically through turbidity, odour, changes in colour and colony morphology and microscopically by monitoring motility, auto-fluorescence, cell shape and size, and number of different cell types. All groups managed to successfully isolate bioluminescent bacteria, actinomycetes, lactic acid bacteria, aerobic methanol oxidizers, hyphomicrobia, myxobacteria, violacein-producing bacteria, cellulose- and chitin-degrading aerobes, aerobic nitrogen fixers, manganese oxidizers, anaerobic fermentative nitrogen fixers, oxygenic phototrophs, anoxygenic phototrophs, chemolithotrophic sulfur-oxidizing aerobes and chemotrophic anaerobes.

Bioinformatics constituted an equally important share of the training sessions and a thorough training in bioinformatics programmes such as ARB, RDP, QIIME, RAST and MG-RAST was offered. During the first half of the Microbial Diversity Course, two group reports and presentations, a mid-term report (1½ weeks into the course) and a final report (3 weeks into the course) were submitted. A couple of symposia were organized on the weekends, focusing mainly on the microbial groups driving climate change.

Microbial mat

The second half of the Microbial Diversity Course was devoted to individual mini-projects; each student had to conduct a research project of their own design based on the techniques and concepts they had learnt during the first half of the course. The aim of my mini-project was to study the variation in the structure and composition of phyllosphere microbial communities with respect to leaf age and to optimize a catalyzed reporter deposition (CARD)-FISH method allowing me to visualize different microbial cell types directly on leaf surfaces without the need to wash the cells off the leaf. This limited the loss of cells by washing and allowed me to observe the arrangements and associations of the microbes with the leaf and amongst themselves. The course ended on the 26 July with a graduation ceremony in which students were given certificates, after having presented their mini-projects and submitting a written report to the course coordinator.

Stereoscope image of fruiting myxobacteria

My participation in the Microbial Diversity Course 2012 was an enormous contribution to my training as a microbiologist and I am grateful for my supportive supervisor Professor Colin Murrell at the University of East Anglia (UK), the Society for Applied Microbiology, the Microbial Diversity Course Co-directors Professors Stephen Zinder and Daniel Buckley, instructors and students for giving me this opportunity and making it a very valuable experience. I thoroughly recommend the Microbial Diversity Course to fellow microbiology students.

Myriam El-Khawand
University of East Anglia

Students into Work Grant reports

am I eligible — can I apply?

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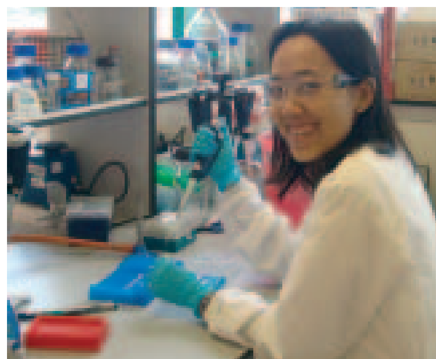
Identification of wild yeasts in brewing samples by combination of PCR and RFLP-based analysis of the 5.8S rRNA gene and the two ribosome internal transcribed spacers



This project was carried out at the School of Biosciences, University of Nottingham, UK in collaboration with VTT Technical Research Centre of Finland and focused on molecular identification of yeast contaminants from an industrial brewery. The aim was to test the use of a molecular technique Internally Transcribed Spacer PCR (ITS-PCR) and RFLP for the reliable identification of potentially problematic non-brewing yeasts in an industrial brewery. Yeasts occurring as contaminants of the brewing process are considered 'wild' yeasts. In practice, they can be distinguished from brewing cultures by their ability to grow on a range of well-defined selective substrates (Jespersen *et al*., 2000). Wild yeast present even at very low concentrations, can cause beer spoilage and result in haze and off-flavour development (Smart & Powell, 2005).

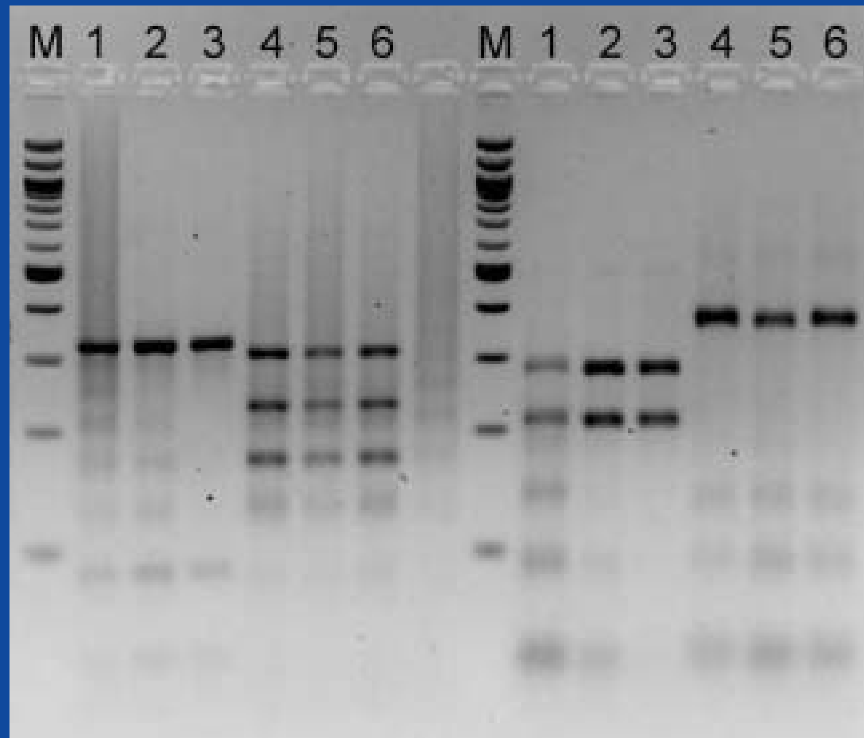
Brewing contaminants are traditionally divided into *Saccharomyces* and non-*Saccharomyces* species. The majority of *Saccharomyces* spp. detected belong to *S. cerevisiae* but other *Saccharomyces* spp. are also recorded (Jespersen *et al*., 2000). Non-*Saccharomyces* spp. usually belong to the *Brettanomyces*, *Candida*, *Debaryomyces*, *Filobasidium*, *Hanseniaspora*, *Kluyveromyces*, *Pichia*, *Torulaspora* and *Zygosaccharomyces* genera (Smart & Powell, 2005). Traditional methods for detection, identification and characterization of wild yeasts are based on chemical, morphological and physiological characteristics. These techniques are widely used in the brewing industry due to their simplicity, however, they are time-consuming (several days or weeks are required) and can produce ambigous results. Therefore, molecular techniques emerge as complimentary or alternative methods with improved speed, accuracy and reliability.

The DNA encoding the ribosomal RNA (rDNA) has been used for the determination of a wide range of yeast and fungal species. This method relies on the conserved nature of rDNA, which means isolates from the same species maintain the same sequences and the differences in the sequence of rDNA indicate phylogenetic diversity. The current method is based on the PCR amplification of the intergenic transcribed spacer (ITS) region, followed by RFLP of the amplified fragments. The amplified region spans from the 3' end of the 18S rDNA to the 5' end of the 25S rDNA, including the 5.8 rDNA and the ITS1 and ITS2 regions (Egli & Henick-Kinh, 2001). No work focused on identifying wild yeasts present in the brewing process has yet been documented. Therefore, the aim of this study was to develop a simple protocol for the DNA extraction from yeasts and the use of a combination of PCR and RFLP to identify wild yeast contaminants to species level.

We isolated wild yeasts found in brewing samples from industrial fermentation vessels (FVs) and conditioning tanks (CTs) and analysed these along with several yeast strains obtained from the National Collection of Yeast Cultures (NCYC, Norwich, UK), Industrial Yeasts Collection, Dipartimento di Biologia Vegetale e Biotecnologia Agroambientale (DBVPG, Perugia, Italy), and VTT Culture Collection (VTT Technical Research Center of Finland, Espoo, Finland). DNA was extracted from single colonies and ITS1 and ITS4 primers were used to amplify the ITS region (Esteve-Zarzoso *et al*., 1999). The endonucleases *Cfo*I, *Hae*III and *Hinf*I were used to digest the PCR products. Samples were visualized by electrophoresis and band sizes estimated by comparison with a 100bp ladder (Figure 1). Results obtained were compared with those from reference and type strains from the culture collections listed above as well as with previously published results.

The ITS-PCR product for *Saccharomyces* spp. is approximately 880bp while for *Candida* spp. size varies from 380bp to 450bp, and 500bp to 650bp for *Pichia* spp. In most cases, strains within a species share the same size for amplified products and similar patterns for restriction fragments. However, some strains including *Issatchenkia orentialis* and *Saccharomyces pastorianus* showed

Figure 1. *Restriction pattern of the PCR-amplified rDNA region of six separate isolates obtained from industrial brewery vessels. Restriction endonucleases were HaeIII (left) and HinfI (right). Fragments were separated by electrophoresis on a 4% agarose gel*



variations in their digestion patterns in some specific strains.

Wild yeast contaminants, 76 in total, were isolated from eight samples collected from conditioning tanks and fermentation vessels in different batches. The results showed that ITS-PCR can be used to detect and identify the majority of wild yeasts present in the brewing process. The predominant wild yeast species identified were *Candida auringiensis*, *Pichia fermentans*, *P. paradoxus*, *P. membranaefaciens*, *P. kluyveri*, *Rhodotorula mucilaginosa* and *S. cerevisiae*. Among these isolates, *S. cerevisiae* made up the largest proportion (60% in the FV and 33% in the CT). Proportionally more *Pichia* species were found in the CT, indicating that different brewery processes support different populations of wild yeast.

This study has demonstrated the feasibility of using ITS-PCR and RFLP in the brewing industry to compliment traditional methods which mostly involve detection rather than identification. The increased accuracy of this molecular method compared with traditional methods may help to understand and control wild yeast contamination in the industrial brewery.

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Biomarkers for post-market monitoring of GMOs in pigs

Having just completed my degree in Biology with Quality Management at Waterford Institute of Technology (WIT), I was delighted to be offered an S*f*AM-funded 10 week work placement. This gave me the opportunity to work as part of a multidisciplinary inter-institutional research team with WIT, Teagasc Pig Production Development Unit and Teagasc Moorepark Food Research Centre. This research team is part of the GMSAFOOD project, which is funded by the European Commission under the Framework 7 Programme and is comprised of a consortium from Austria, Hungary, Turkey, Ireland and Australia. The objective of the GMSAFOOD project is to identify biomarkers for post-market monitoring of GMOs. The research I participated in examined the effects of feeding GM MON810 maize to pigs, with particular emphasis on the intestinal microbiota, intestinal histology and immune status.

The main driver behind this research is the fact that cultivation of GM crops is increasing, with 101 million hectares grown globally in 2006. GM has the potential to increase yield and/or product quality in a range of globally important food crops. However, increased usage of GMOs, both for human consumption and in animal feed has raised safety concerns. Pre-market risk assessment is required in order to obtain EU authorization for a GMO but further evaluation for adverse effects should be considered once the product has reached the market. MON810 maize is an example of a GM crop that has been authorized in the EU. It incorporates a gene encoding an insecticidal protein derived from *Bacillus thuringiensis* (Bt), conferring resistance to the European corn borer, a major insect pest of maize. This 'Bt maize' has been tested for toxicity and allergenicity, mainly in rodents. However, limited studies have been performed in pigs and further pig-feeding trials are required considering that pig meat is the principal meat produced in the EU (17.9 million tonnes in 2005) and that a significant proportion of pig diets will come from GM feedstuffs in the future (even currently it is estimated that 100% of pig diets, at least in some EU countries,

contain GMOs at levels exceeding 0.9%). In addition, pigs are a better human model than rodents, as porcine anatomy, physiology, and immunology are more similar to that of humans. Furthermore, there is an absence of data on the effect of GMOs on the intestinal microflora in any species. This analysis is warranted considering the importance of the gut microbiota for both animal and human health, and in light of the fact that a recent publication has demonstrated antimicrobial activity for the *Bacillus thuringiensis* insecticidal proteins.

A 147-day pig-feeding trial was underway when I commenced my work placement. Weaned pigs (n = 72) had been blocked by weight and ancestry, and randomly assigned to the following dietary treatments (18 pigs/treatment); (1) Non-GM isogenic parent line maize fed to slaughter; (2) GM maize fed to slaughter; (3) Non-GM maize fed for four weeks followed by GM maize fed to slaughter; and (4) GM maize fed for four weeks followed by non-GM maize fed to slaughter. Faecal samples had been collected for microbiological analysis from 10 pigs per treatment (40 pigs) prior to the commencement of treatments and on day 28 (weaner period) to monitor changes in culturable gut microbiota. I was involved in microbiological analysis of the day 56 (grower period) and day 98 (finisher period) faecal samples as well as ileal and caecal digesta samples collected at slaughter at the end of the trial. This enabled me to improve the microbiology skills that I had already developed during my degree. I prepared media and poured and labelled plates and together with the microbiology team I plated the faecal and intestinal digesta samples on a range of media. We enumerated total faecal anaerobic bacteria by spread-plating on Wilkins Chalgren agar in an anaerobic cabinet followed by anaerobic incubation at 37°C for five days. Key intestinal microbiota were also enumerated by pour-plating on selective media; *Enterobacteriaceae* (indicators of pathogenic bacteria) were enumerated on violet red bile glucose agar (VRBG), and lactobacilli (considered beneficial) were enumerated on *Lactobacillus* selective agar (LBS). The VRBG plates were overlaid and incubated overnight at 37°C, while the LBS plates were incubated anaerobically at 37°C for five days. Following incubation, I counted the colonies and calculated the CFU per gram of faeces for each of these microbial groups. In addition, to monitoring effects on unculturable intestinal bacteria, faecal and digesta samples were frozen for 16S rRNA gene sequencing.

The work placement also enabled me to gain experience in other disciplines in which I had only limited experience; namely histology and immunology. Small intestinal samples were taken from the pigs at slaughter for histological analysis to determine the effect of feeding GM maize on intestinal morphology. I learned how to process intestinal samples and embed them in paraffin wax. Once the wax moulds were made I used a microtome to slice the tissue samples for slide preparation. I stained the slides with haematoxylin and eosin stain and using specialized computer software, connected to a light microscope, I was able to capture images of villi and crypts from the duodenum, jejunum and ileum of pigs and measure the villus height and crypt depths. These measurements provide an indication of intestinal health. Another key element of the pig-feeding trial was to monitor changes in immune status in response to feeding GM maize. To measure this, I helped isolate peripheral blood mononuclear cells (PBMCs) from blood, splenocytes from the spleen and intraepithelial lymphocytes from the ileum. All of these primary cells were mitogen stimulated and cultured overnight. The following day I antibody stained the cells and helped to run them on the flow cytometer to measure changes in immune cell phenotypes. I also measured cytokine production by the stimulated PBMCs, splenocytes and lymphocytes using ELISA kits.

I would like to thank S*f*AM, as the Students into Work grant has provided me with the opportunity to gain first-hand experience of working in a number of research laboratories and has helped me to come to the decision that I would like to continue to PhD level. I would also like to thank my supervisors Dr Gillian Gardiner (WIT) and Dr Peadar Lawlor (PPDU) who made it possible for me to undertake this studentship. Thanks are also due to Dr Maria Walsh, Mary Rea and Stefan Buzoianu for their assistance throughout the project.

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Waterford Institute of Technology

The recently assigned *Cronobacter* genus

During the summer break between the second and third year of my Microbiology degree at Nottingham Trent University, I was fortunate enough to be offered the opportunity to apply for S*f*AM funded laboratory experience, and was able to use this to further some voluntary study I had previously undertaken. Support from the Society enabled me to carry out some experimental work with a group of important emerging pathogens — the recently assigned *Cronobacter* genus (Iversen *et al.*, 2008).

Cronobacter is a ubiquitous organism and causes infections in all age groups. Of particular concern are the albeit rare but serious infections of neonates, particularly pre-term or low birth weight infants (Bowen & Braden, 2006) in which it can cause a range of infections including septicaemia, meningitis and necrotizing enterocolitis, which can lead to fatalities (Caubilla-Barron *et al.*, 2007). *Cronobacter* infant infections have been associated with contamination of infant formula, either in processing, packing or administration, though other sources may exist (VanAcker *et al.*, 2001).

Cronobacter strains have been associated with a range of different infections often involving inflammation of host tissue. However, the ability to induce inflammation is common to all host types infected. So, is a strain associated with necrotizing enterocolitis able to induce a higher immune response in enteric cell lines than other cell types? Are meningitis associated strains able to induce a greater response in brain barrier cells than in enteric cells? The main experimental aim of the work was to test a series of strains which cause known pathologies with a range of mammalian cell lines and to measure the immune response in terms of inflammatory cytokine release. The cell lines selected for use were CaCo2 cells (human colonic), HBMEC (human brain microvascular endothlelial cells) and RBMEC (rat brain microvascular endothelial cells).

Cronobacter strains were selected to be associated with a range of infection types, including some associated with meningitis, septicaemia and necrotizing enterocolitis, levels two and three, as well as some strains isolated from infants without causing observable symptoms, and some which were isolated from

neonatal nasogastric feeding tubes.

Unfortunately during the time available for experimentation, we encountered difficulties in culturing the CaCo2 cells and were therefore unable to use these for testing. HBMEC and RBMEC cultured well and were tested using a standardized method. Each bacterial strain was cultured on TSA and then grown in TSB overnight at 37°C before being diluted with a serum-free tissue growth medium, to a standard optical density to be added to tissue culture plates. The tissue cultures were grown using a standard procedure and were inoculated onto 24 well plates 48 hours before each experiment was carried out to provide a good lawn of growth. At T = 0, growth media was removed from the tissue culture, the wells were rinsed with PBS and the diluted bacterial samples were added (500μl per well) in triplicate. At this point tissue cell blanks were also taken off and frozen down. The samples were then incubated for 6 and 24 hours, at each point samples were taken off and frozen down, whilst also being used for serial dilution plates to confirm bacterial growth in each sample.

The frozen samples were studied using BenderMed Systems Human Th1/Th2 11-plex Flow Cytomix kit and the Beckman Coulter Cytomics FC 500 machine, at Nottingham's Queen's Medical Centre hospital. The first obvious result was that bacteria elicited a much higher cytokine release from HBMEC than RBMEC. In HBMEC two cytokines dominated, IL-6 and IL-8, both associated with bacterial meningitis, and it was the expression of these that was examined when comparing strains of differing virulence. We were able to determine that the strain isolated from a case of fatal meningitis induced the highest release of inflammatory cytokine IL-6 and one of the highest IL-8, whereas a strain isolated from an asymptomatic patient in the same outbreak caused the lowest levels of both IL-6 and IL-8. The levels shown were similar to those released following infection with the meningitis causing group B streptococcal bacteria (Tenenbaum *et al.*, 2005) and higher than those when HBMEC were exposed to meningitic *E. coli* (Galanakis *et al.*, 2006). Over the exposure period no significant changes in the viability of the cell lines was determined and all strains of bacteria grew to approximately the same numbers.

The work that I have conducted over the summer has been highly beneficial to me as a student scientist, and I feel that it has shown an area for further study — rat pups have been used as a model to study the pathogenesis of *Cronobacter* species yet the difference in observed cytokine release under identical tissue culture conditions may suggest further differences in live animal studies. I would like to thank the S*f*AM for their support, the QMC for providing access to equipment, and to express my gratitude to all the microbiology staff at Nottingham Trent University, particularly Dr Loughlin without whom I wouldn't have had this great opportunity.

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Laura Tyzack
Nottingham Trent University

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Mucosal microbial communities in the oesophagus

Rich and diverse microbiotas exist in the human gastrointestinal (GI) tract. While the majority of investigations have been performed on lumenal bacterial populations, there has been increasing interest in the role of mucosal communities and how they impact on health and disease. Many of these communities consist of bacteria growing in biofilms associated with the mucosa or the mucus layer in the large bowel, and it is thought that they play a role in various GI conditions, including colon cancer and inflammatory bowel disease. Currently, the majority of studies have been performed on the lower GI tract, and there has been relatively little interest in mucosal populations

associated with the upper gut and oesophagus.

Early microscopic and cultural studies indicated that comparatively low numbers of bacteria ca. 10^2-10^3 per cm^2 were present on the human oesophageal mucosa, and that the predominant culturable bacteria were facultative anaerobes such as streptococci and lactobacilli. More recently, a culture-independent molecular study using oesophageal biopsies indicated that a large and heterogeneous range of bacterial gene sequences could be detected in the normal healthy oesophagus, with the majority belonging to the phyla Firmicutes and Bacteroidetes (Pei *et al.*, 2004).

The spatial location of mucosal bacterial communities enables them to come into close proximity to the oesophageal epithelium, and as with the involvement of *Helicobacter pylori* in gastric cancer, it is possible that there is bacterial involvement in similar conditions in the oesophagus. However, the presence of bacteria on mucosal surfaces could be simply due to passive transfer from gastric reflux, or oral contamination, therefore, it is important to determine if distinct viable mucosal communities occur in the oesophagus, and whether they differ in species composition in health and disease.

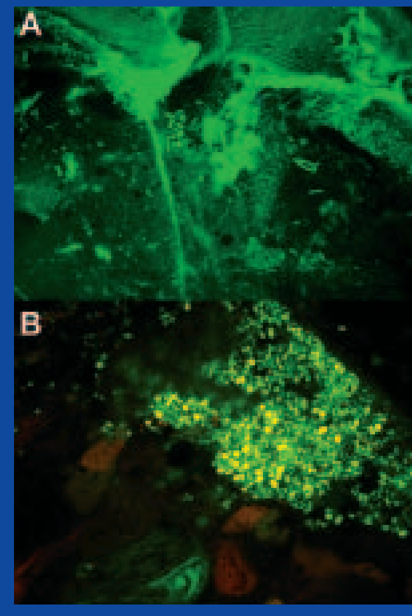
There has been a rapid increase in incidence of Barrett's oesophagus and oesophageal adenocarcinoma over the last 20 years (Van Soest *et al.*, 2005). The term Barrett's oesophagitis (BO) refers to a metaplasia of the lower end of the oesophagus, in which the usual squamous mucosa has transformed into a columnar-lined intestinal type mucosa. Approximately 10% of patients with gastro-oesophageal reflux disease (GORD) will develop Barrett's (Winters *et al.*, 1987); GORD leads to increased exposure of the distal oesophagus to gastric acid and bile, which results in erosion of the mucosa, ulceration and inflammation. The use of proton pump inhibitors to treat patients with GORD results in reduced gastric acid production, and as a consequence, microbial overgrowth in the upper gut. It is believed that all cases of oesophageal adenocarcinoma arise in patients with BO, however, the role of microorganisms in the adenocarcinoma aetiology is unknown. It is, therefore, of interest to determine if there are compositional differences in microbiota structure in BO, and if so, whether they can be linked to the progression of BO to adenocarcinoma.

To this end, we characterized microbial communities in oesophageal aspirates, and those on the distal oesophageal mucosae of seven healthy controls, and seven BO patients, using an extensive range of culture conditions for the isolation of aerobic, microaerophilic, anaerobic bacteria and yeasts (Macfarlane *et al.*, 2007). It was found that significant mucosal colonization occurred, with a diverse range of bacteria being detected in both patient groups. Distinct differences were detected in mucosal communities and aspirate populations, demonstrating that there was *de facto* mucosal colonization by these microorganisms. There was no evidence of *H. pylori* in either group. In total, 46 bacterial species belonging to 16 different genera were found from both the aspirate and mucosal samples. Eighteen different species were isolated from the controls, and 38 from BO patients, with 10 species being common to both groups. Differences were observed in mucosal communities in both the Barrett's (11 genera, 23 species) and control group (7 genera, 12 species) with seven shared species. Prevotellas were only found on the mucosa in both groups, and while lactobacilli were detected in aspirates and normal mucosal samples, they were not isolated from Barrett's mucosae. Of interest was the finding that two species of campylobacter (*Camp. rectus*, *Camp. concisus*) were detected in four of the BO patients, but none of the controls. These organisms are fastidious nitrate-reducing bacteria, and have previously been linked to periodontal infections, enteritis in children and the formation of tumours in animals. They are sensitive to the antibiotics included in selective media for campylobacters, and prefer a hydrogen-enriched atmosphere for growth. In the human body, dietary nitrate is concentrated in saliva, and higher numbers of nitrate-reducing veillonella were also found in Barrett's patients compared with the controls. Under acidic conditions, nitrite can be converted into N-nitroso compounds, which have been implicated as carcinogens in the stomach and lower intestine. Nitrite formation can also lead to the generation of highly diffusible nitric oxide, which inhibits DNA repair enzymes. Therefore, formation of these compounds might contribute to the increased incidence of neoplasia at the gastro-oesophageal junction.

The occurrence of bacterial communities on the mucosa was confirmed by confocal microscopy of oesophageal biopsies. This revealed that in both groups, the majority of bacteria were living, and that the organisms were frequently present in microcolonies or large aggregates on the oesophageal mucosa, with more extensive colonization being seen on the Barrett's mucosa (Figure 1).

These observations demonstrated that the oesophageal mucosa is actively colonized by complex microbial communities in health and disease. The results also showed that qualitative and quantitative changes occur in community structure in disease states such as Barrett's oesophagus. Treatment for adenocarcinoma is usually unsuccessful, and patients have low survival rates. However, recent studies have shown that there is considerable potential for altering the composition of mucosal communities in the GI tract, using functional foods such as prebiotics, probiotics and synbiotics (Macfarlane *et al.*, 2005). Future work

Figure 1. *Live/dead stain (BacLight) of healthy oesophageal tissue showing sparse growth and the presence of a small microcolony (A), and tissue taken from a patient with Barrett's oesophagus showing more extensive bacterial growth in aggregates (B). Green/yellow cells are living, red bacteria are dead*



in our laboratory will focus on how nitrate-reducing campylobacters interact with the oesophageal mucosa at the cellular level, the alterations that occur in the microbiota during the progression of BO to the cancerous state, and the therapeutic potential of functional foods to manipulate these mucosal communities with a view towards establishing protective microbiotas in the upper gut.

An award from the President's Fund was used to travel to the 2008 American Society for Microbiology Meeting in Boston, where part of this work was presented.

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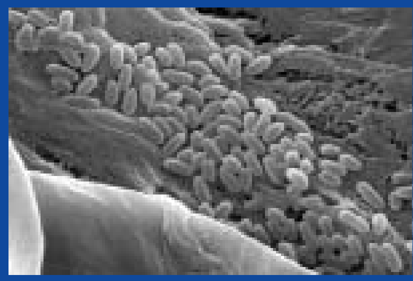
Sandra Macfarlane
Dundee University

Sampling for *Burkholderia* bacteria in the Sabah, Malaysia rainforest

In the summer of 2008 I had planned to attend the 12th International Symposium on Microbial Ecology — ISME 12, in Cairns, Australia (August 17–22, 2008). This meeting coincided with the official opening of the Danau Girang Field Centre (DFGC), managed jointly by Cardiff University and the Sabah Wildlife Department, located in the Lower Kinabatangan Wildlife Sanctuary in Sabah, Malaysia.

This area of Malaysia contains a number of different tropical forest types ranging from undisturbed pristine rainforest to managed secondary forests, as well as agricultural land planted primarily with oil palms. In addition to the forest plants, the Lower Kinabatangan Wildlife Sanctuary is famous for its diverse populations of jungle animals such as orang-utans, proboscis monkeys, Bornean gibbons and Bornean elephants.

Figure 1. Electron micrograph of Burkholderia cepacia colonizing the root surface of a pea plant



As I planned my trip to the ISME meeting, I also thought of the opening of the DFGC field centre and the potential microbial diversity present within tropical rainforests. Several *Burkholderia* species were isolated from rainforest soils and originally characterized as pseudomonads by eminent microbiologists such as Roger Stanier. As part of my ongoing research on *Burkholderia* bacteria, I have established a large collection of isolates. This collection has formed a crucial resource for ongoing studies on the ability to cause infection and the natural biology of these fascinating bacteria (Mahenthiralingam *et al.*, 2008).

Burkholderia species are known for their interactions with plants in the rhizosphere, the area of soil around plant roots. This naturally beneficial interaction leads to the protection of seedling plants from attack by pathogenic fungi in the soil, and has also been shown to promote crop yields in several agricultural plants (Figure 1). The plant-protective phenotype of *Burkholderia* bacteria has been attributed to their ability to synthesize a wide range of antifungal antibiotics such as pyrrolnitrin. However, little is known about the interaction of *Burkholderia* species with rainforest plants and how they may promote the fertility of tropical jungles.

In January 2008, I approached Dr Benoit Goossens, the DFGC manager about the possibility of visiting the jungle field centre and carrying out some microbial sampling. With his assistance, I obtained permission from the Sabah Wildlife Department to carry out a preliminary microbial sampling project to examine the diversity of *Burkholderia* bacteria interacting with the roots of jungle plants. To assist with funding of the visit as an extension to attending the ISME meeting, I successfully applied to the President's Fund, and then waited for the trip with great excitement!

My very first day in Sabah was already an experience of a lifetime. After a two hour drive from the airport, it was then a 40 minute boat ride up the Kinabatangan River to the field centre. Dr Goossens noted that a herd of about 180 Bornean elephants were about one hour from the field centre, so after dropping off my luggage we set off on the river to see them. Being on a small boat within just a few meters of such a large herd of wild elephants was quite amazing. The elephants clearly contribute to maintaining plant diversity in the rainforest through their grazing. Later on as I trekked through the jungle, it was also evident from the elephant dung that they have a fair impact on microbial diversity, especially on the distribution and fruiting of fungi.

Over the next four days I sampled the roots of rainforest plants within a three kilometre radius from the field centre, photographing all the plants as a record and noting their locations using global positioning satellites. On these trips, I encountered many other rainforest animals and insects, the highlight of which was a curious young orang-utan that came crashing through the trees to see what I was digging up. Each night back at the field centre, I homogenized the root samples with a small amount of sterile minimal media, and then dipped charcoal agar transport swabs into the suspension ready for shipment of the specimens back to my laboratory at Cardiff University. The trip was over all too soon, but I left with amazing memories of the wildlife and rainforest plants.

So what of the jungle microbiology? Since the beginning of October 2008, I have been working on the rainforest samples with three final year microbiology undergraduates, Rebecca Weiser, Matthew Bull and Ewa Karpinska, who are carrying out their honours projects in my laboratory. They have been screening the samples for antifungal activity as an indicator of the presence of *Burkholderia* species

bacteria. Several of the samples have already turned out to be positive for this trait as indicated by a zone of clearing of the yeast test organism around the root homogenate microbial growth. The cultivable diversity of microbes from one of these positive samples was separated by serial dilution to single colonies. Molecular speciation of the bacteria within the sample demonstrated the presence of a *Burkholderia vietnamiensis* isolate producing the antifungal activity, and also a *Burkholderia contaminans* strain which was dominant in the original sample. These results have demonstrated that the tropical rainforest rhizosphere is indeed a rich source of *Burkholderia* species. We will now continue to study the samples in detail, teasing apart the full diversity of cultivable *Burkholderia* species present in this unique rainforest collection.

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Eshwar Mahenthiralingam
Cardiff University

Ecotoxicity and biodegradability of the pharmaceutical diclofenac in the aquatic environment

Pharmaceutically active compounds (PhACs) in the aquatic environment have been a growing concern over the last 20 years. Human pharmaceuticals are not entirely metabolized and they are excreted in urine and faeces as parent compounds or metabolites. Many of these PhACs are not completely removed during wastewater treatment. Traces of PhACs have been reported in sewage effluents, river water and groundwater (Halling-Sorensen *et al.*, 1998). Although natural degradation of PhACs can occur in the environment by biotic and abiotic processes, their continuous release via sewage effluents could affect the organisms in the receiving waters.

Most environmental studies have focused on human pharmaceuticals with potential endocrine disruption effects. However, many non-steroidal compounds have also been detected in aquatic media such as ibuprofen and aspirin. Diclofenac is a non-steroidal anti-inflammatory drug prescribed as a painkiller for acute injuries or arthritis. It is used worldwide and has been frequently detected in sewage effluents, surface water and groundwater at concentrations up to a few micrograms per litre (Herberer, 2002). Diclofenac has a low removal rate during the wastewater treatment process and it can be discharged as either the parent compound or its conjugate acyl-glucuronide. These bioactive compounds are stable and may accumulate to toxic concentrations. The interest in diclofenac toxicity was prompted by a report which suggested that this compound is responsible for the decline of vulture populations in Pakistan (Oaks *et al.*, 2004). Further studies have revealed that diclofenac can cause adverse effects on the kidney functions of birds and fish (Hoeger *et al.*, 2005).

Because of its recalcitrance, diclofenac may bioaccumulate in the aquatic environment and may be detrimental to naturally occurring microbial communities. My study aimed to culture actively degrading microbes from sewage treatment plants and to identify the genes responsible for diclofenac degradation. One of my initial challenges was to detect and quantify diclofenac in water samples. In order to extract diclofenac and separate it from all the other chemicals present in sewage waters, solid phase extraction and LC-MS were optimized. These methods proved successful for the detection of diclofenac within the range of reported environmental concentrations. Enrichment cultures were then set up with activated sludge and sewage effluents from local sewage treatment plants. There were no metabolites of diclofenac detected, which suggested that the microbial consortia were unable to degrade diclofenac. Thus, no degradative bacteria were isolated. A rapid decrease in microbial growth was observed, which led me to consider that diclofenac might be toxic to bacteria. Diclofenac toxicity was assessed using the Microtox® test which incorporated *Vibrio fischeri* as a control strain. The bioassay indicated that diclofenac is considered toxic, with an EC_{50} estimated at approximately 10mg/l. The effects of diclofenac on bacteria present in sewage treatment plants were also investigated. A range of representative sewage bacterial strains were exposed to various concentrations of diclofenac. These bacteria exhibited reduced growth rate and changes in morphology.

Overall, microbial degradation of diclofenac seems to be a challenging process. Previous research reported similar results and no clear degradation pathway was established (Quintana *et al.*, 2005). Bacterial degradation alone may not fully account for the natural degradation of diclofenac in the aquatic environment. Other environmental factors such as photo-transformation may account for its removal.

In conclusion, the current testing methods for the evaluation of the risks of pharmaceuticals in the aquatic environment may not yet be entirely suitable for non-target organisms. This study justifies the need to re-evaluate the procedure to encompass the effects on species at all trophic levels.

I would like to thank S*f*AM for awarding me the President's Fund grant. This allowed me to present my work at the 12th International Symposium on Microbial Ecology in Cairns, Australia. This project was funded by the University of Exeter.

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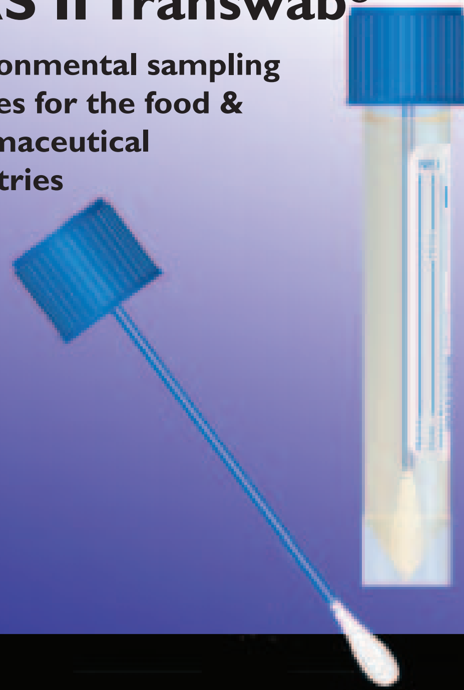

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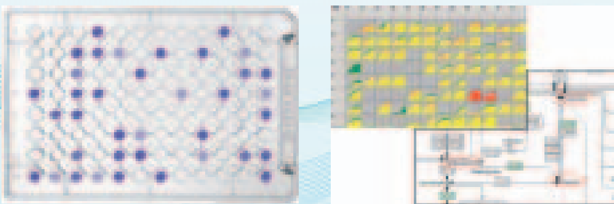

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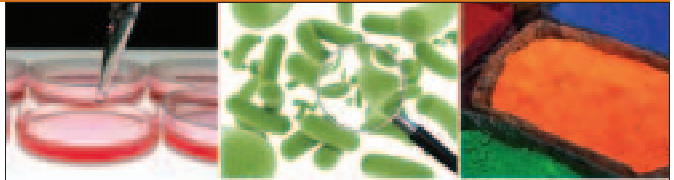

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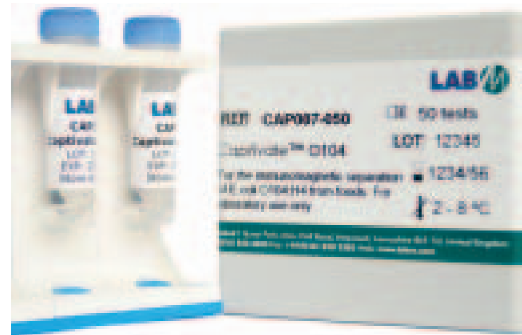

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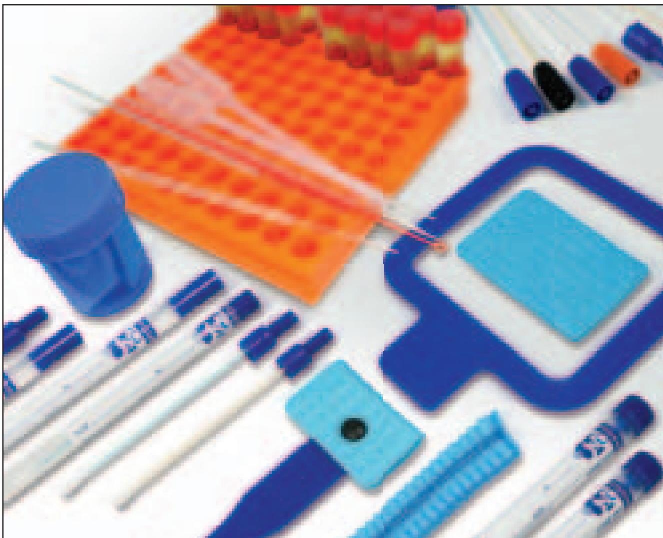

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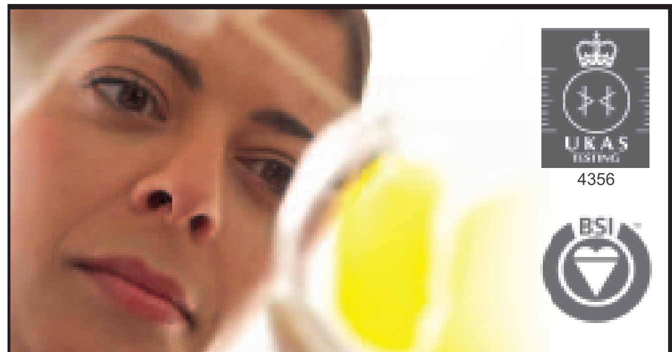

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Designed to fill a gap in the practical reference materials currently available to support clinical laboratory practice for microbiologists, the publication was written by Professor Michael W D Wren, MBE FIBMS, former Consultant Biomedical Scientist in the microbiology department, University College Hospital, and visiting Professor at The University of Westminster. With a foreword by Professor Brian Duerden, Emeritus Professor of Medical Microbiology at Cardiff University and input from Dr Don Whitley, Chairman and founder of DWS, considerable expert knowledge has been combined to make this the most up-to-date reference guide for the modern clinical laboratory. Images were kindly supplied by the Anaerobe Reference Unit, Cardiff.

Now available from DWS, a donation from the sale of each copy will be made to the Society of Anaerobic Microbiology. A preview of the document is available on the DWS website.

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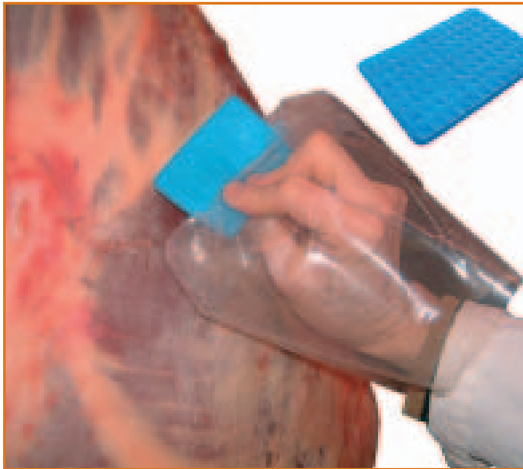

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Carcass Sampling Sponge Kits are simple to use, reliable and cost

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The convenient sampling kit consists of an individually packed 50cm2 blue cellulose sponge, pre-moistened with 10ml of 0.9% unbuffered saline (sodium chloride), MRD or Buffered Peptone Water. Sampling sponges are available sealed within a sterile bag for sample transport to the laboratory (TS/15-B:NaCl; TS/15-I:NaCl), in a blue sterile 120ml plastic container(TS/15-A:NaCl) or use EnviroStick (TS/15-U:NaCl),with its flexible breakpoint handle, ideal for difficult to reach areas.

further information

Visit: www.tscswabs.co.uk
Tel: +44 (0)1706 620600
Email: sales@tscswabs.co.uk

Simple, clean & efficient parasitology

An independent study comparing four commercially available faecal concentrators concluded that the patented FPC® Faecal Parasite Concentrator is very easy to use, provides clean sediment for microscopic examination, and requires the shortest processing time.

FPC® and FPC®-SV are the cleanest, most advanced means of isolating helminth eggs and larvae, protozoan cysts, coccidian oocysts, and microsporidian spores. Excellent results are obtained using either fresh or preserved specimens.

These leakproof, self-contained concentration systems leave no mess and minimize odours. Benefits of this approach also include the ability to standardize the faecal concentration procedure, thus eliminating varying results among different users. The procedure is easy to perform and minimizes personnel exposure to potentially infectious specimens.

Overall benefits include: simplicity, safety, excellent parasite recovery, standardization of the procedure, and minimal odour without funnels, gauze, and potential laboratory spills and contamination.

Evergreen Scientific manufacture a wide range of innovative labware for Life Sciences, Clinical Chemistry, Cyto-Histology, Microbiology, Cryo-Storage, tubes, vials and specimen containers. To find out more about the growing range of Evergreen products and how they can help in your laboratory please visit the BioConnections website, alternatively contact us by email or by telephone.

further information

Visit: www.bioconnections.co.uk
Tel: +44 (0)1782 516010
Email: welcome@bioconnections.co.uk

Cherwell Laboratories — specialists in environmental monitoring of cleanrooms and product testing

Over the years Cherwell Laboratories' product range has been developed to meet the specific requirements of environmental monitoring in cleanrooms and controlled environments, testing of products in pharmaceutical, food and related industries and the validation of processes and operators.

Redipor Prepared Media, manufactured by Cherwell, ranges from general purpose media to more selective agar based media, available in contact plates for surface monitoring and also in 55mm, 90mm and 140mm Petri dishes. Cherwell Laboratories' Redipor range also includes sterile, ready-to-use liquid media and buffers in ampoules, infusion bags and bottles and can work with professionals in pharmaceutical and related industries to make bespoke products.

Alongside Redipor, Cherwell Laboratories supplies a range of active air samplers and carries out calibrations for customers either on site or in Cherwell's well equipped engineering lab. Cherwell complement their own manufactured products with a range of biological indicators, chemical integrators and process indicators and data loggers (for real time process monitoring).

Cherwell Laboratories has recently commissioned additional cleanroom space, designed to accommodate the continued growth of the business and provide the manufacturing facility needed to support the increasing demands of its customers.

further information

Visit: www.cherwell-labs.co.uk
Tel: +44 (0)1869 355500
Email: sales@cherwell-labs.co.uk

Ensuring confidence and accuracy for your testing

Vetqas is the Animal Health and Veterinary Laboratories Agency (AHVLA) independent, accredited, proficiency testing (PT) unit. It is the international market leader in proficiency testing (PT) for veterinary laboratories with over 30 years experience.

Recognized by national accreditation bodies for your ISO 17025 accreditation, our PT schemes provide you with the evidence you need to prove that your tests meet third party quality standards.

Your ability to anonymously compare your performance with other participating laboratories is an important feature of our service which helps to improve consistency between laboratories and countries.

Taking part in a PT scheme shows a commitment to improving performance. This is of great value in maintaining a good reputation for delivering high quality, reliable and accurate results.

The Vetqas PT schemes include:

- Antibody detection
- Microbiology
- Haematology
- Clinical Chemistry
- Histopathology
- TSE
- Virology
- Pathology
- Feed tests

further information

Visit: www.ahvlascientific.com
Tel: +44 (0)1509 670 607
Email: vetqas@ahvla.gsi.gov.uk

NCIMB expands identification services to include qPCR

NCIMB has expanded its range of identification services to include qPCR. This technique is commonly used in clinical microbiology but is useful in any application where very rapid microbial counts are required. For example, it can be used to monitor populations of corrosion-causing sulphate reducing bacteria in the oil and gas industry.

NCIMB has extensive experience of isolating and identifying bacteria and routinely handles a diverse range of samples types including food, pharmaceutical and personal care products as well as cooling or process water, factory slimes, and isolates from personnel monitoring.

Rapid genotypic identification is the main method we use for primary identification of isolates as it can be used to rapidly identify both viable and non-viable organisms, those with complex growth requirements and Gram variable isolates as well as slow growing and/or non-fermenters. Sequenced data is examined against the validated MicroSeq™ database as well as strains in the non-validated but much more comprehensive EMBL public database.

We also apply a range of techniques for strain differentiation including multi locus sequence typing (MLST) which uses sequence analysis of internal fragments of up to seven essential house-keeping genes.

To find out more about our identification services contact Vikki Mitchell.

further Information

Visit: www.ncimb.com
Tel: +44 (0)1224 711100
Email: V.mitchell@ncimb.com

information

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

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

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

International Yakult Symposium 2013

The 7th International Yakult Symposium

The Intestinal Microbiota and Probiotics: Exploiting Their Influence on Health

www.yakultsymposium.com

April 22nd-23rd, 2013

The Queen Elizabeth II Conference Centre, London, UK

The gut microbiota is the focus of this event, with international experts presenting the latest findings about its genomics and physiology, as well as the dynamic, metabolic and immunological aspects of its interaction with the host in the context of health and disease. Related to this, the potential influence of probiotics on the composition and function of the microbiota will also be discussed. As always, this symposium will provide a platform for young investigators and established scientists to present their latest basic and clinical findings on the gut microbiota and probiotics. This will be a unique opportunity to engage in an international and high level scientific debate on this exciting field of research, and to exchange opinions on the current knowledge on gut microbiota and probiotics.

Registration Fee	Until 1st March 2013	From 2nd March 2013
Delegates	€100.00	€150.00
Students (Including PhD students)	€50.00	€75.00

Approved by the Society of Biology for purposes of CPD (18 credits), and by the Institute of Biomedical Science (10 credits).

Considering submitting a poster? Registration fee will be waived for one author of posters selected for presentation

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