Microbiologist

The magazine of the Society for Applied Microbiology ■ June 2013 ■ Vol 14 No 2 ISSN 1479-2699

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INSIDE

- Fungal infection of the eye (ocular mycosis)
- The surprising fungal diversity found in solitary bee nests
- *Aspergillus fumigatus* and aspergillosis: the curse of ubiquity

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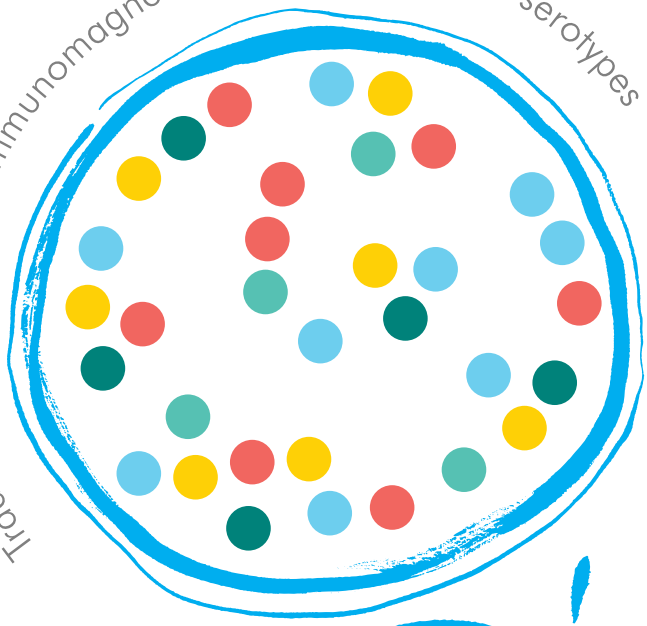

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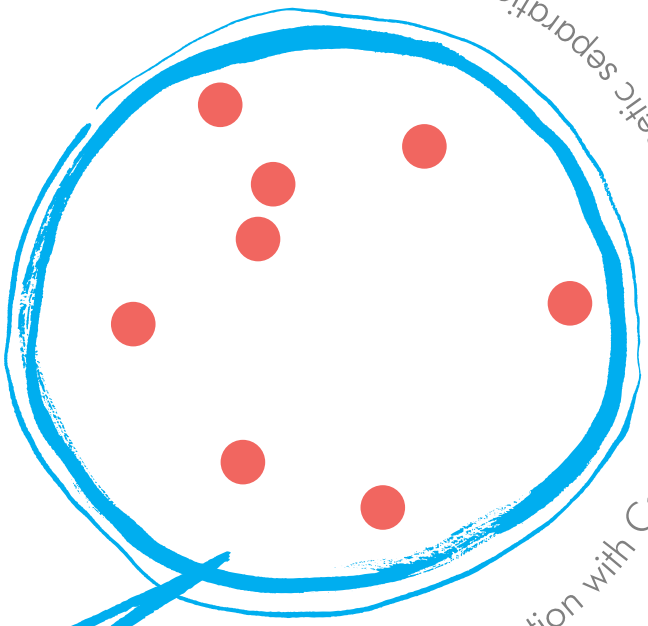

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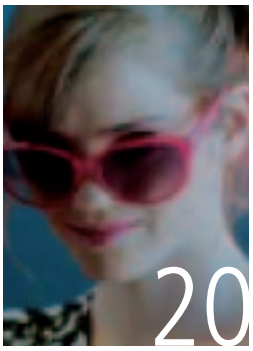

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editorial

Lucy Harper reviews the content of this issue of *Microbiologist*

At the time of writing this Editorial, we have just held our Spring Meeting 2013 which covered the subject "STIs in the 21st century". The meeting was a great success with many delegates commenting on the utility (and in many cases the entertainment value) of many of the presentations. If you weren't able to attend, you can read the tweets from the meeting here: http://storify.com/sfamtweets/sfam-spring-meeting-2013.

STIs is an important topic and one which often appears in the news media. This meeting was no exception as it generated significant media coverage. Professor Cathy Ison was a speaker at our meeting and she kindly took part in several interviews over the course of the day. One such interview was with BBC Radio 4's *Today* programme where she talked about the important and ongoing problem of gonorrhoea resistance. You can listen to the interview here: http://www.bbc.co.uk/news/health-22263030.

Moving on from STIs, in this issue of *Microbiologist* we focus on fungi, as does a really interesting forum from the BBC's World Service (http://www.bbc.co.uk/programmes/p0173gzz). Despite their ubiquity, fungi aren't as well known as they should be. Listening to this forum I learnt that fungi have many uses: fungal enzymes are used to produce cheese and certain species of fungi grow on the outside of cocoa beans, giving chocolate its flavour. And did you know that hundreds of thousands of species of fungi are yet to be named?

In our first feature article, Richard Armstrong examines fungal infections of the eye, saying: "*Although not as important as bacteria or viruses as a cause of eye infection; a large number of fungal species have now been recorded in association with the eye. In addition, several species have been implicated as a cause of eye infection ('ocular mycosis') and some may even cause life-threatening conditions (Thomas, 2003)*." Turn to page 8 to read more.

In our second feature article, we learn about the red mason bee, an important pollinator, and the effect of fungal contamination of their nests. "*Despite the importance of solitary bees as pollinators, relatively little is known about many aspects of their lives, and this includes the microbial communities supported in their nests*," say Adam Hart and Anne Goodenough on page 14. They continue: "*Our aim was to undertake an initial study of the fungal community present in the individual nests, contained within bee houses, to further knowledge of solitary bees in general, and red mason bees in artificial bee houses in particular*."

Finally, we take a look at the clinical side of fungal infection. In his article on aspergillosis, Richard Barton writes: "*This ubiquitous eukaryotic pathogen, present so extensively in the environment and occasionally causing disease in humans, continues to present academic and clinical laboratory scientists, and clinicians in a variety of disciplines with a challenge to understand, detect and effectively treat affected patients*." Read more on page 17.

This issue of *Microbiologist* is the last one that I'll be editing for some time now, as I'll be handing over the ropes to Nancy Mendoza who will soon* be joining *Sf*AM as my maternity cover. I hope she gets as much pleasure from editing the magazine and working with all the editors, authors, proofreader and production company as I have over the last nine years**. I'd like to thank everybody who's helped to make this part of my job such a fun, stimulating and often enlightening experience.

*correct at time of writing.
**I began editing the *Microbiologist* whilst working as a postdoctoral scientist at Aston University.

contribute

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

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

Lucy Harper

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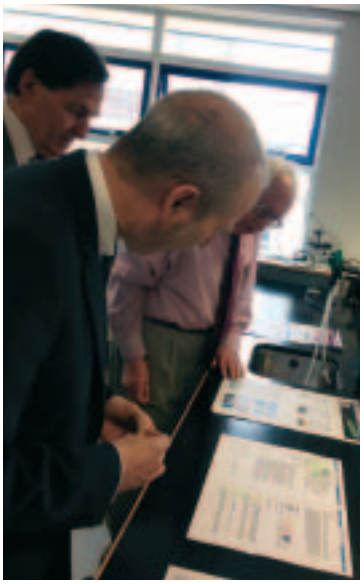
president's column

S*f*AM President, **Professor Martin Adams** discusses the importance of social media in promoting applied microbiology

I have to admit that in a moment of heady abandon some years ago, I set up a Facebook page for myself. Regrettably, that was about as far as I ever got and I am told that if you visit my page today, all that is evident is the odd clump of tumbleweed blowing lazily across the screen and the eerie sound of the whistling wind broken occasionally by a lonely, distant train whistle.

Fortunately, not everyone is like me and it is very apparent that social media are now a permanent and valuable part of modern life. It was in recognition of this that when S*f*AM sponsored the most recent annual competition for schools, organized under the auspices of the Microbiology in Schools Advisory Committee (MiSAC), we decided on the theme "Facebug: a social media network for microbes". Needless to say, the original idea was not mine and I am indebted to our excellent communications team for coming up with the idea, explaining it to me very slowly and developing it into a workable competition theme. It was a good choice, the competition was an unprecedented success and it was a joy to be involved in the judging. There is a full report on the competition and the winning entries on page 28 which I urge you to read, but I thought I would use this column as an opportunity to say a little about our work with schools, how this might develop and MiSAC in particular.



MiSAC has been around since 1969 when it was founded to promote the teaching of microbiology in schools and colleges. The committee itself comprises volunteers from its various sponsoring educational and scientific organizations (S*f*AM included). Over the years, on a modest budget it has made an enormous contribution, producing new ideas for the educational use of microorganisms, including practical activities, answering queries, giving talks, contributing to training courses, interacting with examination boards, science suppliers, publishers and industry, and advising on the safe use of microorganisms in schools. The annual competition is one of its flagship activities and has been a regular feature since 1985. You can find out more about MiSAC, what is available and what is currently going on by visiting the website http://www.misac.org.uk/.

Independently of MiSAC, many S*f*AM members are active proselytizers for microbiology in the wider community and in schools in particular. As a Society, we have supported some of these activities financially through our grants programme and some notable examples have been described in the *Microbiologist* over the last couple of years; for example, the World of Microbiology programme from Aston University and our association with the Germ Wars project in Dundee. We also become aware in a more *ad hoc* fashion of numerous other examples of our members' work with schools, and this appears to be increasing. One reason for this is that microbiology is one of many subjects which is not a core discipline in the school curriculum and universities now recognize the importance of promoting it in schools if they are to ensure a future stream of good applicants for undergraduate courses.

This has demonstrated to me the huge amount of expertise and commitment to school activities among our membership and that perhaps we are not operating most efficiently or taking full advantage of this. For someone starting to work with schools there are plenty of materials already out there, from MiSAC, from our colleagues at the Society for General Microbiology and elsewhere, so there is not always the need to start from the very beginning. But what is important is that there is also a need for continuous innovation and development with regard to school materials and activities. New ideas and approaches can stimulate and provide a fresh impetus, as was demonstrated in this year's MiSAC competition.

For these reasons, it is important that ideas and experiences are shared, and we innovate rather than duplicate when it comes to the range of materials and support available to schools. One important aspect of the World of Microbiology project we are funding is the "training the trainers" component, whereby old hands and relative novices to this area can come together, share their ideas and experience and possibly develop new ones. I also hope that we will be able to strengthen our support for MiSAC beyond what we currently do and for this we will need the help of individual members. We do not want the situation where "*Full many a flower is born to blush unseen*". Please check on what MiSAC have produced; if you have anything you have developed or are currently working on — a practical activity, a fact sheet or something completely new then it may well deserve wider exposure. Please let me know (m.adams@surrey.ac.uk).

Martin Adams
President of the Society

ceo's column

Philip Wheat reports on the latest developments within the Society

Once again it is a pleasure to announce this year's annual *Environmental Microbiology* Lecture. The venue this year will be the Institute of Civil Engineers situated in Westminster, London, and will be held on the 28 October 2013 beginning at 6.30 pm. The lecture this year will be given by Professor Víctor de Lorenzo, Madrid, Spain, whose presentation is entitled: "***Programming soil bacteria to do amazing things***" full details of which can be found on page 38 of this issue.

Víctor de Lorenzo is a chemist and microbiologist. He works as Professor of Research at the National Centre of Biotechnology in Madrid, where he has been employed since 1996 after running a large number of Molecular Microbiology and Environmental Biotechnology activities at the Pasteur Institut (Paris), the University of California (Berkeley), the University of Geneva and the Federal Center of Biotechnology (Braunschweig). His research exploits the advanced molecular biology and genetic engineering of soil microorganisms (e.g., *Pseudomonas putida*) for the sake of biomonitoring, bioremediation — and wherever possible, valorization of chemical pollution in the environment.

The lecture is free of charge and open to all members to attend and you should receive an invitation from the President of the Society in this issue. So that we can finalize arrangements for the event, please can you respond as soon as possible. Once again, I would like to remind you that should you require financial assistance to attend, why not apply for a **Scientific Meeting Attendance Grant** (full details here: http://www.sfam.org.uk/en/grants--awards/scientific-meeting-attend-grant.cfm). If you are unable to attend the lecture it will be available online within 72 hours of the event.

As I am writing this column (end of March 2013), membership of the Society is once again at an all-time high with the total number of members currently standing at over 2400. We think that this increase in membership reflects its tremendous value for money. I would like to thank all existing members who have actively recommended joining the Society to potential new members. We try to ensure that the needs and the expectations of our members are met or indeed exceeded. One way we do this is through surveying the membership with questionnaires, the most recent of which we conducted in 2012. In my last column of the *Microbiologist* I stressed the importance of the information gleaned from such surveys in helping to shape the future direction of your Society.

One example of the Executive Committee listening to the needs of the membership can be illustrated in the launching of yet another innovative grant — **PhD Studentship Grant**.

This grant is designed to support research in applied microbiology and offers potential funding for up to three years. The applicant for the grant must have been a Full Ordinary Member of the Society for at least two years to be eligible to apply. The applicant (acting as the supervisor) and the nominated student must both provide summary *curriculum vitae* together with an outline of the proposed project. Projects should address important issues of applied microbiology which align with the objectives of the Society. Applications from academics (who are members) in a Higher Education Institution will be considered for the award. Collaborations with non-academic research institutions are also eligible. The value of the award will be up to £22000 p.a. This will be made up of tuition fees up to the UK/EU rate (up to £4000 p.a.), a maintenance fee for the student (currently £13000 p.a.) and finally up to £5000 p.a. as bench fees for the host laboratory. Although there will be a commitment of funding for three years this will be on condition that satisfactory annual progress reports are received from both supervisor and student.

It is proposed that the first award will be made for the student to begin their studies in October 2013. The deadline for receiving applications has been set for 30 June 2013. Full details of this new award and indeed all grants can be found by visiting http://www.sfam.org.uk/en/grants--awards/index.cfm.

Finally, on a personal level for members that do not know, the Editor of the *Microbiologist*, Dr Lucy Harper, is about to go on maternity leave! On behalf of all the Executive Committee, members and staff I would like to wish Lucy and her fiancé David all our best wishes, and I look forward to welcoming her back next year, when they will be proud parents of a baby boy!

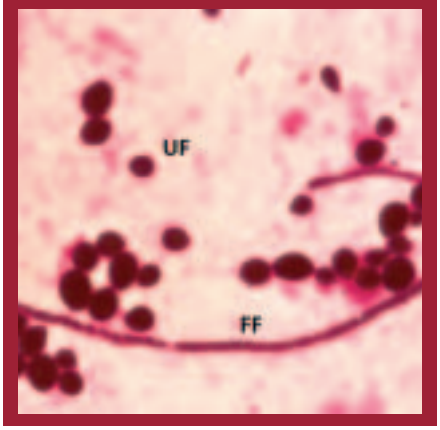
Philip Wheat
Chief Executive Officer

Fungal infection of the eye (ocular mycosis)

Richard Armstrong investigates the diverse microorganisms responsible for eye infections and the latest treatments

The eye surface is rich in nutrients and supports a diverse range of microorganisms, including many bacteria and fungi, and which constitute the normal ocular flora (Armstrong, 2000). The ocular surface can usually resist ocular infection both as a result of the mechanical action of the eyelids, which physically remove potential pathogens, and the washing effect of tears which contain the enzyme lysozyme. Nevertheless, infections of the external structures of the eye are common, especially by bacteria (Armstrong, 2003), and result from either the acquisition of a virulent microorganism or uncontrolled growth of an existing organism due to lowered host resistance. By contrast, the globe of the eye is relatively impermeable to microorganisms, but if breached by trauma or surgery, the contents of the eye can also provide a suitable medium for microbial growth. In addition, infections within the eye can be a consequence of systemic disease and transmitted to the eye via the blood stream or lymphatic system.

Figure 1. *Cells of Candida albicans, a dimorphic fungus showing unicellular (UF) and filamentous (FF) forms*



Although not as important as bacteria or viruses as a cause of eye infection; a large number of fungal species have now been recorded in association with the eye. In addition, several species have been implicated as a cause of eye infection ('ocular mycosis') and some may even cause life-threatening conditions (Thomas, 2003). Ocular mycoses are being reported more frequently as a consequence of new medical practice and the increased numbers of immunocompromised patients in the population, e.g., patients receiving radiation treatment or chemotherapy. This article describes the most common conditions caused by fungi which can affect the different structures of the eye, the importance of fungal contamination of materials as a source of eye infection, and the methods available for treatment.

Types of fungi in eye infection

Estimates of the number of fungi involved in eye infection range from 60 species (30 genera) (McGinnis, 1980) to 105 species (35 genera) (Wilson & Aiello, 1998). Members of all the major groups of fungi are involved. Hence, species of *Mucor* (Zygomycota) have high growth rates and produce enormous numbers of aerial spores. The majority of yeast-like fungi, such as *Candida albicans* (Figure 1), which infect the eye, are members of the Ascomycota. Classified within this group is also the common eye-associated genus *Aspergillus* (Figure 2), a frequent fungus in many environments worldwide. Species of *Aspergillus* produce enormous numbers of spores and are also an important cause of allergy and lung infection. In addition to *Aspergillus*, species of *Fusarium*, *Penicillium* and *Cephalosporium* (*Acremonium*) can

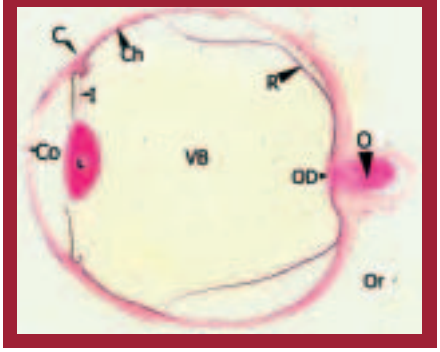
Figure 2. *Aspergillus: a common filamentous fungus to attack the eye showing the characteristic spore heads (CN = conidiophore, CSC = columnar spore chains)*

be involved in eye infection. The unicellular yeast *Cryptococcus* is a member of the Basidiomycota and a pathogen characteristic mainly of immunocompromised patients. A further group associated with the eye is the 'dematiaceous' fungi and includes a number of genera characterized by dark pigmentation of the hyphae caused by the deposition of melanin. At least 20 species from 11 genera have been recorded including species of *Bipolaris*, *Curvularia* and *Exophiala*. Finally, entities of uncertain classification, such as the common fungal-like aquatic organism *Pythium insidiosum*, have been recorded in association with the eye.

Diagnosis of ocular mycoses

Diagnosis of a fungal infection involves the recognition of typical clinical features followed by direct microscopic observation of the organism responsible in scrapes from the eye or biopsy samples. Any fungi isolated by these methods can then be

Figure 3. *Regions of the eye that can become infected by fungi (C = conjunctiva, Ch = choroid, CO = cornea, I = iris, O = optic nerve, OD = optic disc, Or = orbit, R = retina, VB = vitreous body)*



cultured and identified. Further histopathology, immunohistochemistry and DNA-based tests may be necessary to confirm a diagnosis of an infection attributable to a specific fungus.

Normal ocular mycoflora

Although there have been many reports of bacteria in association with normal eyes (Armstrong, 2000), fewer studies have included fungi. In a UK study, fungi were recorded from the eye surface in 2.9% of healthy individuals tested, the incidence being higher in older subjects (Williamson *et al.*, 1968). Most species recorded were non-pathogenic and could have been transmitted to the eye by aerial contamination, but some pathogenic species were also present. Species of *Penicillium* are usually the most commonly isolated from the surface of normal eyes. In areas of the world where a low socio-economic status is combined with poor hygiene however, fungi are found more frequently in association with the eye, especially in rural and agricultural communities. In Ahvaz, Iran (Feghi *et al.*, 2010), for example, 172 swabs were taken from patients attending hospital during the period 2007–2008, 11 being positive for pathogenic fungi. *Cladosporium* was the commonest genus recorded, followed by *Drechselera*, *Alternaria*, *Fusarium*, *Epicoccum* and three yeasts, *viz.*, *Cryptococcus* species, *Candida albicans* and *Rhodotorula rubra* (Feghi *et al.*, 2010).

Ocular mycoses

Many structures of the eye may become infected by fungi and these are illustrated in Figure 3. The most frequent fungi associated with these structures are listed in Table 1 (Armstrong, 2000). A few fungi are 'generalists' infecting several different structures, while others, exhibit a distinct preference for a specific region. Only a relatively small number of fungi however, have been repeatedly isolated from the eye, or have been recovered from more than one ocular site. For the purpose of this article, they will be divided into those fungi which affect: (1) eyelids and associated structures, (2) conjunctiva, (3) cornea, (4) sclera, (5) uvea, (6) vitreous body, (7) retina, (8) optic disc and nerve, and (9) orbit.

Table 1. Major eye infections caused by fungi

Region	Condition	Fungi
Lids	Ringworm	*Microsporum, Trichophyton*
	Eyelid lesions	*Cryptococcus, Candida, Sporotrichum*
Lacrimal apparatus	Inflammation	*Aspergillus, Leptothrix, Sporotrichum, Rhinosporidium*
Conjunctiva	Conjunctivitis	*Leptothrix, Sporotrichum, Candida, Trichophyton*
	Ulcerating nodules	*Rhinosporidium*
Cornea	Keratomycosis	*Aspergillus, Fusarium, Cephalosporium,* Dermatiaceous fungi, *Pythium insidiosum*
Sclera	Scleritis	*Cryptococcus, Metarrhyium anispliae*
Uvea	Uveitis	*Aspergillus, Candida, Coccidiodes.* Rarely *Paecilomyces, Cephalosporium*
Vitreous	Abscess	*Aspergillus, Cephalosporium, Fusarium, Candida*
Retina	Retinitis	*Candida*
	Local lesions	*Cryptococcus*
Optic disc	Papillitis	Phycomycetes, *Cryptococcus*
Optic nerve	Optic neuritis	Phycomycetes, *Cryptococcus*
Orbit	Orbital cellulitis	*Aspergillus, Mucor, Pythium insidiosum*

Eyelids and associated structures

The most important fungi to affect the eyelids are species of *Trichophyton* and *Microsporum*. These fungi cause flaking skin lesions which then become inflamed. Particularly notable is *T. schoenleini*, which develops as a raised crusted area around the eye lashes. Species of *Cryptococcus*, *Candida* and *Sporotrichum* can also infect eyelids causing characteristic lesions. In addition, the tear glands and associated canals may be infected by species of *Aspergillus*, e.g., *A. fumigatus* or *A. flavus* causing 'dacryocystitus', a condition which causes obstruction of the nasolacrimal duct. Patients exhibit 'epiphora' (running over of tears on to the cheeks) and a black disc-like elevated region on the lower lid. In addition, species of *Leptothrix*, *Sporotrichum* and *Rhinosporidium* can invade the lacrimal sac.

Conjunctiva

The conjunctiva is the outer membrane of the eye covering the white fibrous sclera. The membrane is continuous with that of the transparent cornea and extends onto the surface of the upper and lower lids, and is a region especially vulnerable to infection. Fungal infection of the conjunctiva is relatively rare. In children and young adults however, infection by *Leptothrix* may cause a unilateral inflammation of the conjunctiva ('conjunctivitis') characterized by distinct yellow or grey areas under the epithelium. This condition does not usually lead to an ulcer although there may be considerable infiltration by blood cells. Conjunctivitis can also be caused by the fungus *Sporotrichum* resulting in the formation of small, yellow and occasionally ulcerating nodules on its surface, while in *Rhinosporidium* infection, yellow to pink nodules are formed which may develop into tumours. *Candida* and *Trichophyton* can also cause conjunctivitis with or without involvement of adjacent areas of skin.

Cornea

The cornea is the transparent region of the eye through which light is transmitted to the retina through the pupil. Common risk factors for infection of the cornea by fungi (termed

'keratomycosis' or 'fungal keratitis') include surgical trauma, the wearing of contact lenses in association with poor hygiene and alterations in lacrimal secretion, and is a potentially serious sight-threatening condition (Zago *et al.*, 2010). Keratomycosis is chronic and often begins as a grey-coloured, superficial necrosis of the cornea with a dull, dry surface and surrounded by a sharp yellow line. The condition may be accompanied by pain, corneal infiltrates, pus in the anterior chamber of the eye, and inflammation of the iris ('iritis'). Perforation of the cornea is rare, but if it occurs, the fungus can invade the vitreous body, uvea and retina.

The most common species involved in keratomycosis is believed to be *A. fumigatus*, followed by members of the Mucoraceae. In developing countries however, infection by *Fusarium* species is more common. For example, in cases of infectious keratitis in elderly patients in Brazil, 6.1% were caused by fungi, mainly species of *Fusarium* (Passos *et al.*, 2010). In addition, in China (Qiu *et al.*, 2005), 61 fungal strains were isolated from eyes with the signs and symptoms of fungal keratitis. The most common genera were *Fusarium* and *Aspergillus*, followed by *Alternaria*, *Trichophyton*, *Curvularia*, *Chrysosporium*, *Cephalosporium* and *Scedisporium*. Dermatiaceous fungi have also been implicated in fungal keratitis and may be the third most frequent cause after *Aspergillus* and *Fusarium*. *Pythium insidiosum* can also cause keratitis in tropical and temperate regions.

Fungal keratitis was believed to be relatively rare among contact lens wearers, but in a recent outbreak of *Fusarium* keratitis in the USA, 283 out of 695 identified cases were contact lens wearers (Gower *et al.*, 2010). In the UK, the British Ophthalmologic Surveillance Unit identified 0.32 cases of fungal keratitis per year and in 56% of these cases, *Candida* was the only fungus isolated from the eye (Tuft & Tullo, 2009). Filamentous fungi however, are often a more common cause especially in male patients following trauma. Recent studies also suggest that species of *Alternaria* and *Paecilomyces* can cause keratitis in patients wearing frequent replacement contact lenses and who were using a multipurpose solution, i.e., for soaking, wetting and disinfecting the lenses (Yildiz *et al.*, 2010). Hence, if there are signs of corneal infiltration, especially in contact lens wearers, it is recommended that mycological assessment should now be performed as a matter of routine.

Sclera

The sclera is the white fibrous outer layer of the eye and infection by fungi of this region of the eye is unusual. However, scleral ulceration caused by *Cryptococcus* has been reported in individuals who are HIV-positive (Garelick *et al.*, 2004). In addition, the first case of 'sclerokeratitis', i.e., a scleral necrosis adjacent to an area of corneal infiltrate, and caused by the fungus *Metarrhyium anisopliae*, has been reported in Australia (Amiel *et al.*, 2008).

Uvea

The uvea is the term given to several internal structures of the eye including the iris and ciliary body, together with the choroid which constitutes the vascular layer of the eye. The iris is the light-sensitive diaphragm which controls the amount of light entering the eye, while the ciliary body supports the lens and is involved in making fine adjustments in focusing. Infection of these regions by fungi and causing an inflammation ('uveitis') is relatively rare. The most frequent organisms involved are *A. fumigatus* and *Candida*, although *Coccidiodes* may also be implicated. An endogenous chronic uveitis can also be caused by fungi.

A more general infection of the anterior structures of the eye is often referred to as 'endophthalmitis'. Recently, a case of a 70-year old male was reported with anterior endophthalmitis following an intraocular lens implant. A 'fluffy fungal-like growth' was observed which was ultimately identified as the fungus *Paecilomyces variotii* (Anita *et al.*, 2010). Species of *Cephalosporium* have also been reported occasionally as a cause of endophthalmitis (Fincher *et al.*, 1991).

Vitreous body

The vitreous body is the gel-like connective tissue which occupies the posterior segment of the eye. Inflammatory reactions within the vitreous ('vitritis') can result in liquefaction, opacification and shrinkage, the tissue ultimately becoming necrotic (Armstrong, 2000). A fungal infection of the vitreous progresses more slowly than a bacterial infection and often remains localized, the most common genera being *Aspergillus*, *Cephalosporium*, *Fusarium* and *Candida*.

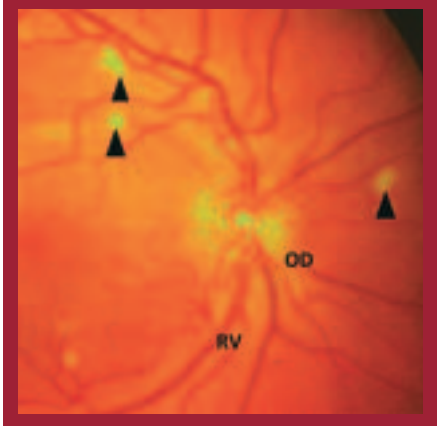
Retina

The retina, the light-sensitive layer at the back of the eye, contains the photoreceptor cells (rods and cones) which react directly to light and initiate the process of vision. Inflammation of the retina ('retinitis'), caused by fungi, may occur in patients with a debilitating systemic disease, especially if the patient is also receiving chemotherapy or immunosuppressant drugs and is usually attributable to *Candida* (Figure 4). Focal lesions of the retina may be present, including the optic disc, and usually with an overlying vitritis. In addition, intravenous drug users and patients who have had long-term venous feeding are at risk of infection of the retina. Such infections can present with endophthalmitis, vitritis or focal lesions, affecting the retina and optic disc (Figure 4). Localized lesions caused by *Cryptococcus* may also occur.

Optic disc and nerve

The optic nerve leaves the eye at the optic disc ('blind spot'), thus conveying visual information from the eye to the brain. Infections spreading from the eye, orbit or brain, can affect the orbit, resulting in inflammation of the optic disc and nerve ('optic neuritis'). Optic neuritis can also be caused by members of the Zygomycota and the yeast *Cryptococcus*.

Figure 4. *Infection of the retina by Candida showing focal lesions (arrows) (OD = Optic disc, RV = retinal vessels)*



Orbit

Fungal infections of the orbit comprise a small minority of orbital infections ('orbital cellulitis'), but can have a serious impact on vision (Kuruba *et al.*, 2011). Fine needle aspiration cytology can help in making an early definitive diagnosis of this condition. Most fungal invaders of the orbit are opportunistic saprophytes. In some HIV-positive patients however, an invasive infection caused by *Aspergillus* may occur (Kronosh *et al.*, 1996). Sinonasal 'aspergillosis', with extension to the orbit, often exhibits a relentless progression and can lead to complete visual loss (Kuruba *et al.*, 2011). Infection of the orbit by *Mucor* ('Mucormycosis') may be associated with diabetic ketoacidosis (Lee *et al.*, 1998). This condition occurs most commonly in immunocompromised children who may present with blurred vision, orbital pain and problems with eye movement ('ophthalmoplegia'). In addition, two aggressive cases of orbital cellulitis have been reported in the USA associated with *Pythium insidiosum*. Chronic inflammation of the orbit can also be observed in a few cases and is usually of unknown cause, but may be associated with fungi.

Fungal contamination

Fungi are ubiquitous in nature and consequently, they can frequently contaminate cosmetics, skin lotions and ocular medical materials. In shared use cosmetics available to the public, for example, fungi were present in approximately 10% of products tested (Mislivec *et al.*, 1993). Of these, 3.9% contained fungal or opportunistic pathogens. Contamination of contact lenses by fungi is being increasingly observed. Whereas the bacterium *Haemophilus influenzae* can be isolated in large numbers from patients wearing disposable hydrogen lenses, on a six-night extended wear schedule, fungi are usually present in much smaller numbers (Hickson *et al.*, 1996). In addition, in a separate study of hydrogel contact lenses (Hart *et al.*, 1993), no fungi were isolated but bacteria were isolated from 38% of lenses tested. Background infections of the cornea are also a complication of photorefractive surgery (Faschinger *et al.*, 1995), some cases being traced to a disposable contact lens worn post-operatively overnight. The fungus involved was usually a species of *Aspergillus*, most commonly *A. fumigatus*. Ocular problems may also occur in patients fed intravenously through feeding lines (Nightingale *et al.*, 1995), 24% of such patients developing a *Candida* infection (most probably caused by *C. albicans*, *C. parapsilosis*, *C. glabrata* or *C. guillermondi*). The consequence of this infection may be uveitis or endophthalmitis, which may lead to blindness.

Treatment

The majority of antifungal agents (Table 2) are classified into two groups: (1) polyenes such as amphotericin B and (2) azoles such as econazole and fluconazole. In addition, the cytosine analogue flucytosine has been used to treat some eye infections. Polyenes and azoles both disrupt membrane function, but their mode of action is markedly different.

Polyenes

Polyenes, which bind directly to the unique fungal sterol ergosterol, continue to be regarded as important in the treatment of fungal eye infections. Amphotericin B, for example, is effective against *Candida*, but may penetrate ocular tissues poorly and this substance, and its solubilizing agent, can also be potentially toxic. Natamycin is better tolerated, and although originally thought to suffer from lack of penetration, is actually an effective treatment after topical application. The range of organisms effectively treated by natamycin continues to be controversial, but it is often the treatment of choice against filamentous fungi which cause keratitis. As natamycin is too toxic to be used intravenously, amphotericin B is often used for orbital infections and endophthalmitis.

Azoles

Azoles bind to cytochrome P-450, a group of fungal enzymes, resulting in a decrease of ergosterol synthesis and an increase in membrane permeability. All azoles, with the exception of fluconazole, depress the activity of the immune system. Several compounds have been used including thiabendazole, itraconazole, clotrimazole, miconazole, ketoconazole, fluconazole and econazole. Most are effective against *Candida*, while econazole is particularly effective against filamentous fungi. Azoles are frequently used as eye drops since they often exhibit good penetration of ocular tissues. These compounds are well tolerated although econazole, miconazole and ketoconazole have been reported to irritate eyes in some cases (de Luise, 1983). Itraconazole, thiabendazole, ketoconazole and fluconazole are absorbed significantly by ocular tissue following oral administration, fluconazole penetrating the eye better than ketoconazole or itraconazole. Econazole and miconazole can also be used intravenously, but levels reaching the eye are usually too low to treat systemic mycoses and these treatments are not available in the UK. Fluconazole is probably the azole of choice for intravenous use, achieving high levels of penetration of ocular tissues within a few hours of a single dose (Savani *et al.*, 1987). Posaconazole, a structural analogue of itraconazole, was approved for use in the USA in 2006 (Schiller *et al.*, 2007). It can be given orally and administered even if the patient has poor kidney function. Hence, it can be

Table 2. Treatment of ocular mycoses (IVA = intravenous administration)

Agent	Effective against	Administration	Side effects
Amphotericin B	*Candida*	topical, IVA	impaired renal function
Natamycin	Broad spectrum	topical	IVA toxic
Thiabendazole	*Candida*	topical, oral	none reported
Clotrimazole	*Candida*	topical	none reported
Miconazole	Broad spectrum	topical, IVA but not in UK	eye irritant
Ketoconazole	Broad spectrum	topical, oral	eye irritant
Econazole	Filamentous fungi	topical	eye irritant
Fluconazole	*Candida*	topical, IVA	none reported
Posaconazole	Broad spectrum	oral	none reported
Flucytosine	*Candida*	topical, oral	possible skin lesions
Itraconazole	*Aspergillus*, keratitis	oral	gastro-intestinal problems

used in high-risk patients, with rare species, or fungi resistant to conventional treatments, or if the patient cannot tolerate other antifungals. Several new azoles are also becoming available and include isavaconazole, ravaconazole and altaconazole. In some cases, a combination therapy, e.g., topical amphotericin B eye drops and subconjuctival injection of fluconazole may be more efficient at treating keratomycosis, compared with the eye drops alone (Mahdy *et al.*, 2010).

Flucytosine

Flucytosine is highly toxic to fungal cells and can interfere with various aspects of fungal metabolism. It is most effective against *Candida* and certain filamentous fungi, although is generally ineffective against species of *Aspergillus* and *Fusarium*. It is well tolerated as eye drops, a treatment which is often more effective than a subconjunctival injection. Oral flucytosine is also readily absorbed by ocular tissues.

Conclusions

The incidence of fungal infection of the eye is relatively low compared with that attributable to viruses and bacteria. Fungal infections of the eye are however, increasing in frequency largely as a result of immunosuppression. A wide variety of such infections have been described worldwide, species of *Fusarium*, *Aspergillus*, *Candida* and dematiaceous fungi being the most commonly reported. A limited number of antifungal compounds are available to control ocular mycoses and resistance to these compounds has been growing in recent years, especially to azoles. Several mechanisms of resistance have been identified, including modification of sterol synthesis pathways by the fungus, modification of enzymes to reduce the binding of azoles to fungal components, and increased efficiency of removal of the azole within fungal cells. Resistance to amphotericin B has also been reported, but it continues to be an important treatment for life-threatening conditions and severe eye infections. Natamycin is often the treatment of choice for filamentous fungi causing keratitis and topical amphotericin B for *Candida* keratitis. Continued monitoring of the behaviour of ocular fungi will be essential in the future, as the proportion of immunocompromised patients is likely to continue to increase.

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The surprising fungal diversity found in solitary bee nests

Solitary bee nests are home to a rich fungal community, which varies substantially on a nest-by-nest basis

Many animals, including birds, mammals and reptiles, as well as numerous invertebrates, have the "nesting habit". Most nests are temporary structures that are constructed specifically for breeding. Birds are perhaps most associated with nest building, and building skills need to be good as a poorly constructed nest may mean that few or no chicks survive to adulthood. Mammals also construct nests for breeding purposes, with good examples being otter holts and squirrel dreys, while other species such as dormice construct nests for resting or hibernation. In all cases, well-constructed nests offer protection from the elements, camouflage from predators and thermal stability.

Although most species only use nests at certain times of the year, some animals live permanently within a nest structure. In many cases, permanent nests can be large and elaborate (Hansell, 2007), and the size of the animal architect is not necessarily a good indication of the size and complexity of their nest structure. For example, within the so-called "social insects" (ants, termites, and some bees and wasps), there are many examples of complex nests that have come about through the collective efforts of hundreds, thousands or even millions of individual insects living and working together socially. Indeed termites — widely regarded as the champion insect builders — often construct huge earthen mounds, up to several metres high, with a system of chambers for rearing young as well as complex internal constructions which allow ventilation and temperature control (Wilson, 1971).

The orders of insects with the most pronounced nesting behaviour are the *Hymenoptera* (the order that includes the ants, bees and wasps) and *Isoptera* (the termites). Social wasps are superb nest architects, constructing large intricate nests from wood pulp mixed with water to form natural papier mâché (Wilson, 1971). Likewise, bees are talented nest makers, with the familiar honeybee constructing its wax honeycombs in tree cavities and the popular bumblebees making their wax storage pots in abandoned rodent nests. A less well-known group of bees, the tropical stingless bees, go to greater lengths combining wax with mud and tree resin to produce extravagant nest structures and elaborate nest entrances (Roubik, 1989). Although the *Hymenoptera* are frequently thought of as social insects, living together in colonies with queens that reproduce and workers that carry out the colony's labour, many lead solitary lives – indeed of the 20,000 species of bees across the world, only around 1,000 (5%) are social. Most of the solitary bees also have the nesting habit, working on their own to build a nest suitable for

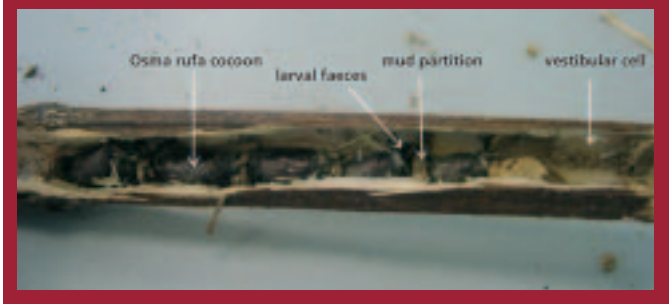
rearing larvae either in the ground (mining bees) or within vegetation structures such as hollow plant stems. Within the mine or cavity, the female bees make individual cells in some cases using mud (mason bees) or leaves (leafcutting bees) to subdivide the nest cavity. Into each cell, the female packs pollen and nectar collected from plants, and then lays an egg. The egg hatches and the larva feeds, growing and developing until it pupates and later emerges from the nest as an adult bee (O'Toole, 2000).

In order to feed the larvae developing in the nest structures, bees of all kinds collect pollen and nectar from flowering plants. In undertaking this activity they transfer pollen between flowers, which pollinates them and allows seed and fruit production. The insect pollination is vital for the continued survival of many plants and is of great economic importance; a number of agriculturally significant products, including apples, pears, tomatoes, beans, squashes and soft fruits, rely on bees for pollination. Recently, there has been widespread attention given to the decline of pollinators, both within the UK and internationally (Parliamentary Office of Science and Technology (POST), 2010). The reasons for this decline are likely to be complex and multifactorial, but certainly include habitat degradation (Kluser & Peduzzi, 2007). The honeybee has increasingly become the flagship species for pollinator decline, largely because it has reasons for decline over and above those affecting all insect pollinators. The potential effect of pesticides and the burden of disease are both suggested to play a part in recent honeybee decline (POST, 2010) and considerable research effort and funding is in place to find solutions to the "honeybee crisis". However, despite the focus on honeybees, many other bees, including solitary species, are important pollinators. In fact, in many cases, individual solitary bees are better pollinators than honeybees because of the former's habit of collecting pollen quite loosely on hairs covering the body rather than storing it neatly in "pollen baskets" found on the legs of honeybees and bumblebees. One common UK solitary bee species, the red mason bee (*Osmia rufa*), is estimated to do the pollination work of more than 100 honeybees (O'Toole, 2000). Consequently, understanding as much as possible about the ecology and biology of the often-overlooked solitary bees is an important component of integrated pollinator conservation.

For any nesting animal, including the red mason bee, the nest environment is a critical factor in its overall reproductive success, and thus in the long-term success of the species. Often, the conditions that make a good nest for the nesting animal (sheltered, warm, not too dry and not too wet) also provide ideal conditions for microbes. As we discussed in a previous article (Goodenough & Hart, 2011), the relationships between microbes and nest-hosts are ecologically complex, with some acting as potential or actual pathogens, some being beneficial, and others having little ecological effect on their host. As a result, there are a diverse suite of generalist and specialist microbes associated with nests, and describing that diversity is the first step in understanding microbe-host interactions (Goodenough & Stallwood, 2009).

Despite the importance of solitary bees as pollinators, relatively little is known about many aspects of their lives, and this includes the microbial communities supported in their nests. A popular, and often recommended, method of helping solitary bees is installing bee houses in gardens and parks.

Figure 1. *An Osmia rufa nest in a cow parsley stem, split open to show longitudinal brood cells containing cocoons. Each cell is separated from its neighbour cell by a mud partition. The vestibular cell is situated between the last brood cell and the entrance and is sealed with a thick mud plug. This empty cell is common in many solitary bee and wasp species (photo by K. Wisniewska)*



These typically consist of nest tubes (often simply drilled lengths of bamboo cane, plant stems or cardboard tubes) packed together into a holding structure (the "bee house"). The microbial community of these bee houses has not been investigated and so to address this, the Bee Guardian Foundation (www.beeguardianfoundation.org), an education and conservation group dedicated to helping bees of all species, provided us with small solitary bee houses used by the red mason bee for microbiological study. Our aim was to undertake an initial study of the fungal community present in the individual nests, contained within bee houses, to further knowledge of solitary bees in general, and red mason bees in artificial bee houses in particular.

The study bee house comprised 10 "nest tubes" (ca. 10cm long) made from dry cow parsley stems *(Anthriscus sylvestris* — a natural nesting site for solitary bees). Some of the stems had been used by red mason bees as a nesting site for rearing young (Figure 1), and some stems were empty (i.e., unoccupied). This initial study was a preliminary descriptive investigation — a "look see" — which also served to pilot sampling techniques. Regrettably, to sample the stems within the bee house in a complete and robust manner, we had to open the individual stems. Since this meant that the contents of occupied stems (typically bee larvae or pupae) would no longer be viable, we decided to limit our investigations to this one small bee house, thereby keeping the loss of bees to an absolute minimum. Whilst sampling the stems for fungi, we also collected pollen samples that will be analysed separately to provide an indication of the flowers on which the bees forage. This ensured that as much scientific benefit as possible could be gained from the inevitably lethal sampling procedure.

The bee house was collected from an orchard located in the rural district of Horsley, near Stroud in Gloucestershire, UK, in late June 2010 and microbial investigations were carried out over the subsequent two months. Prior to this, the bee house had been at the same location since March 2010 and during late spring and early summer a number of red mason bee females had used the bee house, filling up five cow parsley stems with nests. The remaining five cow parsley stems were unoccupied.

Stems containing *Osmia rufa* brood cells were recognized by the mud seal placed across the end of the tube by the

Figure 2. *The red mason bee (Osmia rufa). Bees are closely related to wasps, but unlike wasps, bees are usually covered in branched hairs that aid in the collection of pollen. The three simple eyes (or ocelli) are clearly visible on top of the head in this image (photo by the Bee Guardian Foundation, www.beeguardianfoundation.org)*

nesting female. Each cow parsley stem, whether occupied or not, was opened with a sterile scalpel. Each occupied stem was filled with individual cells subdivided by a fine wall made from mud collected by the female (a habit giving the mason bee its common name) as shown in Figure 1. The contents of each nest cell and any cocoons present were swabbed thoroughly with sterile rayon-tipped swabs moistened with 9% saline. Between two and four samples were taken from each nest (depending on the number of brood cells). A similar procedure was applied to the five unoccupied cow parsley stems, taking three samples from along their length (one from each end and one in the middle). The swabs were agitated in 10ml of 9% saline, following which sabouraud dextrose and potato dextrose agar plates were inoculated with this liquid. The plates were incubated at 24°C for seven days (to allow the development of slow-growing fungi) and then samples were monitored every 24 hours so that CFUs could be studied. To give an idea of relative diversity, isolates were differentiated on the basis of colour, shape (form, elevation and margin) and size. Isolates were identified to genus level macroscopically wherever possible.

In total, 50 different species of fungi were present and the results from each stem were pooled. Thirty-one of these species were only found in occupied cow parsley stems, 11 were only found in unoccupied stems, and eight were common to both classes. Of the 39 found in occupied stems, only one species was found in more than one stem (despite these stems being held tightly next to each other in the bee house and being gathered, when the house was originally built, at the same place and time) indicating a high level of stem individuality with respect to fungal communities. Despite this variation, there was no significant difference in the number of CFUs between occupied and unoccupied stems. *Cladosporium* was identified as the dominant fungal genus, with multiple individual species within this genus being present in occupied and unoccupied stems. *Mucor* and *Aspergillus* were also present in occupied stems. We also identified *Microsporum*, the causative agent of ringworm infection in many mammals and birds.

The project, though very preliminary, showed that it is possible and straightforward to sample fungi from solitary bee nests and that the nests are home to a rich fungal community, which varies substantially on a nest-by-nest basis. The fact that nearly three times as many unique fungi were found in occupied stems than unoccupied stems is interesting and strongly suggests that the presence of the nesting female is associated with higher fungal diversity. The materials brought into construct the nest cells and plant materials to feed the larvae, would also be potential fungal reservoirs. The presence of 11 fungal species in unoccupied stems that were not found in occupied stems is intriguing and may indicate that "background" fungal species are outcompeted by bee-based fungi in occupied nests, and thus are only found in low-competition environments. This pilot study has produced intriguing results and it is clear that further work, with a larger sample size and more directed identification of fungal species, as well as sampling and culturing of bacteria, should be a priority. Such a study will tell us much about the nesting ecology of bees and may give insight as to how we can help conserve them.

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acknowledgments

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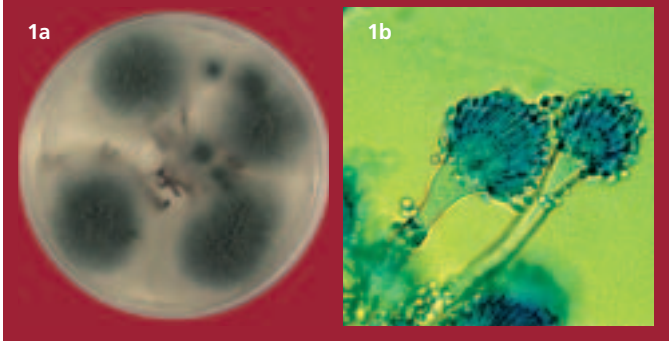
Aspergillus fumigatus and aspergillosis: the curse of ubiquity

Figure 1a. *The characteristic blue green colony of A. fumigatus.* ***1b.*** *The conidial head of A. fumigatus with a single layer of phialides producing small round conidia*



Aspergillus fumigatus is a fungus whose spores are one of the more common members of the airborne flora around the world (Figure 1). It is the cause of a range of diseases that presents particular challenges in diagnosis and treatment.

In the air and in the patient

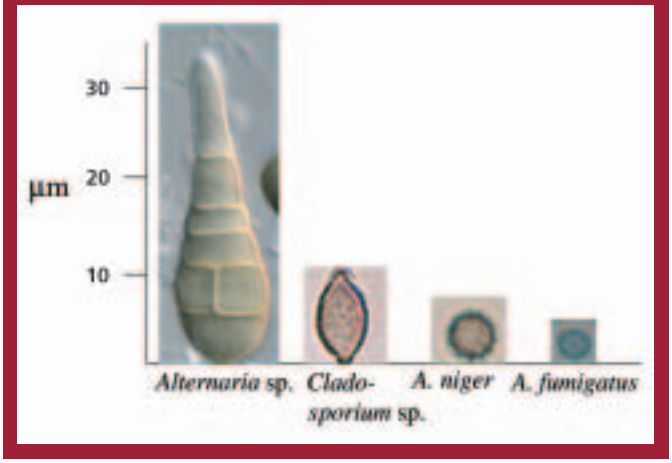
In December 2006, at the Hospital General Universitario Gregorio Marañón in Madrid, Spain, a patient underwent heart surgery and was then nursed in a post-operative room. Unbeknown to medical staff at the time, the air in the room had 175CFU/m^3 of *A. fumigatus*. The patient developed an infection of the mediastinum, the tissue present between the heart and lungs that would have been exposed during surgery. *A. fumigatus* was isolated from mediastinal tissue taken post-surgery and seen in the tissue by histopathological analysis. The strain from the patient and one of the strains isolated from air sampled at that time were subsequently analysed, by examination of several regions of their DNA, and found to be indistinguishable. Despite surgical drainage and antifungal treatment the patient died. In the months prior to this incident the level of *A. fumigatus* recorded in this room was 0CFU/m^3. In the 15 months following this incident a further six cases of aspergillus mediastinitis, or the more common pulmonary invasive aspergillosis, were recorded in every case during or just prior to the recording of the presence of *A. fumigatus* in the environment, ranging from 20–400CFU/m^3 (Peláez *et al.*, 2012).

The epidemiology of the *Aspergillus* disease

The curse of this ubiquity is not felt by most healthy humans, nor by most unhealthy humans, but by two particular patient groups: those with existing lung pathologies and those with severe immune or host defence defects (Kousha *et al.*, 2011). As the conidia of *A. fumigatus* are airborne, in most cases the lungs are the first point of contact following inhalation. Where lung function or integrity is compromised there is an increased risk of some form of aspergillosis. In patients with asthma or cystic fibrosis, forms of allergic or inflammatory reaction can occur. In patients with chronic lung disease, such as bronchiectasis (dilation of the bronchioles) or chronic obstructive lung disease, chronic pulmonary aspergillosis has been described. People with cavities in their lungs from prior tuberculosis, for example, are at risk of these cavities filling with a ball of matted *Aspergillus* hyphae known as an aspergilloma. Once inhaled, the conidia of *A. fumigatus* are usually rendered non-viable through phagocytosis by alveolar macrophages and failing that, by neutrophils infiltrating the lung tissue from the blood. Where neutrophil levels are low, seen typically in leukaemia patients following chemotherapy or stem cell transplantation, there is an increased risk of invasive aspergillosis (IA). IA is the most feared form of *Aspergillus* infection where up until 20 years ago mortality was estimated to exceed 90% (Denning & Stevens, 1990). While markedly improved, mortality remains high (Neophytos *et al.*, 2009).

Any infection is the result of an interaction between a microorganism and human host defences. *A. fumigatus* is a classic opportunistic pathogen that is unable to infect a healthy human host, but takes the opportunity to exploit defects in this defence. Not all fungi cause opportunistic infections, so why is this species such a relatively common cause of fungal disease? Its ubiquity in the airborne flora is one of the most important reasons, though the conidia of *A. fumigatus* are not the most common spores in the air. The spores of fungi such as *Cladosporium* sp., and *Penicillium* sp., are present in the air at a much higher level than *A. fumigatus*, though *A. fumigatus* is more likely to be isolated from either sputum specimens or lung tissue biopsies (Mullins & Seaton, 1978). While disease caused by *Cladosporium* and *Penicillium*, including sensitization-related pulmonary disease, may be important, *A. fumigatus* is still the most important cause of serious fungal pulmonary disease. In part, this is likely to be due to the ability of *A. fumigatus*, along with a few other *Aspergillus* sp., to grow at the human body

Figure 2. *The relative sizes of some of the common airborne fungal spores*



temperature of 37°C. The natural habitat for *A. fumigatus* is decaying vegetation. Compost heaps can generate considerable heat and result in temperatures of 40–45°C, the maximum temperature *A. fumigatus* can tolerate; levels of *A. fumigatus* are particularly high around composting units (Kaarakainen *et al.*, 2011).

Another factor in the virulence of *A. fumigatus* is the size of the conidia. Their diameter of 1–3μm has been estimated to render them capable of penetrating deep into the lungs and into the alveolar space. Conidia from a related species, *A. niger*, and fungi such as *Alternaria* and *Cladosporium* are also common in the environment, but account for a much lower level of disease than *A. fumigatus*. This may be due to the fact that the *A. niger* conidia are significantly larger at 3–5μm and more likely to impact on the surface of the upper airways tract (Figure 2). Thus, the combination of the extensive presence of *A. fumigatus* in the atmosphere, its small size and thermotolerance probably account, to a large extent, for its prominence as a fungal pathogen.

Diagnosis of aspergillosis

The ubiquity of *A. fumigatus* in the environment is perhaps a curse not only for susceptible patients, but also for the clinical laboratory scientist. The most straightforward approach to diagnose pulmonary aspergillosis is to culture respiratory specimens such as sputa and broncho-alveolar lavage (BAL) specimens in order to isolate *A. fumigatus* or other *Aspergillus* sp. Here, the problem is to determine whether a positive culture represents a true infection, transient colonization or even laboratory contamination. In a report on a newly commissioned medical mycology laboratory, the level of *A. fumigatus* in the rooms where this organism was handled rapidly overtook *Penicillium* sp., as the predominant organism isolated from the air (Sautour *et al.*, 2012).

Visualization of *A. fumigatus* hyphae within the specimen by microscopy, often using a stain specific for the cell walls of fungi such as calcofluor, may help confirm that the culture is from an established fungus. But microscopy is labour intensive, relatively insensitive and the numbers of sputa and BAL samples coming into hospital microbiology laboratories daily means that this approach is unworkable. This problem is nowhere more pronounced than in people with cystic fibrosis (CF). Mutations in the CF transmembrane conductance regulator gene result in the accumulation of sticky mucus that lines the airways. *A. fumigatus* is isolated in up to 60% of people with CF, whilst it is thought that only 10–20% will be suffering from forms of aspergillosis requiring treatment (Bakare *et al.*, 2003). Ironically, considering an infection that is supposed to take advantage of deficits in host immunity, many of the signs and symptoms of aspergillosis, like many infections, are a function not of fungal activity, but of the host's excessive and ineffective response to the fungus. Thus, antibody responses to *A. fumigatus* are seen in forms of aspergillosis in immunocompetent patients. This response while not protective, aids the clinician in distinguishing if a positive culture is colonization or infection (Baxter *et al.*, 2012).

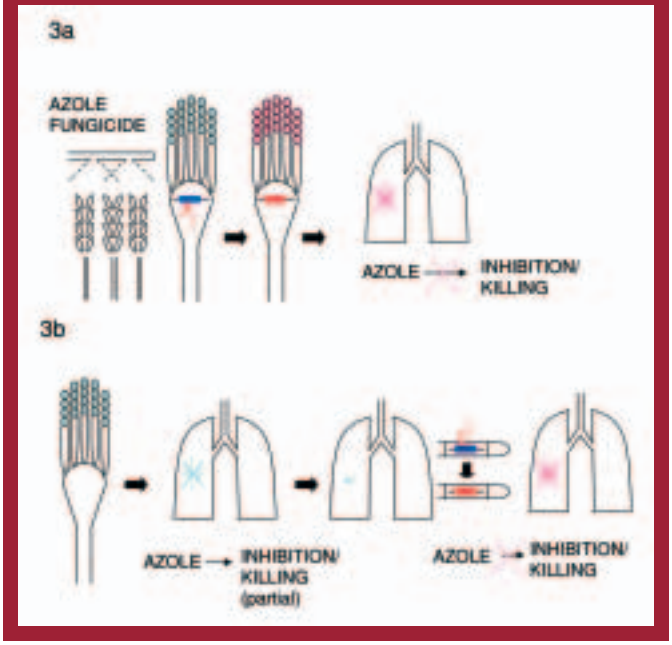
Diagnosis of invasive aspergillosis (IA)

Prompt diagnosis of IA is even more important than the forms of aspergillosis seen in relatively immunocompetent individuals. IA is a rapidly progressive disease and without prompt treatment is usually fatal. IA presents some further challenges as susceptible patients with low neutrophil counts often fail to mount good antibody responses, making this approach to diagnosis unhelpful. Interestingly however, there is mounting evidence that patients with antibodies to *A. fumigatus* prior to the initiation of chemotherapy are at increased risk of IA, and that this analysis may prove to be a useful predictor of this disease (Du *et al.*, 2012). Thus, the approach to diagnosis in this setting is to detect the fungus itself. Culture of sputa or BALs is a key tool but insensitive; typically only 50% of cases will be positive, though for this kind of patient any positive culture will be taken very seriously. Curiously, although during invasive pulmonary aspergillosis the disease spreads to other tissues such as the skin, kidney and brain, *A. fumigatus* is very rarely cultured from blood. This may simply relate to the very short time the fungus is in the blood before lodging in another site. Other diagnostic markers for IA are antigens of *A. fumigatus*, the most important of which is a cell wall carbohydrate, galactomannan (GM). GM is present in the cell wall of *Aspergillus* sp., and to some extent in other fungi. GM can be detected by a commercial ELISA kit in both BAL and blood specimens in patients with IA (Mennink Kersten *et al.*, 2004). GM testing is an important activity in any microbiology laboratory supporting patients at risk of IA. However, because of the high mortality of IA, many centres, caring for patients with haematological malignancies or undergoing stem cell transplantation, treat patients prophylactically with antifungals to reduce the incidence of IA. It has been discovered that where patients have breakthrough IA on prophylactic antifungals, the GM test has reduced sensitivity, thus limiting the usefulness of this test (Marr *et al.*, 2005). Hence, an alternative approach to diagnose IA is the detection of *A. fumigatus* DNA in BALs and particularly in blood. Several laboratories have published success stories regarding the value of PCR-based DNA detection for the diagnosis of IA (White *et al.*, 2006), but the problem with this kind of method has been that, up until recently, there has been a lack of standardization. A European consortium has recently published sets of guidelines on DNA extraction and PCR methods which should help standardize this approach in future (White *et al.*, 2010).

Treatment of aspergillosis

Aspergillosis can often be treated with antifungal therapy, though in some cases treatments to suppress over-reactive immune responses are given instead or in addition (Kousha *et al.*, 2011). Antifungals active against *A. fumigatus* include tri-azoles such as itraconazole and voriconazole, polyenes such as amphotericin B and the more recently developed echinocandins, such as caspofungin. It is the use of such antifungals, particularly lipid formulations of amphotericin B, which have led to a reduction in mortality due to IA in at-risk patients from 90% in the 1980s to 25% in more recent studies (Denning & Stevens, 1990; Neofytos *et al.*, 2009). However, the approach to treating IA in haematological malignancy varies widely. Some centres opt to aggressively treat any neutropenic patient, whose fever is not responding to antibacterial antibiotics with antifungals, assuming a fungal infection so-called empiric therapy. Other centres prefer to apply a range of diagnostic tests with a low threshold to

Figure 3a. *The proposed mechanism of development of resistance to azole antifungals in the Netherlands, the blue box represents the CYP51A gene, the red box the mutated CYP51A gene.* ***3b*** *The mechanism of azole resistance seen in Manchester and other centres*



initiating therapy but if all are negative, hold back from empiric antifungal therapy. Both approaches are valid but the former is expensive and is known to expose many patients to unnecessary treatment (Freemantle *et al.*, 2011). Trials to compare such approaches are currently nearing completion (Morrissey *et al.*, 2011). Another problem with antifungal therapy that has arisen relatively recently is the presence of resistance to azole antifungals in some strains of *A. fumigatus*. Interestingly, this was originally observed in two European centres arising in two very different ways. Studies in Nijmegen in the Netherlands showed increasing rates of itraconazole resistance in their isolates of *A. fumigatus*, some of which were from patients who had never been treated with antifungals and some from the environment (Snelders *et al.*, 2008). This led them to conclude that this resistance was being driven by the use of agricultural azole fungicides (Verweij *et al.*, 2013). In Manchester, UK, resistance to azoles has also been observed at the National Aspergillosis Centre, where many patients with chronic aspergillosis are referred for treatment often following many years of only partially successful treatment. Here, development of resistance from the exposure of *A. fumigatus* cells in the lungs to sub-inhibitory levels of drug has driven resistance by the more traditional route (Figure 3) (Howard *et al.*, 2009). Azole resistance in *A. fumigatus* and its impact on the effectiveness of therapy is being closely monitored as these agents are the mainstay of oral therapy in many forms of aspergillosis.

This ubiquitous eukaryotic pathogen, present so extensively in the environment and occasionally causing disease in humans continues to present academic and clinical laboratory scientists, and clinicians in a variety of disciplines with a challenge to understand, detect and effectively treat affected patients.

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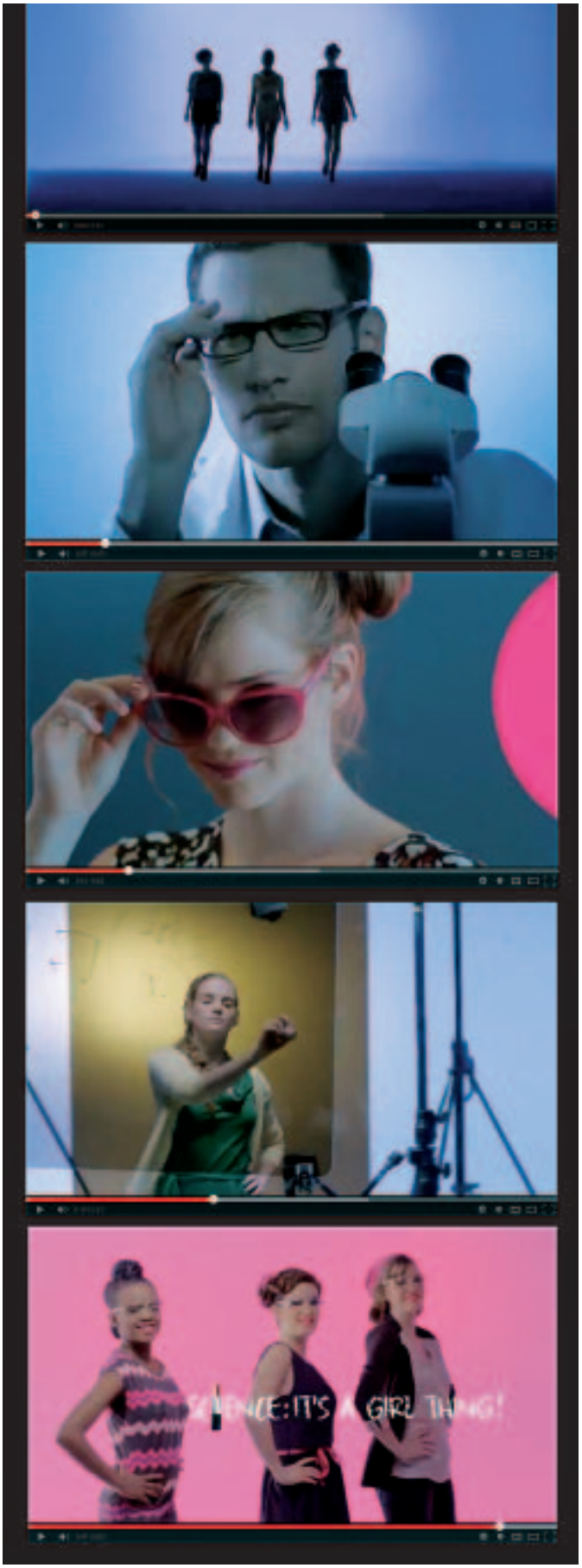


Women in Science: visible and vocal

That video...

The year 2012 will not easily be forgotten with many memorable events such as the Queen's Diamond Jubilee, the London Olympics, Andy Murray winning a grand slam and in June 2012, the EU Commission becoming embroiled in controversy about girls…It's funny, but who would have guessed that it was an online storm about a video that has united so many people. I suspect one day we'll all sit around and laugh when we think back to the day we first saw *that* video, but when the EU Commission's video 'Science: It's a Girl Thing' hit the airwaves, not many people were laughing. In fact, there was outrage from across the globe — a simple internet search reveals adjectives like 'disgraceful,' 'offensive' and 'fiasco' associated with the video on YouTube, and there was a universal feeling that the EU Commission had somewhat missed the point. When questioned about the video in Dublin at last year's European Science Open Forum, Chief Scientific Advisor to the EU, Professor Anne Glover was keen to point out that the video was trying to reach girls that wouldn't normally have an interest in science, but unfortunately the video that was labelled 'ineffective' did offend a rather large number of girls and women in science, technology, engineering and mathematics (STEM) because regardless of intention, it appeared to reinforce a stereotype that girls are more interested in heels, handbags and lipstick than physics, chemistry and biology. Since then, the subject of women in science has provoked some strong responses — especially online. A recent story focused on a popular Facebook science page (with more than 4.4 million likes) which is run by a girl. The reaction by some was one of surprise: yes — a girl is behind this site! Imagine it, a girl liking science and posting funny pictures, observations, and asking questions, and people actually liking it! Initially, the revelation that the page owner was a girl was met with a host of sexist comments, but these quickly turned into something else as people began to question their own views, having assumed a gender profile and so the debate turned once again to stereotypes.

Representation of women (in science) in popular media

Many years ago, I remember watching the BBC science programme *Tomorrow's World* and of all of the presenters over the years, the only two I can recall by name are Judith Hann and Vivienne Parry. It's odd that they are the only two I

remember. As I recall Parry replaced Hann who had been the longest serving presenter on the show. But I think the reason I remember them is because they were women and as a young girl becoming fascinated with things of a scientific nature, images of these impressive women were somehow burned into my subconscious. Sadly though, if I think about many of the science and/or technology programmes I have watched since then, I'm hard-pressed to name any women that feature, which is a bit odd since I know they are out there. In fact, look around carefully, you may be sitting/standing next to one… Sadly, this lack of representation helps to perpetuate the stereotype that scientists are male. Even the BBC's Dara Ó Briain's *Science Club* came in for a bit of stick on Twitter when the first episode aired; some were irritated at the lack of women (Figure 1) but thankfully in the following episodes, more female scientists were featured.

Clearly, the representation of women in popular media is key to ensuring that women in science are visible and that voices and opinions are heard. Television and print media such as daily newspapers are conduits that we are all exposed to regularly, so it is interesting to note that data collected from three daily newspapers shows that news articles featuring science and/or technology are predominantly written by men (Figure 2) with women contributing less than 30% of the items written from June 2011 – July 2012 (Matias & Evans, 2012). To me, this is a sad number since more than 30% of women read newspapers (Bakker, 2013) and therefore, the nation could be getting an unbalanced view. I think it is important to show young people that both men and women are engaged in science regardless of whether that is doing science, teaching science or writing about science.

Depressing statistics

Like me, you have probably read a lot of media coverage recently about women in science and quite frankly, it is all rather depressing. I recently attended a lecture on International Women's Day given by Professor Lesley

Figure 1. *Twitter responses to the first episode of Science Club*

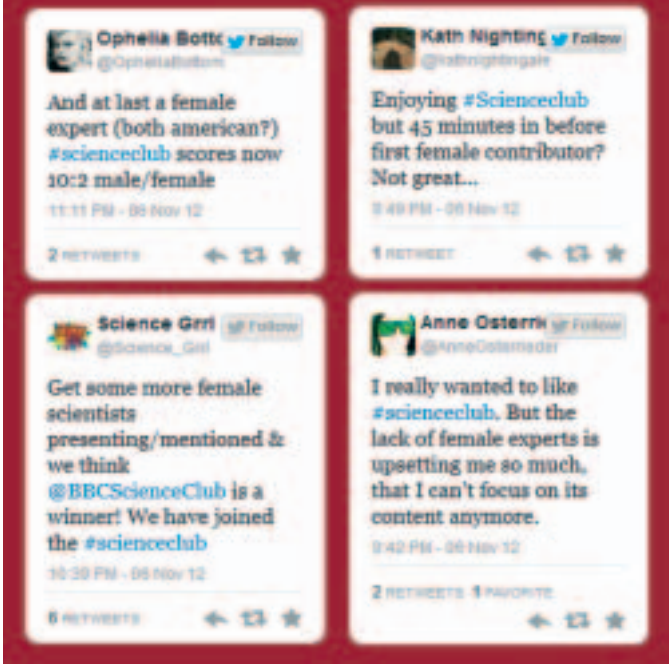
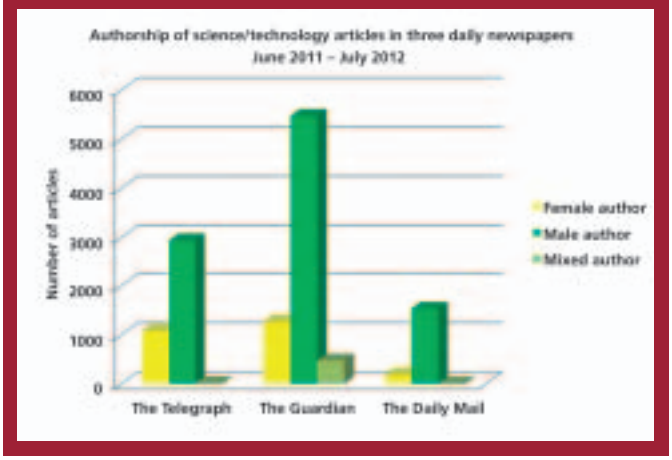


Figure 2. *Authorship of science/technology articles in three daily newspapers June 2011– June 2012 (Matias & Evans, 2012)*



Yellowlees, the current President of the Royal Society of Chemistry and Vice-Principal and Head of the College of Science and Engineering at the University of Edinburgh. Professor Yellowlees is an impressive role model and has been vocal about the 'macho culture' and 'old boys club' that is perceived to be part of the problem preventing women from progressing in STEM careers. But as I sat and listened to all of the statistics around retention of women (you can read examples here: http://www.wisecampaign.org.uk/about-us/wise-resources/uk-statistics-2012; http://www.royalsoced.org.uk/ 1027_Report.html; http://ec.europa.eu/research/science-society/document_library/pdf_06/she-figures-2012_en.pdf) especially in academia, I began to wonder if we will ever really be able to understand all of the complexities around this so-called 'leaky pipeline'. For a more detailed examination, I would recommend you take a look at Professor Athene Donald's blog (http://occamstypewriter.org/athenedonald/), which contains a number of eloquent and astutely written blog posts about gender and academia. The fact remains however, that some women leave STEM careers. But, there are many who remain, and if we celebrate the science performed by these women, rather than the women themselves, then we can show young people (i.e., the scientists of the future) that there are no barriers. Hence, the rest of this feature will celebrate some role models and examples of women taking responsibility for their own visibility.

Putting the Grrl into Science

Amidst all the shock and outrage about *that* video, one group of people decided to 'take up arms' and show that science isn't for girls, or boys, or men, or women, or cats, or dogs, but in fact, science is for EVERYONE. In the space of just four months, a group of people collectively known as ScienceGrrl pulled together pledges of funding and put together a series of photo shoots to create a calendar for 2013, which would showcase a range of different scientists at different stages of their careers. A successful online campaign saw the calendar distributed all over the world and proceeds from sales have gone towards supporting projects to inspire, such as funding young people to attend Mission Discovery (http://isset.org/mission_discovery/). Since then, Science Grrl

(http://www.sciencegrrl.co.uk/#) has gone from strength to strength and continues to grow its network of like-minded scientists, both male and female. ScienceGrrl also intends to work collaboratively in four key areas: 1) resources for schools, 2) informing policy, 3) partnerships with key organizations that share similar interests, and 4) improving visibility of women in STEM, particularly in mainstream culture. S*f*AM are proud to report that Communications Manager, Dr Lucy Harper was recently elected to the ScienceGrrl Committee at their recent AGM and it certainly looks like 2013 is going to be an exciting and busy time for both Lucy and ScienceGrrl!

Soapbox Science

It is clear that the voice of women in science is growing louder. While there is still some way to go to ensure that women are adequately represented at the top of scientific professions and in mainstream media, women are increasingly visible because they have taken charge and are able to reach far wider audiences using social media such as Facebook and Twitter. One such event that gives women a visible platform for their voice is Soapbox Science (http://soapboxscience.org/), an event showcasing female scientists that is now in its third year and as the name suggests, features women on soapboxes in a similar format to that usually seen at Speakers Corner in Hyde Park. The event runs for one day during the summer on the Southbank in London and gives the public the opportunity to get very close to some of the UK's leading scientists. One of the key features of this event is that it is science laid bare — no slides, no animations, just the opportunity to engage, chat, question and even heckle the scientists taking part! This year's event on 5 July will feature two microbiologists, Professor Laura Piddock and Professor Hilary Lappin-Scott who, given the number of microbiology stories that make headlines, are sure to be quizzed on all things infection related!

Support from S*f*AM

S*f*AM has always sought to support our members and offer benefits to ensure that our members can engage with microbiology. However we recognize that in some cases there are barriers which may prevent members from doing so. One of our popular grants is our **Scientific Meetings Attendance Grant**, and we are pleased to announce that we have modified this grant to ensure that all members can take advantage of financial support for meetings. S*f*AM recognizes the need to provide assistance to ensure that all members are able to 'stay in touch' during a period in which they might not normally be able to access funding, so for the first time, as part of the **Scientific Meetings Attendance Grant**, members who are on a period of parental leave (maternity or paternity leave) will be able to apply for costs associated with childcare during the meeting they plan to attend. We hope that this will enable members to balance their work-life commitments and continue to contribute to microbiology while on parental leave. As a first step to ensure all of our members remain visible, S*f*AM is committed to ensuring that the range of assistance we offer is equally available to all.

The future

The evidence is clear and the statistics tell us that despite increases in the number of women studying STEM subjects at university, women only comprise 13% of all those working in STEM occupations (Botcherby & Buckner, 2012). However, the momentum is growing and there is both recognition of the 'problem' and more importantly the will to do something about it. Organizations such as ScienceGrrl provide a supportive network to inspire the next generation to think beyond gender and stereotypes, and events such as Soapbox Science ensure that our celebrated female scientists are both heard and seen. As employers begin to address issues around equality seriously, and scholarly societies such as S*f*AM ensure all members can access support, then we can all be optimistic that the scientists of the future will not see barriers; in the future it should not be necessary to label scientists as 'female' or 'male' because being a scientist will be a natural choice for anyone with an enquiring mind.

websites

- WISE Campaign: http://www.wisecampaign.org.uk/about-us/wise-resources/uk-statistics-2012 Royal Society of Edinburgh, Tapping All Our Talents, Report on Women in STEM: http://www.royalsoced.org.uk/1027_Report.html
- European Commission, She Figures 2012: http://ec.europa.eu/research/science-society/document_library/pdf_06/she-figures-2012_en.pdf
- Professor Athene Donald's blog: http://occamstypewriter.org/athenedonald/
- ScienceGrrl: http://www.sciencegrrl.co.uk/
- Soapbox Science: http://soapboxscience.org/

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Clare Taylor
S*f*AM Executive Committee Member

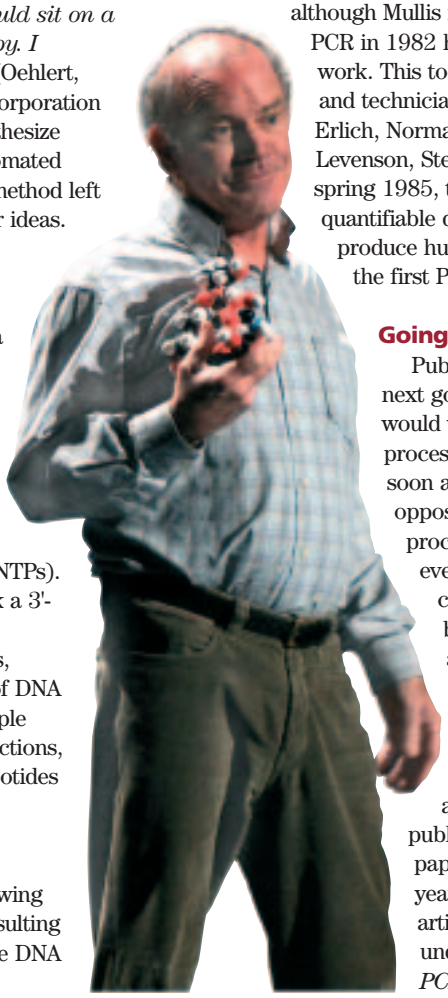
The origins of the polymerase chain reaction

It's almost impossible to think of any other technique in biology which has had a similar impact to that of the development of the polymerase chain reaction (PCR), but 30 years ago PCR was just an idea in the mind of Dr Kary Mullis. Mullis (see picture below), who was a rather enigmatic scientist, eventually went on to be awarded the Nobel Prize for Chemistry in 1993 for his discovery which has revolutionized virtually every area of life science, from diagnostics to forensics, fundamental biological research and even archaeology.

The idea of PCR

The concept of PCR, in which a single molecule of DNA can be used to generate billions of copies of itself using a simple enzymatic method, is so simple that most people's first response when introduced to the technique is, "*Why didn't I think of that, it's so simple*!" However, it did take one man to have that eureka moment. Mullis himself claims, in his *Scientific American* paper, that he came up with the idea "*during a moonlight drive through the mountains of California*" but he has also implicated the influence of psychedelic drugs; "*Would I have invented PCR if I hadn't taken LSD? I seriously doubt it. I could sit on a DNA molecule and watch the polymers go by. I learned that partly on psychedelic drugs*" (Oehlert, 2006). At the time, Mullis worked for Cetus Corporation of Emeryville, where he was employed to synthesize oligonucleotide probes, but the advent of automated instruments to replace the laborious manual method left him a fair amount of time to think about other ideas. It was around then he started thinking about harnessing the natural power of the DNA polymerase enzyme to determine a specific nucleotide, from a long fragment of DNA, as a method of sequencing.

Fred Sanger had invented the dideoxy chain-termination method for sequencing DNA back in 1977. This method required a single-stranded DNA template, a DNA primer, a DNA polymerase, normal deoxynucleosidetriphosphates (dNTPs) and modified dideoxynucleosidetriphosphates (ddNTPs). The ddNTP chain-terminating nucleotides lack a 3'-OH group required for the formation of a phosphodiester bond between two nucleotides, causing DNA polymerase to cease extension of DNA when a ddNTP is incorporated. The DNA sample was divided into four separate sequencing reactions, containing all four of the standard deoxynucleotides (dATP, dGTP, dCTP and dTTP) and the DNA polymerase. Then, only one of the four dideoxynucleotides (ddATP, ddGTP, ddCTP or ddTTP) was added to each reaction and, following DNA extension from the bound primer, the resulting DNA fragments were separated by size and the DNA sequence determined.



Picture: Kary Mullis, the inventor of PCR. Courtesy of Erik Charlton, Menlo Park, USA

Mullis contemplated using the same concept. However, he would omit the ordinary unmodified dNTPs so the extension of the primer would terminate immediately after the addition of the ddNTP. The process would be similar to Sanger sequencing, with the DNA strand being denatured by heating and an oligonucleotide would be hybridized to a complementary sequence on one of the strands. If the mixture was separated into four tubes, containing four types of ddNTPs, he could determine the identity of the corresponding base in the template strand. During his moonlight drive he suddenly realized that by adding a second primer, "bracketing" the targeted base pair, he would effectively have an internal control. Further musings on his drive led him to contemplate running the reaction as a "mock reaction" initially, without ddNTPs to eliminate stray traces of dNTPs prior to adding the ddNTP to perform the sequencing step. It was at this moment he suddenly realized the potential of the technique. Both DNA strands in the target and the extended nucleotide would have the same sequence and the "mock reaction" would have made two copies of his sequence. His familiarity with reiterative loop processes made him immediately realize that if he repeated the process he would double the number of copies again and further repeats would increase the numbers exponentially from 2, 4, 8, 16, 32, 64, etc., with 20 repeats producing potentially a million copies. From this eureka moment came the concept of PCR.

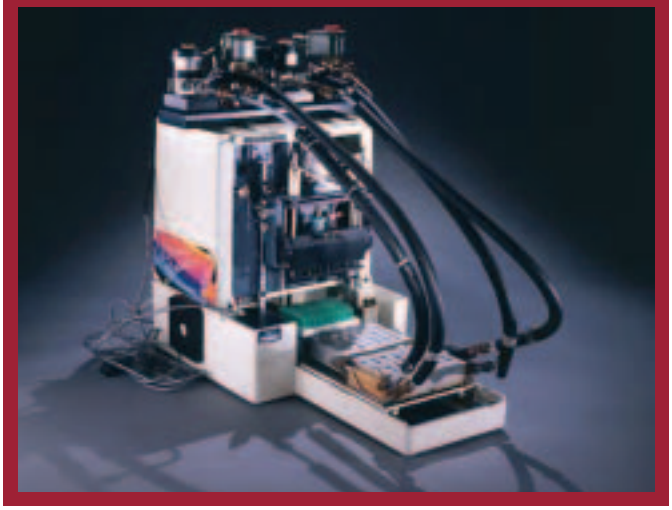
Making PCR work

It is one thing having a great idea but then the hard part is to make it actually work. This is where the controversy starts because although Mullis invented or perhaps discovered the concept of PCR in 1982 he wasn't alone in making the idea actually work. This took the efforts of many other Cetus scientists and technicians working alongside Mullis including Henry Erlich, Norman Arnheim, Randall Saiki, Glen Horn, Corey Levenson, Steven Scharf, Fred Faloona and Tom White. By spring 1985, the team had demonstrated with reliable and quantifiable data that they could amplify genomic DNA to produce hundreds of thousands of copies and Cetus filed the first PCR patent application in March.

Going public with PCR

Publication in a peer-reviewed journal was the next goal and most of the team agreed that Mullis would write the full paper describing the PCR process, and then an applications paper would follow soon afterwards. However, Mullis was fiercely opposed to publishing any paper describing the process and wanted to keep it as a trade secret. He even proposed selling tubes of PCR reagents, the contents of which would remain secret and the buyer would just add their own DNA sample and be amazed by the amount of DNA produced! By September 1985, the rest of the team knew PCR had to go public and Mullis was given a deadline of 1 November for the PCR "fundamentals" paper, after which the applications paper would be submitted for publication. In the end, Randy Saiki's applications paper appeared in *Science* in December of that year and PCR became known worldwide. The article concludes with one of the great understatements of science; "*The ability of the PCR procedure to amplify a target DNA*

Figure 1. *Mr Cycle. The first automated PCR instrument (1985). Courtesy of the Smithsonian Institution*

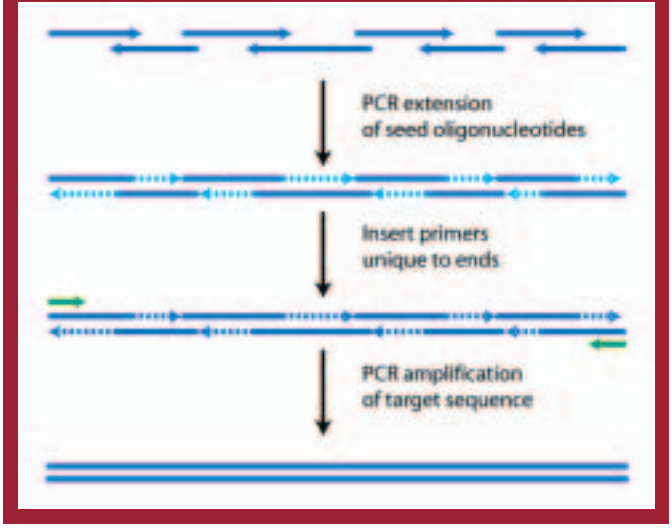


segment in genomic DNA raises the possibility that its use may extend beyond that of prenatal diagnosis to other areas of molecular biology". In December, Mullis submitted his paper to *Nature* but the whole team were stunned when it was rejected as technical and unoriginal. It was hastily resubmitted to *Science* who also rejected it, leaving Mullis extremely bitter about the publication of the earlier applications paper. What should have been the first comprehensive description of PCR was eventually published belatedly in *Methods in Enzymology* in 1987.

From research method to commercial product

To make PCR a marketable commodity there were several improvements needed before it could be properly exploited. At this time, PCR was incredibly laborious requiring samples to be moved between a nearly boiling water bath to one at 37ºC and adding more polymerase, with this process being repeated over and over. The solution was the first automated PCR machine or thermal cycler which was a modified Perkin-Elmer Cetus Pro/Pette liquid handler, dubbed "Mr Cycle" (Figure 1), developed by a PerkinElmer/Cetus Instruments (PECI) collaboration in 1985.

Figure 2. *Schematic of PCR reaction*



Samples were placed in uncapped microcentrifuge tubes in the front block and this block was connected via a switching valve to two water baths (at 37ºC and 94ºC). The rear block held solutions of DNA polymerase and was connected to a 4ºC water bath. It was programmed to incubate the samples at 37ºC and 94ºC by activating the switching valve between the water baths, and pipette aliquots of polymerase enzyme into the samples in the front block. The PerkinElmer/Cetus collaboration led to the introduction of the first commercial DNA Thermal Cycler (TC-1) in 1988.

The final piece in the jigsaw was to identify and purify a thermostable polymerase which could withstand the 95ºC DNA denaturing temperatures. The answer came from the thermophilic bacterium *Thermus aquaticus*, originally isolated in 1969 by Thomas D. Brock and Hudson Freeze of Indiana University. Cetus scientists obtained the bacterium from the American Type Culture Collection and went on to isolate and then patent the heat-stable *T. aquaticus* polymerase giving it the name *Taq* polymerase. This development was the final step in the PCR story which led to the first PCR reagent kits becoming commercially available in November 1987. The availability of a commercial PCR machine and PCR reagents saw an explosion of interest in the PCR technique and soon laboratories all over the world were using PCR for a wide range of applications. *Taq* polymerase was even declared as "molecule of the year" by *Science* in 1989. In the 26 years since the introduction of PCR we have seen further development and innovation in the technology, including the introduction of many modifications of the basic PCR method including real-time PCR and fully automated diagnostic instruments utilizing PCR technology.

Conclusions

The simplicity of the concept of PCR (Figure 2) leads one to conclude that it was inevitable that it would be discovered at some point. This has led some observers to question if Mullis really deserved the Nobel Prize he received. Yes, he was the person who invented the concept, but it took the efforts of many others to make it work in an experimental system. One is left asking whether it is the inventor of an idea or those who actually make it happen who deserve the most credit.

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Andrew Sails
SfAM Meetings Secretary

bioFocus

Mark Downs reports on the Voice of the Future 2013 event

www.societyofbiology.org

Holding policymakers, particularly parliamentarians and the Government, to account is integral to any democratic society. We all like to think that there is the opportunity to change thinking and, particularly from the perspective of a scientist, to ensure that policies are based on evidence. Of course, as we all know, accountability is often variable and not as effective as it might be. Information is often imperfect and voters are sometimes unsure as to the best way to influence a debate.

The science community needs to ensure that accurate information is available and that it is presented in a way which is helpful to our elected representatives. There are many initiatives to try to ensure this happens and a clear willingness from biologists to play their part. Science communication has become an integral part of any scientific training. There is plenty of room for improvement, but I think the media appetite for science and the political willingness to engage in science and evidence is encouraging. However, no matter how good the evidence is, or how strong the arguments are, they are of little value unless politicians know where to turn to and what the community thinks about key policy debates.

Many organizations are working hard to address this and the Society of Biology and our member organizations are now, thankfully, the starting point for debate on many occasions. But it is difficult to represent the views of the diverse landscape of biology across members of all ages and backgrounds working in very different areas. And, it is probably in the area of representing early career scientists where we have been least focused in ensuring politicians understand their views and concerns. To try to redress that, the Society of Biology ran a unique event for the first time last year when we turned the tables on the Science and Technology Select Committee and gave young members from across our organizations the opportunity to quiz politicians directly. None of us were sure how well this would be received and the witnesses who we proposed were inevitably nervous about the way in which the event may have turned out. In the end, it was a huge success, so much so that this year we have been able to run it again, learning from our experience of last year.

Our "Voice of the Future 2013" event took place on 20 March, when members of the Science and Technology Select Committee, the Minister for Science and Universities, the Shadow Science Minister and the Chief Scientific Adviser agreed to be quizzed by a new selection of early career scientists.

As a member organization who contributes to the funding of the pan-science work Dr Stephen Benn, here at the Society of Biology, carries out in Parliament, *Sf*AM was able to benefit from close involvement and a dedicated allocation of places for members to bid for. We were delighted to see Sabrina Roberts, Christiana Adesanwo, Joanna Kronda, Cassie Olateju and Emmanuel Adukwu participate, each with the opportunity to ask a question of their choosing and, even better, to be able to engage a very wide audience through the BBC's live broadcast of the event. If you would like to see this or look in more detail at the questions asked, please visit: http://tinyurl.com/c52joy8 **or** http://www.societyofbiology.org/ newsandevents/news/view/533.

Horsemeat in the food chain, the challenge of climate change, openness and transparency in Government advice and, from an *Sf*AM perspective, the challenge of developing new antibiotics, were just some of the questions raised by the early career scientists. The Rt Hon David Willetts MP was put on the spot about the Government's approach to funding research and tuition fees, spending some time explaining why he felt the situation in the UK was just as good, if not better, than many of our competitors: listen to the answer to make up your own mind!

As well as direct dialogue between the three rotating groups of young scientists who took turns to ask their questions, there was also a significant interaction from around the UK and indeed further afield through social media, with many followers commenting through forums such as Twitter. This element of the day is captured in Storify: http://tinyurl.com/cn826u6.

There is no-one-size-fits all solution to ensuring everybody is engaged effectively in policy debates and ensuring accountability. Nonetheless, unique events such as this can make a useful contribution to raising the profile of science, demonstrating the range and types of scientist in professional bodies, and in the push for more evidence-based policymaking. It also provides, for many of the attendees, a first taste of the parliamentary process and how to get involved.

It was a good event for Parliament too and it is important that we recognize the willingness of our representatives to engage in these issues. For so many busy people, including the Speaker of the House, to give up time on budget day bodes well for the future as a positive reflection on our parliamentary system at a time when it is often criticized. After all, who could imagine the Congress in the United States offering to be quizzed live on television by groups of young American scientists in their own backyard?

> I found the Voice of the Future event to be a valuable experience which I both enjoyed and learnt from. It was a great way to interact with the MPs responsible for setting scientific policies as well as interacting with other young scientists from a wide variety of backgrounds. I found that a lot of topics, such as the small number of women in science, were common themes throughout different parts of the country as well as many different disciplines.
> *Cassie Olateju*

> I found the event very informative and a good channel for dialogue between the scientific community and policymakers. It was also important that the young and early career scientists put forward their concerns regarding topics such as careers and employability, women in science, immigration, and other pressing scientific issues before the Minister and MPs. This was indeed an enjoyable event which offered great networking opportunities.
> *Emmanuel Adukwu*

> A fantastic day at the House of Commons that spurred my interest in science policy. The highlight for me was getting the opportunity to ask Sir John Beddington a question.
> *Sabrina Roberts*

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

The workshop ▼

Early morning at the children's book festival ▼

Return of the

Readers may remember from a previous issue of *Microbiologist* (**Vol. 13**, No.1), the success of the Manchester Metropolitan University (MMU) Monsters, Maths and Microbiology team in our attempts to use zombies, werewolves and vampires to convey principles of disease epidemiology to the general public.

We had developed an 'activity mat' that participants could work on, identifying parameters of an outbreak of some manifestation of "undeadness" according to different scenarios. These parameters were entered into our 'SimZombie' programme (www.matthewcrossley.com/sims; Crossley & Amos, 2011), which presented the spread of the outbreak via a population of 2000 individuals (dots) that changed from live (green) to dead (red) over time.

We tested the activity with staff and students at MMU, and with members of the public at a pub quiz event during the 2011 Manchester Science Festival. This gruesome subject matter was also the theme of two meetings of the Bad Bugs Bookclub (*I am Legend* by Richard Matheson and *World War Z* by Max Brooks). It was clear to us that the activity had great potential for use by a younger audience, with their obvious morbid fascination with all things zombie — evidenced by the rapidly increasing number of novels on the subject.

The Manchester Children's Book Festival, brainchild of Carole Anne Duffy, Poet Laureate and MMU Professor, was launched in 2010. Our science offering explored the parallels between vampirism and infectious disease transmission via the *Twilight* novel (Stephenie Meyer), combining readings, discussion (particularly about influenza), and demonstrations of disease transmission (by contact, not by biting!). The workshop was set up as a biology laboratory (for those who haven't read the book, Edward and Bella — the main characters of the novel — first met over a microscope whilst investigating the cell cycle), and participants received information on hand hygiene, and departed with some useful information and a cute vampire bite tattoo transfer.

Unlike vampires, zombies exude very little charm, and rather more pus, providing an excellent opportunity to explore disease spread in the absence of romance! Charlie Higson, comedian and zombie author (*The Enemy*, *The Dead*, *The Fear*, *The Sacrifice*) has noted in his public lectures the resultant interest in zombies amongst boys. So, zombies present a new audience, a new monster and some more microbiology! An ideal topic for the 2012 Manchester Children's Book Festival! Katie Carolan, undergraduate microbiology student, supported the work as her final year project. She identified four novels (*The Enemy* by Charlie Higson, *Warm Bodies* by Isaac Marion, *Rot and Ruin* by Jonathan Maberry and *Boneshaker* by Cherie Priest) of interest to early secondary school pupils (around 12 – 14 years), and picked scenarios from each that demonstrated the behaviour of the zombies and the uninfected populations. These scenarios formed the case studies that would be explored in our 'epidemiology lab' workshop. Katie, myself, Matt Crossley (computing PhD student and SimZombie expert) and Van Le (public engagement intern) donned our white coats and assumed our roles. Newsfeeds were projected onto a screen

◀ *Left and below: simulated zombie bite created by our make-up students*

▲ *activity sheet used to stimulate discussion around outbreak scenarios*

zombies!

showing the emergence of the zombie pandemic 'outside'. Next we led our participants (in four groups) through their scenarios, reading from the novels, so that we could discuss and identify parameters of their zombie outbreak. These parameters were then entered onto their activity sheets, ready for entry into SimZombie.

We invited four media make-up students from Stockport College, who spent the whole day creating more and more zombies amongst the visitors to the family fun day — truly a plague of increasing numbers of ghouls. They also created a very realistic bite that was hidden under Matt's lab coat until the end of the workshop.

In our event planning, we had noticed that there were really only two basic scenarios to deal with zombies in all the novels — isolate the uninfected, or isolate the zombies. Matt created visual models of these options on SimZombie, including events described in the novels — zombies invading the uninfected haven, or zombies escaping from their isolation. In the first case, the zombies always 'won', once they'd gained access to the uninfected. In the second, it was possible to contain the zombies as long as not too many escaped and the enclave was well guarded. This, of course, shows parallels with immunization and quarantine!! Immunization only works if herd immunity (protection) is maintained; likewise quarantine. We were rather pleased with ourselves for noticing these important prevention strategies, as well as their limitations!

Meanwhile, Matt's arm was beginning to ache. He revealed his bite to the workshop participants, and we then had to find out if he had infected anyone. Not resorting to transmission by biting in this case, we had laced Matt and his wound and his worktable with wash and glo fluorescent gel, so that everyone and everything he had touched also glowed.

We ended the session by quarantining his table forever, and hurrying the other groups from the room. Perhaps they are still there, locked in the classroom...

further information

■ This activity has proved really versatile. We have adapted it for a range of audiences — teachers, adults, families and children — and we have used different delivery formats — zombie awareness 'bootcamp', pub quiz, school activity, drop-in and workshop. We are planning to develop a learning pack in the near future that summarizes all of our work. If interested in trying anything out, please get in touch! We would like to thank the Society for Applied Microbiology for providing funding for this work through their **Public Engagement Grant**. For more information visit: http://www.sfam.org.uk/en/grants--awards/public-engagement-grant.cfm.

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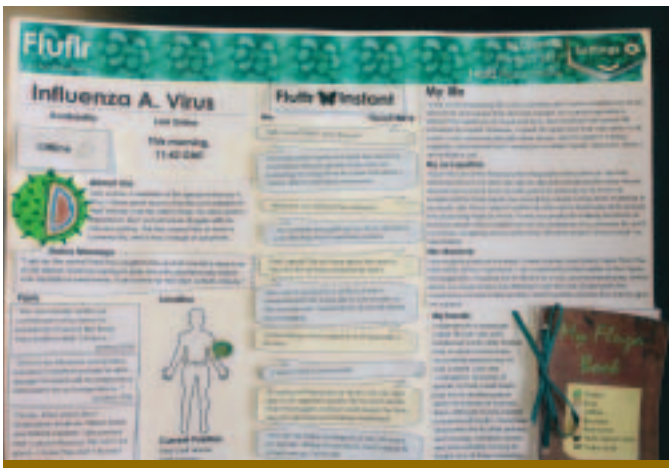
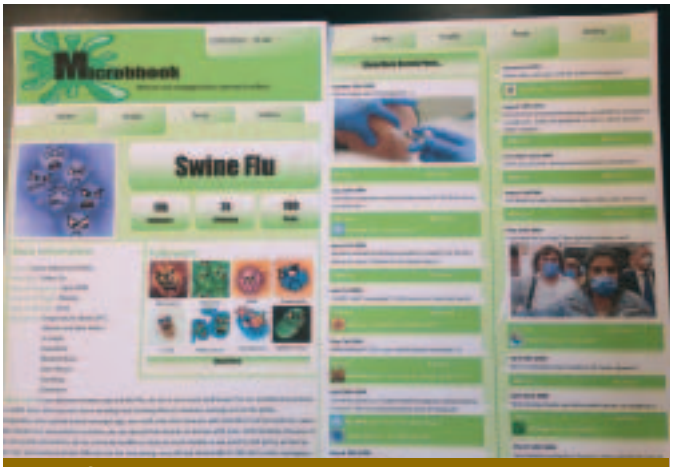
Joanna Verran
Manchester Metropolitan University

MiSAC Competition
Social media – a gateway into schools

Judging the entries



KS3 - first prize



KS4 - first prize

KS4 - second prize

This year saw the 25th anniversary of the MiSAC competition for schools which S*f*AM were proud to support. The competition theme was social media, and judging by the competition entries, this really sparked the imaginations of many of the students. We asked for each entry to represent the social media profile of a microbe that has an effect on human health and for each profile to describe how the bug in question causes this effect.

And the response was immense! One of the largest number of entries in the competitions' history was received from students in two categories: Key Stage 3 (KS3) and Key Stage 4 (KS4) (Secondary 1/2 and 3/4 respectively in Scotland) — some 700 entries involving almost 1000 students.

A wide range of microbes was chosen — mainly pathogens and their association with infectious disease — describing their effects, spread, prevention, eradication and history in equal measure. It was clear that fun was had in creating the entries which were imaginative, topical and in many cases extremely witty. One status update for the fungus *Penicillium chrysogenum* read: "Finally moved house! We have been trying to move out of the soil for a long time. We were looking at cultivated land and decayed vegetation, but in the end we found some wonderful temperate forest floor!"

The technical capabilities of entrants were also clearly evident, with many of the entries displaying the genuine look and feel of the social media networks we all use, such as Facebook, Twitter and Flickr.

The judging process was made easy by the inventive nature of the entries. I was lucky enough to be part of the judging panel alongside members of MiSAC, S*f*AM President Martin Adams and *The Times* Science Correspondent, Tom Whipple.

MiSAC would like to thank all participants and their teachers who encouraged them and provided MiSAC with some great feedback about this years' competition. Winners and their schools share £1,000 prize money and a certificate of entry was provided for all students who enter the competition.

Lucy Harper
S*f*AM Communications Manager

journalWatch

News about the Society's journals

Highlights from the SfAM journals

Microbial Biotechnology

Now Open Access!!!

Evolution of *Bacillus thuringiensis* Cry toxins insecticidal activity.

In this review, we discuss how Cry toxins have evolved insect specificity in nature and analyse several cases of improvement of Cry toxin action by genetic engineering. Some of these examples are currently used in transgenic crops. We believe that the success in the improvement of insecticidal activity by genetic evolution of Cry toxins will depend on the knowledge of the rate-limiting steps of Cry toxicity in different insect pests, the mapping of the specificity binding regions in the Cry toxins, as well as the improvement of mutagenesis strategies and selection procedures (http://onlinelibrary.wiley.com/doi/10.1111/j.1751-7915.2012.00342.x/full).

***Corynebacterium glutamicum* promoters: a practical approach**.

In this mini-review, the main aspects of promoter studies and their use to analyse *C. glutamicum* are discussed. These include definitions of the consensus sequences of the distinct promoter classes, promoter localization and characterization, activity measurements, the functions of transcriptional regulators and examples of practical uses of constitutive, inducible and modified promoters in biotechnology. The implications of the introduction of novel techniques, such as *in vitro* transcription and RNA sequencing, to *C. glutamicum* promoter studies are outlined (http://onlinelibrary.wiley.com/doi/10.1111/1751-7915.12019/abstract).

***Microbial Biotechnology* – Crystal Ball 2013**

In the *Microbial Biotechnology* Crystal Ball feature, leading researchers speculate on the technical and conceptual developments that will drive innovative research and open new vistas over the next few years (http://onlinelibrary.wiley.com/doi/10.1111/1751-7915.12014/abstract). www.microbialbiotech.com.

Environmental Microbiology

Advances in computational analysis of metagenome sequences.

Second-generation sequencing technologies are revolutionizing the study of metagenomes. Whole-genome shotgun sequencing of metagenomic DNA may become an attractive alternative to the current widely used ribosomal RNA gene studies. Large data sets of short sequence reads are mapped onto a custom microbial reference sequence. This paper looks at how parallel sequencing of metagenomic DNA allows deep insights into the composition and the genetic repertoire of polymicrobial communities (http://onlinelibrary.wiley.com/doi/10.1111/j.1462-2920.2012.02843.x/abstract).

Antibiofilm polysaccharides.

Bacterial extracellular polysaccharides have been shown to mediate many of the cell-to-cell and cell-to-surface interactions that are required for the formation, cohesion and stabilization of bacterial biofilms. However, recent studies have identified several bacterial polysaccharides that inhibit biofilm formation by a wide spectrum of bacteria and fungi both *in vitro* and *in vivo*. This review discusses the composition, modes of action and potential biological roles of antibiofilm polysaccharides recently identified in bacteria and eukarya. Some of these molecules may have technological applications as antibiofilm agents in industry and medicine (http://onlinelibrary.wiley.com/doi/10.1111/j.1462-2920.2012.02810.x/full).

Special Issues available online now: Plant-microbe interactions; Environmental ecology of pathogens and resistances; Baeza. www.env-micro.com.

Environmental Microbiology Reports

The Clermont *E. coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. There is extensive genetic substructure within the *E. coli* species. In 2000, a simple triplex PCR method was described by Clermont and colleagues, enabling an *E. coli* isolate to be assigned to one of the phylo-groups A, B1, B2 or D. Here, a new PCR-based method is developed that enables an *E. coli* isolate to be assigned to one of the eight phylo-groups and which allows isolates that are members of the other cryptic clades (II to V) of *Escherichia* to be identified. The development of the method is described and the method is validated (http://onlinelibrary.wiley.com/doi/10.1111/1758-2229.12019/abstract).

***Nosema ceranae* (Microsporidia), a controversial 21st century honeybee pathogen**.

Nosema ceranae, a microsporidium recently detected in the European bee all over the world, has been implicated in the global phenomenon of colony loss, although its role remains controversial. A review of current knowledge is presented, considering a biological or veterinarian point of view. For authors, the disease produced by *N. ceranae* infection cannot be considered a regional problem, rather a global one, as indicated by the prevalence of this parasite in multiple hosts. Not only does this type of nosemosis cause a clear pathology on honeybees at both the individual and colony levels, but it also has significant

effects on the production of honeybee products (http://onlinelibrary.wiley.com/doi/10.1111/1758-2229.12024/full).

***Environmental Microbiology Reports* – Crystal Ball 2013**

In the Crystal Ball feature, leading scientists in the field of environmental microbiology consider the technical and conceptual developments that they believe will drive innovative research and open new vistas over the next few years (http://onlinelibrary.wiley.com/doi/10.1111/1758-2229.12021/full). www.env-micro-reports.com.

Journal of Applied Microbiology

Supramolecular reactive sulfur nanoparticles: a novel and efficient antimicrobial agent.

Antimicrobial resistance continues to be an inexorable threat for biomedical and biochemical researchers. Despite the novel discoveries in drug design and delivery, high-throughput screening and surveillance data render the prospects for new antimicrobial agents as bleak as ever. The advent of nanotechnology however, strengthens pharmacology by offering effective therapeutics to treat this problem. Here, we focus on various aspects of the physico-chemical features of sulfur nanoparticles (SNPs) and their biochemical interactions with microbes. This review also illustrates the effects of SNPs on plants and animals in terms of cytotoxicity and biocompatibility (http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2672.2012.05422.x/abstract).

Types of cell death and methods of their detection in the yeast *Saccharomyces cerevisiae*

The occurrence of programmed cell death in unicellular organisms is a subject that is of great interest to theoreticians and experimental scientists. Evolutionarily conserved genes and metabolic pathways have confirmed its existence in yeast, protozoa and even bacteria. In the yeast *Saccharomyces cerevisiae*, at least three main types of cell death are described: apoptosis, necrosis and autophagy. Several laboratory methods previously used to detect the types of cell death of higher eukaryotes, and later developed and successfully used for the analysis of yeast cells, are critically reviewed. Their advantages and limitations are described (http://onlinelibrary.wiley.com/doi/10.1111/jam.12024/full).

Virtual Special Issues available online now:

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www.journalappliedmicro.com.

Letters in Applied Microbiology

A multiplex RT-PCR for rapid and simultaneous detection of viruses and viroids in chrysanthemum.

Chrysanthemum plants are subject to serious viral disease, so detection and identification of viral pathogens is important to prevent virus spread. A reliable one-step multiplex RT-PCR was developed to simultaneously detect two viruses and two viroids: *chrysanthemum virus B*, *tomato Aspermy virus*, *chrysanthemum stunt viroid* and *chrysanthemum chlorotic mottle viroid*. In addition, the detection limit and the efficiency of single and multiplex RT-PCR assays were investigated. The results showed that the multiplex RT-PCR assay proved to be as sensitive as the single one. In conclusion, this technique is potentially useful in the routine diagnosis of chrysanthemum viruses and viroids (http://onlinelibrary.wiley.com/doi/10.1111/lam.12007/abstract).

Analysis of microbial diversity on deli slicers using PCR and DGGE technologies.

Cross-contamination of pathogenic and spoilage bacteria from food-contact surfaces to food products is a serious public health issue. The objective of this study was to assess the extent and distribution of microbial diversity of deli slicers by identification of pathogenic and background bacteria. Slicer-swab samples were collected from restaurants in Kansas and Texas in the United States. Bacterial distribution was similar for all surface areas, while the blade guard exhibited the greatest diversity. This study provides a profile of the microbial ecology of slicers using DGGE to develop more specific sanitation practices and to reduce cross-contamination during slicing (http://onlinelibrary.wiley.com/doi/10.1111/lam.12021/full).

Featured Editor's Choice article:

Real-time PCR-based rapid and culture-independent detection of *Salmonella* in dairy milk — addressing some core issues.

This investigation reports on an optimized real-time PCR technology for the detection of *Salmonella* strains in milk under field conditions. This study examined the development of a SYBR Green based real-time PCR assay for culture-independent detection of *Salmonella* in milk. The methodology is based on the immobilization of PCR components to increase the stability of kits and to make this assay simple and user-friendly. In addition, a new DNA extraction method for whole milk samples was developed and data on the stability of the master mix reaction, reliability and rapidity of the method are provided. This proposed new assay may facilitate the routine culture-independent detection of pathogens in dairy milk under field conditions (http://onlinelibrary.wiley.com/doi/10.1111/lam.12046/abstract). www.lettersappliedmicro.com.

Melissa McCulloch
Wiley-Blackwell

StatNote 33

In the 33rd of a series of articles about statistics for biologists, **Anthony Hilton** & **Richard Armstrong** discuss: **Intra-class correlation coefficient (ICC)**

In a previous StatNote (Hilton & Armstrong, 2008), the degree to which one variable was linearly related to another was measured using Pearson's product moment correlation coefficient ('r'). The intra-class correlation coefficient (ICC or r_i) is a less well-known coefficient and used primarily for measuring correlation between two variables when the data are paired or organized into groups. A useful analogy is with the unpaired and paired 't' tests (Hilton & Armstrong, 2005) to compare the differences between two means. Hence, an unpaired 't' test is used to compare the means of two independent samples, while the paired 't' test is appropriate if two measurements are made on the same experimental unit or are paired, e.g., by age, location or some other variable. Similarly, the ICC is used to test the degree of correlation between paired measurements, e.g., to compare the degree to which siblings may resemble each other in a quantitative feature, the correlation between the abundance of a bacterium isolated from the left and right eyes of patients, or the degree of reproducibility of a measurement made either by different observers or methods. This StatNote applies the ICC to two scenarios. First, to assess the repeatability of two types of agar in estimating the viable count of a bacterium in broth culture and second, whether there is a correlation between the counts of a bacterium, *viz.*, *Propionibacterium acnes* in the left and right eyes of a sample of patients.

Scenarios

Repeatability study

We return to the scenario first described in StatNote 22 (Hilton & Armstrong, 2010). A hypothetical substitute product for nutrient agar, Magi-plate™, has become available on the market. This product is offered as a dehydrated powder which, when reconstituted with distilled water, triggers an exothermic reaction of sufficient energy to sterilize the media without autoclaving. The molten agar is simply poured into sterile Petri dishes and when set, offers a culture medium with an application range identical to nutrient agar. The method could be an attractive feature for environmental microbiologists on field studies, where access to autoclaving facilities may be limited.

An initial trial investigated the repeatability of nutrient agar and Magi-plate™ to estimate the viable count of *E. coli* in a broth culture. Twelve flasks containing 10ml of sterile nutrient broth were inoculated with one colony of *E. coli* and incubated with shaking for six hours at 37°C. Following incubation, serial 10-fold dilutions were prepared from each flask in fresh nutrient broth and the surface of a nutrient agar plate and a Magi-plate™ inoculated in duplicate (plate A & B) with 0.1ml of each dilution. In each case, the inoculum was spread over the surface of the media using a sterile spreader and the plates incubated at 37°C for 24 hours. Following incubation, the dilution bearing the largest countable number of colonies was selected for estimation of the viable count in the flask. Counts were corrected for volume, dilution plated, and the CFU per ml of the original culture calculated for replicate plates A & B for each culture media. For the purpose of this scenario, the data comprise two separate estimates (A, B) of the counts of bacteria in 12 separate cultures by each of the two methods (nutrient agar, Magi-plate™) and are clearly paired. The data are presented in Table 1.

Table 1. Viable counts (10^6 per ml) of bacteria from 12 samples determined by two methods (Nutrient agar, Magi-plate™) each on two occasions (A, B).

	Method			
	Nutrient agar		*Magi-plate™*	
Culture vessel	*A*	*B*	*A*	*B*
1	1.55	1.54	1.52	1.58
2	1.6	1.61	1.4	1.4
3	1.52	1.66	1.36	1.42
4	1.79	1.45	1.58	1.39
5	1.2	1.12	0.95	1.02
6	1.47	1.7	1.21	1.24
7	1.39	1.58	1.27	1.32
8	1.84	1.78	1.47	1.57
9	1.86	1.8	1.52	1.44
10	1.29	1.37	1.01	1.19
11	1.99	1.89	1.81	1.93
12	1.5	1.49	1.43	1.63

ICC: Nutrient agar = 0.78; Magi-plate™ = 0.88

Left and right eye study

Propionibacterium acnes is a Gram-positive bacterium that forms part of the normal flora of the skin, mouth, large intestine, ear canal and eye. In the eye, *P. acnes*, is often found in association with the surface of the conjunctiva, i.e., the outer membrane of the eye covering the white fibrous sclera, and extends onto the surface of the upper and lower lids. Although involved in the development of acne, *P. acnes* can also cause a variety of post-operative infections including complications of cataract surgery, one of the commonest types of surgery performed in the UK. *P. acnes*, however, is a very slow-growing bacterium and may cause an eye infection up to a year after cataract surgery. The result is an inflammation of the iris, ciliary body and choroid, termed 'uveitis', which is usually accompanied by inflammatory deposits on the inner surface of the cornea and lens implant.

An investigator wished to determine whether both eyes were equally at risk of this type of complication following cataract surgery by measuring the degree of correlation between colony counts of *P. acnes* obtained from the right and left eyes of a series of 12 subjects. A topical anaesthetic (0.5% proparacaine) was first applied to the eye and a swab of the surface of the right and left eyes taken with a soft-tipped applicator previously moistened with a sterile medium. The applicator was inoculated onto a medium composed of enriched thioglycollate, 5% sheep's blood and chocolate agar, and incubated anaerobically. The density of *P. acnes* on the surface of the eye is usually very low and dilution of the sample prior to inoculation is not necessary. Following incubation, the number of colonies of *P. acnes* from both eyes of each subject was recorded. As in the first scenario, the data

are clearly paired, one measurement being from each eye of each patient.

How is the analysis carried out?

Theory

The ICC was one of the earliest correlation methods to be applied to paired data. The main difference between the ICC and the conventional Pearson's 'r' is that all data are pooled to estimate the mean and variance of the ICC. A further difference is that, unlike the ICC, the magnitude of Pearson's 'r' depends on the variability between subjects and where this variation is greater, larger values of 'r' will result (Bland & Altman, 1996). In addition, in a paired study, the pairs of values are considered to be 'unordered', e.g., in twin or sibling studies, there is no meaningful way to determine within a twin pair which individual should come first. Reversing the order of any pair would give a slightly different correlation if Pearson's 'r' was used. The ICC avoids this problem because it estimates the average correlation among all possible orderings of the pairs of observations.

ICC is calculated from a 'random effects' model of the analysis of variance (ANOVA) (Hilton & Armstrong, 2007). A commonly used notation to describe the basic model of an ANOVA is to use the subscript 'i' to denote the group or class (i.e., the treatment group), 'i' taking the values '1 to a', and the subscript 'j' to designate the members of the class, 'j' taking the values '1 to n' (hence, 'a' groups and 'n' replicates per group). Within class 'i', the observations x_{ij} are assumed to be normally distributed about a mean μ with variance s^2. This linear model can be written:

$$x_{ij} = \mu + a_i + e_{ij} \quad \text{.....(1)}$$

Hence, an observed value x_{ij} is the sum of three parts: (1) the overall mean of the observations (μ), (2) a treatment or class deviation 'a', and (3) a random element 'e', drawn from a normally distributed population. In a random effects model however, it is the components of variance that are important, i.e., the component of variance of a_i is σ_α^2 and e_{ij} is σ_ε^2. The ICC can then be defined as follows:

$$\text{ICC} = \sigma_\alpha^2 / \sigma_\alpha^2 + \sigma_\varepsilon^2 \quad \text{..... (2)}$$

The ICC detailed in this article is only one of several ICCs that have been described, but is the most efficient, and also has the advantage that it can be used to analyse data where there are more than two observations per subject. The best method of calculating this ICC is from the sums of squares (SS) of a one-way ANOVA of the data as follows:

$$\text{ICC} = \text{MSS}_B - \text{SS}_T / (M - 1)\ \text{SS}_T \quad \text{..... (3)}$$

Where M is the number of groups being compared, SS_B is the SS between culture vessels or subjects and SS_T is the total SS of the data (Bland & Altman, 1996).

Interpretation

The repeatability of the measurements of bacterial counts determined by the two types of agar is shown in Table 1. The ICC for the nutrient agar (0.78) is less than for Magi-plate™ (0.88) suggesting that the latter may result in more reproducible results. These results are in accord with those of the Bland and Altman method of measuring 'agreement' discussed in StatNote 22 (Hilton & Armstrong, 2010), which suggest that the limits of agreement were smaller for the Magi-plate™ method.

A comparison of the counts of the anaerobic bacterium *P. acnes* in the right and left eyes of 13 subjects is shown in

Table 2. Counts of the anaerobic bacterium Propionibacterium acnes in the right and left eyes of 13 subjects.

Number of bacterial colonies		
Subject	***Right eye***	***left eye***
1	6	4
2	0	1
3	0	0
4	5	4
5	3	2
6	0	0
7	1	1
8	7	6
9	4	3
10	11	9
11	2	4
12	0	0
13	1	3
ICC between left and right eyes = 0.91		

Table 2. The value of ICC = 0.91 suggests a highly significant correlation in bacterial counts between the left and right eyes of a subject. The implication of this result is that if an infection caused by *P. acnes* follows cataract surgery in one eye, ophthalmologists should be alert to the possibility that there will also be a high probability that the fellow eye could be affected.

Conclusion

The ICC is a method of measuring correlation when the data are paired and therefore, should be used when experimental units are organized into groups. A useful analogy is with the unpaired or paired 't' test to compare the differences between the means of two groups. In studies of reproducibility, there may actually be little difference between the ICC and Pearson's 'r' for 'true' repeated measurements. If however, there is a systematic change in the measurements made on the first compared with the second occasion, then the ICC will be significantly less than 'r', and less confidence would be placed in the reproducibility of the results.

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Winter Meeting 2013 report

Royal Society, London, UK, Wednesday 9 January 2013

Food mycology

Emerging technologies in applied microbiology

This year's Winter Meeting was held on the 9 January at the Royal Society, London. Jointly arranged with the British Mycological Society it covered two fascinating topics; Food mycology and Emerging technologies in applied microbiology.

The meeting opened with the Denver Russell Memorial Lecture, which was awarded to the immediate past President of the Society, Geoff Hanlon. In many regards Geoff's work has paralleled the career of the late Denver Russell, for example, by training in pharmacy and later pursuing interests in biocides. Geoff gave a fascinating presentation on the use of microbial systems to study the metabolism of important drugs. In humans, many drugs are metabolized by cytochromes in the liver. Some fungi produce monooxygenases, which are functionally similar to cytochromes. Screening a range of different fungi identified several organisms capable of metabolizing 2-arylpropionic acids, a class of drugs that includes ibuprofen. Like many drugs, profens have a chiral centre and it was important to identify which enantiomer was metabolized. Work in this area led to the development of an optimized system for producing a single enantiomer from a racemic mixture of profens. This approach has great potential for the production of pure drug compounds that do not suffer from toxicity due to alternative enantiomers.

The next talk was given by the immediate past President of the British Mycological Society, Naresh Magan, who described the dangers of fungal toxins in foods. Increasingly, food manufacturers are being driven to reduce the use of preservatives in foods. There is a risk that reducing the concentrations of preservatives too far will allow the growth of toxin-producing fungi, leading to outbreaks of food poisoning. Using DNA microarrays, it was shown that intermediate concentrations of some preservatives can trigger the up-regulation of fungal toxin genes, potentially leading to dangerous concentrations of toxin accumulation even without extensive fungal growth. As the food industry evolves, it will be important to ensure that toxigenic fungi are not inadvertently given a foothold in products for human consumption.

Mark Wilcox from the HPA in Leeds concluded the morning session with an introduction to the PLEX_ID system. The system was first developed for rapid detection of bioterrorism agents, and involves the amplification of microbial genes by PCR, followed by the identification of amplified fragments by mass spectrometry. Work at Leeds has shown that the system can be used for the rapid identification of microorganisms in a wide variety of clinical samples. The use of targeted PCR also allows the detection of antibiotic resistance genes and provides rapid insight into the optimal antibiotic therapy to prescribe to a patient. The system is still in development and the next generation model is eagerly awaited.

Nick Jakubovics

Martin Adams presenting Geoff Hanlon with a framed print

Following a delicious lunch delegates attended one of two afternoon sessions. The first speaker in the '***Food mycology***' session was Ulf Thrane, University of Denmark. Ulf continued where the morning's lecture had left off with his presentation; '***Metabolomics and taxonomy aspects of food spoilage moulds***'. Ulf discussed the presence of mycotoxins in food and the difficulties in identifying the fungi responsible for the toxin. Ulf explained that by using secondary metabolites it was possible to generate information about the fungi responsible for the toxins and potentially identify it. Ulf continued by talking about the challenges and confusion created by dual nomenclature for fungi, and that the recent changes to fungal classification were a step in the right direction. Ulf concluded by expressing his hope that in the future, classification of fungi would move towards 'one fungus, one name, one metabolite profile'.

The next speaker in the session, Sonia Marin, presented her work on '***Modelling growth of mycotoxigenic fungi***'. Sonia discussed the use of mathematical models to ensure the quality and safety of food products. Sonia's work looked at predicting spoilage to ensure the quality of products. Sonia explained that fungal quantification was not usually a good indication of the existing levels of mycotoxins in foodstuffs and so, to ensure the quality of the food remained high, the aim had to be to prevent mycotoxigenic fungal growth. Sonia concluded her talk by saying that probability models could be applied to either growth or toxin production and used within food safety management systems.

After a quick tea break we came back for the final part of the afternoon session. Phil Voysey, Campden BRI, was the penultimate speaker of the day and gave a fascinating insight into '***Spoilage fungi in the factory environment***'. Phil started by explaining that spoilage of food products by yeasts and moulds is a major concern, but that relatively little was known about this area of research. Phil explained that by increasing our knowledge of this area it could be used to extend the shelf life of foodstuffs. Phil's research had involved environmental sampling within the factory environment over a two-year period. These samples were then characterized by genus and analysed on a molecular level. Phil explained that this work could be used to provide a better understanding of spoilage fungi in the factory environment and help in the development of control measures.

Paul Dyer, the final speaker of the day, discussed '***Spoilage fungi and sex in the food environment***'. Paul began by reminding us that not all fungi were bad and that some fungi played a beneficial role in the development of food products. Paul explained that many foods depended on fungi to give them flavour. The fungi *Aspergillus oryzae*, the base for miso, is so revered in Japan that there is even a cartoon character named after it! Paul then went on to discuss the importance of sex within fungal species in the food industry. Paul and his team had been investigating the possibility of sexual reproduction within species, known only to reproduce asexually. By creating conditions favourable to sexual reproduction, Paul and his team hoped to improve the strains by creating a better flavour or a faster growth rate. This research was still ongoing but they already had some promising results. The session was bought to a close by the chair, Naresh Magan, who thanked the speakers for four fascinating talks before wishing us all a safe journey.

Clare Doggett

*Sf*AM Meetings Secretary, Andrew Sails, chaired the afternoon session addressing '***Emerging technologies in applied microbiology***'. The first speaker, Nick Loman from the University of Birmingham, gave an interesting talk on the '***Applications of next generation sequencing in microbiology***'. Nick explained how the sequencing of whole bacterial genomes has greatly helped in the understanding of bacterial pathogenesis and evolution. However, the recent development of high-throughput sequencing, which is faster and cheaper than conventional sequencing, has enabled the investigation of tens to hundreds of isolates in a single experiment. These improved sequencing platforms now generate data quickly enough and cheaply enough to have an impact when investigating outbreaks. Large-scale sequencing experiments have improved the understanding of the population structure of pathogenic bacteria. Nick explained this provides an informed epidemiological investigation of outbreaks within hospitals and local communities, suggesting potential chains of transmission in the healthcare environment. Genome sequences can also provide a real-time view of bacterial evolution, including changes associated with host adaptation and the development of antibiotic resistance phenotypes. The ever-decreasing costs of sequencing now means that the genome sequences from bacterial communities in clinical samples can be sequenced directly without the need for culture, yielding vital diagnostic information and permitting the detection of novel or previously undetectable pathogens. Nick discussed how whole-genome sequencing has already transformed clinical microbiology, with particular reference to how he played a role in the crowd sourcing of the Shiga toxin-producing *E. coli* 0104:H4 genome, during the 2011 'sproutbreak' in Germany and France.

Andrew Fox, HPA Royal Preston Hospital, then presented '***Molecular methods in food and water microbiology***'. He

highlighted how the detection of pathogens in food, water and environmental samples requires timely confirmation for effective public health action. The current protocols used by the Health Protection Agency, Food, Water and Environmental (FW&E) Microbiology Laboratories require culturing plus additional biochemical and phenotypic tests. As these conventional methods can take five to seven days, there is a need for a rapid identification of pathogens, particularly in outbreak situations.

Andrew explained how RT-PCR-based methods provide rapid detection and identification of *Campylobacter coli*, *Campylobacter jejuni*, *Listeria* spp., *Listeria monocytogenes*, *Salmonella* spp., *E. coli* 0157 and verocytotoxin genes 1 and 2 from directly enriched food matrices, and isolates from bacterial cultures of all sample types. RT-PCR is a closed tube system which minimizes DNA contamination. As it is semi-automated there is also a reduction in contamination and human error. This assay is as sensitive and specific as traditional culture-based methods for screening food samples therefore, allowing culture-based testing to be focused on positive samples. As culturing is no longer required, the direct addition of DNA extract / isolate suspension into the lyophilized PCR assays significantly reduces the detection and identification time for foodborne pathogens. The molecular testing of pathogens from food, water and environmental samples using PCR provides accurate results with high sensitivity and specificity. This has led to improved public health management due to the rapid response to outbreaks.

'***MALDI-TOF in microbiology***' was the topic of the third talk, given by Wolfgang Pusch from Bruker Daltonix GmbH, based in Germany. Wolfgang gave an overview of MALDI-TOF and its use in microbial identification for the 21st century. The material being analysed is put on to a sample plate, placed under a high vacuum and a laser is used to cause desorption / ionization. The time it takes for the ions produced to reach the detector is converted into molecular weight. This technology is able to identify microorganisms in minutes from liquid and solid cultures. It works on the principle that each species has a unique proteomic fingerprint. The fingerprint produced by the microorganism under investigation is matched against a reference library of over 2,150 species and the result is based on score-based pattern matching. MALDI-TOF can give rapid results in identifying microorganisms in the food, pharma and environmental monitoring industries.

The final talk of the session was '***Automating the bacteriology laboratory***', presented by Neil Bentley from the HPA Cambridge. Neil discussed how the Carter Report in 2006 indicated that the pathology services in England should consolidate multiple sites to provide large laboratories, each serving a number of NHS hospitals, to increase savings. In response to this review, the management at the HPA Cambridge set up a strategy to modernize and future-proof their laboratory. Neil highlighted that historically, bacteriology laboratories have had limited automation with staff performing a number of manual tasks. When total system automation became available, it was purchased and installed in their laboratory. Neil discussed how there were a number of issues when this was installed, for example, staff responding negatively to the changes, delays to work during the installation and fluctuations in currency which affected budget. However, automation has had a positive impact upon staff and the running of the department, as staff were able to concentrate on tasks for which they were trained, instead of the time-consuming manual tasks, they were able to focus more on the patient rather than the process, therefore enhancing their roles within the department. Even though the workload has increased by 6%, this system has enabled an increase in productivity through the reduction in bench reading times, quick negative authorizations and automatic zone reading for antibiotic susceptibility. Future initiatives include further automation of the department, for example, the addition of urine microscopy and inoculation to provide an effective and efficient service.

Samantha Law

1 - 4 July 2013

Summer Conference 2013

IBMS CPD ACCREDITATION 15 POINTS

- **Lactic acid bacteria and bifidobacterium**
- **Actinobacteria**

■ Including the *Journal of Applied Microbiology* (JAM) Inaugural Annual Lecture

Hilton Cardiff Hotel, Cardiff, UK ■ Monday 1 July – Thursday 4 July 2013

DELEGATE FEES UNCHANGED FOR 2013!

Programme

Monday 1 July 2013

11.00 – 17.00 **Workshop session:** public engagement

18.00 – 19.00 ***Journal of Applied Microbiology*** **Inaugural Annual Lecture**
When the sleepers wake: the germination of bacterial spores
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 **Intestinal microbiology in early life**
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 **Marine actinobacteria and drug discovery**
William Fenical, University of California, USA

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**

SfAM Award Lectures

Chair: **Martin Adams**

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

16.05 – 16.40 **SfAM New Lecturer Research Grant Lecture. HIV-1 maturation inhibitors: a novel class of antiretroviral drug**
Catherine Adamson, St Andrews University, UK

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**

For the latest programme please visit: www.sfam.org.uk/en/events/index.cfm/summer_conference

Environmental Microbiology lecture 2013

Programming soil bacteria to do amazing things

On the 28 October 2013, the annual SfAM *Environmental Microbiology* Lecture will take place at the Institute of Civil Engineers, 1 Great George Street, London. Attendees will hear from Professor **Víctor de Lorenzo**, CNB-CSIC (Madrid, Spain) who will discuss the enzymatic activity of environmental microbes and how such properties can be harnessed and put to invaluable use. The vast majority of this treasure trove hasn't yet been exploited, and in order to do so, understanding of a vast number of fundamental biological processes is needed. Genetic engineering successes and failures will be presented to illustrate what needs to be done in order to merge genetic engineering with environmentally-friendly technologies.

Professor de Lorenzo is a Spanish chemist and microbiologist. He works as Professor of Research at the National Centre of Biotechnology in Madrid, where he has been employed since 1996 after running a large number of Molecular Microbiology and Environmental Biotechnology activities at the Pasteur Institut (Paris), the University of California (Berkeley), the University of Geneva and the Federal Center of Biotechnology (Braunschweig). His research exploits advanced molecular biology and genetic engineering of soil microorganisms (e.g., *Pseudomonas putida*) for the sake of biomonitoring, bioremediation, and wherever possible, valorization of chemical pollution in the environment.

Professor de Lorenzo is also active in promoting a European-wide debate with the various stakeholders interested in the beneficial application and dissemination of modern research in biology. **http://www.cnb.csic.es/~meml**.

All members will have received an invitation to the lecture with this issue of *Microbiologist* and for those who are unable to attend, the lecture will be available online soon after the event.

Institute of Civil Engineers, 1 Great George Street, London ■ 28 October 2013

benefits

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

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

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

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

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

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

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

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

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

membership options

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

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

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

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

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

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

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

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

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

JOIN US!

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

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.

Bangladesh

B. Sabikunnahar

Belgium

T. Callwaert

Brazil

A. de Souza Sant'Ana

Canada

G. Nish

Chile

F. R. Cerda Leal

Costa Rica

A. Castillo-Zeledon; M. Suarez-Esquivel

Greece

A. Balkoni; M. Gavra; E. Gyftogianni; K. Kondylis; V. Kontaratou; M. Mezili; M. Souri; L. Tsounis

India

S. Goal; A. Kocharekar

Italy

G. Di Consoli; N. Monaco; G. Spano

Japan

M. Fakuda; N. Kimura

Malaysia

Z. A. Bashir

Mexico

C. A. Garza; L. Guzman; A. Sanchez Reyes; G. Valencia

Nigeria

T. Adebisi; V. O. Adegeye; A. Akinpelu; B. Ayodeji; M. I. Dania; O. Daniel; E. Nwoke; I. O. Olotu; S. Saibu; K. Sowunmi

Northern Ireland

A. De Baroid

Pakistan

I. Ali; N. Siddiqui; H. Z. Wadood

Peru

M. Gutierrez-Correa

Poland

P. Golinska; J. E. Karczewska-Golec

Portugal

N. Velosso

South Korea

S. Gim

The Netherlands

A. Abdelkhaliq; D. Fei; E. M. Pothius; M. Spus; Y. R. Wardhana; P. Zeballos Rebaza

Turkey

I. Akyol

Uganda

P. Musoke

UK

A. Abdel Latif; R. Akoy; H. Alhammod Alhilli; P. Almedia Powell; T. Anwar; M. N. Asogwa; E. Ballou; M. Barden; N. Barnes; E. J. Beattie; M. A. Beese; K. Belfield; J. Berry; C. Bloor; C. Bowen; M. A. Breban; C. Broadbent; L. Buddrus; O. Burns; M. Carpenter; A. Carter; P. G. Cartwright; F. Cheesman; Y. Cheng; S. Chetal; M. Cho; S. Y. Chong; C. Cooper; H. Cox; A. Crooks; H. Cruickshank; G. D'Arcy; M. L. Dawes; E. Dias; L. Doolan; A. Dunn-Sale; R. Eagles; C. Eldridge; S. Esse; J. Evans; C. Fairbrother; M. Falage; J. Faucher; S. Fiaz; C. Foster; C. Frapwell; L. Furness; J. U. Gaddi; S. L. Gallop; E. L. German; R. Goomany; S. Gower; R. Greenup; S. Gregory; M. Hair; L. Hall; K. Hambly; S. J. Hardwick; P. Harvey; H. Hassan; R. Hilmi; J. Holman; M. E. Horan; J. Howson; N. Humphreys; R. Issa; E. Jameson; A. M. Jayanth; O. J. Jowett; A. Kadamparambil Muhammad Unni; V. Kulesh; T. Lancaster; A. Lavender; G. Leung; S. J. Lonsdale; W. L. Low; A. Mabiala; R. R. Makharita; F. Mallerve; C. Marden; J. Mattock; R. Meek; P. J. Millichap; S. Mohamad; H. Mohamed; R. Norman; M. Norman; I. Norton; D. Nurnberg; C. O'Callaghan; N. Okonkwo; A. Olalemi; P. Olley; H. Onyeaka; M. Ostrovsky; K. Page; R. Patel; J. Paterson; S. Pereira; E. Pettit; K. Pishtsheva; H. Plant; J. Porciuncula; M. Price-Hayward; I. L. Princewill-Ogbonna; S. Procopiou; P. Prodromakis; J. Ramsamy; J. Redfern; C. Renshaw; K. J. Robertson; G. Robson; S. Rowe; T. Russell; M. Rycroft; K. Salek; C. Sanders; J. Score; N. Siddique; E. Sozzi; K. Sriskanda; C. Stevens; T. Stewart; S. S. Sumar; C. Summerson; M. Swaine; V. Thies; R. Thompson; C. Trevelyan; N. Van Veggel; M. Van-de-Velde; N. Wallmsley; F. C. Webber; T. Williams; H. Wing; H. Wood; E. Yankova; K. Yates; P.-E. Zimunya

USA

S. Azeem; R. Beger; A. Gorton; R. Gray; C. M. Hall; E. Levin; E. Lucassen; A. Taken

Losses

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

SfAM Communications Award
Call for nominations!

Have you been spreading the word about an important applied microbiology topic? Do you know someone who has? Then nominate them for the 2013 SfAM Communications Award.

Perhaps you've seen a really interesting artwork with microbes as its theme, or read an elegant piece of writing about infectious disease spread? Maybe you've read a popular science book about environmental microbes or you've watched a television programme covering the role of microbes in food production?

This award aims to recognize individuals who have communicated and engaged the public with applied microbiology (either their own work or that of others) and can demonstrate measurable outcomes of the nominated work.

There will be provision for awards in each of two categories:

- **Category 1: Scientist**
- **Category 2: Professional communicator**

Each award is worth £500 and nominations should be in writing using the online form at the link below, providing detailed information about relevant media/communications work of the nominee. Nominees will be invited to receive their award at a dedicated event during 2013.

The closing date for nominations is **14 June 2013. For more information and to nominate yourself or somebody else, visit:** http://www.sfam.org.uk/en/grants--awards/sfam-communications-award.cfm.

Obituary
Ronald Board

The Society is sorry to announce the death of Professor Ronald G. Board. Professor Board had an international research reputation as a food safety microbiologist and advised both governments and food companies. His research interests included *salmonella* in poultry and yeasts in cold meats / sausages. He was an active member of the Society for Applied Microbiology as a Journal Editor of the then *Journal of Applied Bacteriology* (1973 –1981) and later as Editor with special responsibility for reviews (1988 –1991). He was instrumental in helping the Society to find a new publisher for the journals (Blackwells) in 1981 and was made an Honorary Member of the Society in 1992.

SfAM AGM AGENDA 2013

The 82nd Annual General Meeting of the Society for Applied Microbiology will be held on Wednesday 3 July at 4.45 pm at the Hilton Hotel, Cardiff.

1. Apologies for absence

2. Approval of minutes

Approval of minutes published in the September 2012 issue of *Microbiologist* of the 81st Annual Meeting held in Edinburgh in 2012.

3. Matters arising from the previous minutes

4. Report of the Trustees of the Society 2012

(i) Report of the President.

(ii) Report of the General Secretary.

(iii) Report of the Meetings Secretary.

(iv) Report of the Treasurer.

5. Adoption of the 2012 Annual Report

6. Election of new Members

(including Honorary Members), deaths and resignations.

7. Nomination and election of the General Secretary

8. Nomination and election of the Vice President

9. Nomination and election of new Executive Committee Members

10. Any other business

Scientific Meeting Attendance Grant or President's Fund?

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

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

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

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

In the loop pecs

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

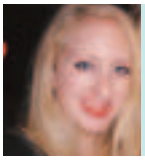
Student activities at the Summer Conference

Where: **Cardiff, Wales**

When: **1 – 4 July**

Summary of events

- **Monday 1 July 2013**, following the *Journal of Applied Microbiology* Lecture, icebreaker event.
- **Tuesday 2 July 2013**, 5.00 – 6.00pm, Student session 'Career options — what next?'
- **Wednesday 3 July 2013**, 5.30 – 6.00pm, open PECS Committee meeting.



Jenni Drever-Heaps
PECS Publications Officer

Every year the Postgraduate and Early Career Scientists (PECS) Committee put together a selection of events and activities for the students attending the Summer Conference and this one is no exception!

If you are shy or apprehensive about networking then why not attend the icebreaker session being held on Monday night before the quiz. The icebreaker provides the perfect opportunity to meet other delegates in a fun, stress-free environment and will provide a fantastic starting point to develop new contacts throughout the conference.

The PECS Committee thought long and hard about what the student session should focus on this year. 'The future' is always an important consideration in anyone's life. In science there is a vast diversity of careers, but figuring out what path to take can be very difficult. That is why we decided to bring in a panel of experts in various scientific occupations to give you the opportunity to ask any questions you might have about your future options. If you are thinking about a possible career in academia or even going into industry but aren't sure how to approach it, then come along to the student session and get some answers.

For those wanting an alternative to the conference evening events we have a list of things to do in Cardiff. Ellen Evans is one of the latest recruits to the PECS Committee and also happens to be a Cardiff resident so has put together a few ideas. Just outside the conference accommodation, the Hilton Hotel, there are numerous bars on Greyfriars Road and on the east side of town, including the Live Lounge that often has live bands and DJs playing until late into the night. There are plenty of places to walk and sit outside, let's just hope that the weather is favourable! If it is raining, the iconic Wales Millennium Centre often has free concerts/performances and exhibits in the foyer, with a lovely café and gift shop for Welsh souvenirs. The bay area is easily accessible; there are regular buses from the Hilton, aqua buses from the city centre and trains departing from Queen Street, and in the bay there is a Hollywood Bowling alley with a student deal of 2 bowling games for £7.50. If you have not registered to attend the conference dinner maybe you might like to try some of the restaurants and bars in the Cardiff Fashion Quarter; an area located opposite the Castle entrance on Womanby Street. You could try Fire Island, Pica Pica and Zero Degrees for food, or 'Clwb Ifor Bach' (Welsh club) and the Full Moon for music.

On Wednesday, the PECS Committee will be having an open meeting that all students and early career scientists are welcome to attend. This is a prime opportunity to have your say about what you would like to get out of future student-led events. If you are reading this and would be interested in having more input into the PECS group then we would also like to invite you to our steering group, just let one of us know at the conference and we will put you on the list. The steering group has direct involvement with the Committee, contributing ideas about organizing events and the direction in which the PECS Committee should be moving, this could be a great opportunity to add some extra-curricular activities to your CV.

If you have any questions regarding the student events or anything else, why not visit the PECS pages on the S*f*AM website for details about who we are and how to contact us. We really look forward to see you all at the conference!

Meandering through microbiology

Sally Cutler describes her varied and eventful microbiological career

Early signs of a future biology-related pathway started to emerge during my childhood. Instead of mucking out the horses in the field, I was often to be found studying the insect life living within the equine faeces. A brief exploration into understanding the language of poultry made me realize that perhaps I should start to study something smaller but, at this time, I did not know what.

My school did not even attempt to introduce the world of microbiology to me. However, I had become aware that biology was my strength and that I wished to go to university. The careers advisors of the time did suggest I should be more realistic in my aspirations and that a job as a secretary might be more appropriate. Even today, I still fail to heed the advice of others, trying to forge my own way forward. So, as a girl who had gone through the local secondary modern, I applied to various universities to study microbiology (the degree path was more a process of elimination rather than having identified a passion for microbes at this stage). Even this stage was challenging with one of my interviewers asking me what I thought were the major problems facing the world and how I would go about solving them. I now realize microbiology was the answer. I, however, rambled on about overpopulation. At this stage of my life, I naïvely thought that if I could understand the working of microbes, then maybe I would be able to advance to understanding higher animals.

I successfully secured a place to study microbiology at University College London. I soon realized that I adored the subject — a passion that has stayed with me. I was drawn towards the medical aspects of microbiology. Before graduating, I participated in a field trip to the Sinai desert (then Israel) which also inspired me with a desire to travel. Although not involving microbiology, it did allow the collection of scorpions using mark and recapture methods to estimate the level of these and other arachnids in the area in which we were camping. Upon graduating, I was convinced that diagnostic medical microbiology was the direction for me. Filled with enthusiasm to launch my career, I was met with the situation that many graduates of today have to wrestle with — finding that first job! After almost six months of trying, success! I was offered a job in diagnostic virology under the late John Bertrand at St Thomas' Hospital but, as with buses, my job offers came in pairs, and within a couple of days, I was also offered a job in diagnostic bacteriology at the Royal London Hospital. After much soul-searching, I chose the latter.

So, day one arrived, working as a junior B medical laboratory scientific officer (now biomedical scientist). As a graduate entrant among a group of largely non-graduate staff, my reception was a little frosty, but by the afternoon of day one, I was soon wrestling with the delights of plating out purulent sputum! The experience was rewarding in several ways, I was living and breathing microbiology, and was spotted by my now husband. After a couple of years and having gained my Fellowship of the Institute of Biomedical Science, I moved to Stoke Mandeville Hospital to broaden my experience. I additionally undertook a Masters in Medical Microbiology at the University of Surrey on a day release basis. This taught me that maximizing full-time education was the best way to study rather than trying to juggle study and a full-time job. Hindsight is a wonderful thing! Having been at Stoke Mandeville for nearly four years, I had started to become slightly disillusioned with diagnostic microbiology. I could see little in the way to progress my career and still work "hands-on" with microbes. The realization had also dawned on me that to succeed as a scientist, I needed a PhD.

Having decided to change my direction from diagnostic microbiology to research, at last an opportunity was in sight to undertake a part-time PhD, whilst technically managing the Lyme borreliosis reference unit based within the Microbiology unit at Charing Cross

Hospital. Although this setting was still within a diagnostic unit, my tasks were to develop, assess and implement the Lyme borreliosis assays being used to test referred samples, and in my "spare" time to conduct research into the consequences of Lyme borreliosis during pregnancy. This, I later realised, was not a feasible study within the UK given our reduced case burden compared with other European countries. This left me with a predicament regarding the PhD, but prompted me to weave together various studies that had been conducted under the overarching theme of epidemiology of Lyme borreliosis in the UK. I adored the challenges that research presented, especially learning new techniques and applying these to address specific research questions. I also developed a passion for working with *Borrelia*, and expanded this interest into the relapsing fever borreliae, facilitated through a trip to Ethiopia. Following this, I was able to cultivate *Borrelia recurrentis*, something that had evaded microbiologists since the days of Robert Koch. Subsequently, I was able to extend these studies to *B. duttonii* in Tanzania. These studies truly brought home the impact of microbiology with the first job of the morning being recruitment of patients from charitable hostels and having to see the reality of those who had not survived the night.

Between these studies, I also found another role in life, that of being a mother. Thus, I gained full experience of having to juggle both roles (probably badly) and having to put up with comments such as, "*Mum, why can't you have a normal job like the other mums so you can be there to collect me from school?*" I survived these criticisms and three children, though the challenges of childcare did not make things easy. Financial constraints were being imposed and through restructuring exercises I found myself in a vulnerable position; running a national facility without the financial support to sustain it had left me sitting on a "time bomb". I was relocated to the Hammersmith Hospital where I was given several months to find external income or move on. Regrettably, the funding was not forthcoming, but in the eleventh hour I received a call from the Veterinary Laboratories Agency (now AHVLA) regarding a short-term opportunity to help deliver on a Lyme borreliosis project that was shortly coming to term. Whilst within this short-term post, a permanent post of research team leader for the VLA brucellosis research team became available and I was successfully appointed to this role.

As my research interest had started out with zoonotic infection, it appeared a natural progression to broaden this to other forms of zoonoses. The only difference was that many of my "patients" now had four legs rather than two. This post was rewarding. However, much of the role was managing projects and staff rather than getting my lab coat on to undertake the research myself. That said, there were huge opportunities facilitated by the resources available at such an institute. The negative side of this story is that the supported research was primarily to inform policy decisions rather than being able to follow interesting science. This, coupled with limited eligible sources for research funding, made me consider that an academic environment might provide greater freedom of research directions.

Having been at VLA for nearly five years, I moved on to an academic role, initially as a Senior Lecturer, then Reader in Medical Microbiology at the University of East London, where I have worked for the last five years and my passion for microbiology research drives me forward. Additionally, passing this enthusiasm on to others who can take research efforts forward is also hugely rewarding.

Upon reflection, my goal has been to undertake medical microbiology, which I have succeeded in doing under various guises. To date, my career has spanned diagnostic medical microbiology through to research from both human and veterinary aspects. Although I miss being at the bench myself, delivering research through the hands of others is also rewarding. Furthermore, feasting upon our emerging knowledge of microbes and their host interactions will always have me entranced. Whether I have understood how microbes work, my teenage aspiration, I must confess still eludes me.

Sally Cutler
*Sf*AM Executive Committee Member

Students into Work Grant reports

am I eligible — can I apply?

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

For further information visit: **http://www.sfam.org.uk/en/grants--awards/students-into-work-grant.cfm**

The persistence of *E. coli* and *Enterococcus* species in water and sediments from fresh and saltwater systems in East Sussex

Studies indicating the survival and persistence of faecal indicator bacteria (FIB) in aquatic sediments, have questioned the reliability of microbiological water quality surveys. The significance of these findings, particularly from a public health perspective captured my interest. I wanted to investigate the topic myself in my local area, and I took the opportunity to do so during my dissertation at the University of Brighton. Thanks to a recommendation from my lecturer, Dr Jonathan Caplin and funding from the Society for Applied Microbiology, I was able to pursue this line of research full time for a further 12 weeks.

E. coli and *Enterococcus* are the current FIB of choice. FIB are important tools in the monitoring of drinking and bathing water, and the control of disease. Tests for FIB are frequently carried out all across the world to determine the degree of contamination and assess how safe the water is for human contact. The EU Bathing Water Directive states threshold levels of FIB in bathing waters, categorizing water as poor, good and excellent quality (European Union, 2005). Recent research has brought to light the idea that FIB may be surviving in underlying sediments as well as overlying water (Craig *et al.*, 2002, 2004; Haller *et al.*, 2009). If this is the case, then sediment disturbances such as rainfall may affect counts of FIB in the overlying water, producing false-positive results. Furthermore, these findings question the suitability of *E. coli* and *Enterococcus* as FIB (Alm *et al.*, 2003; Craig *et al.*, 2002, 2004; Lee *et al.*, 2006).

This project aimed to investigate the survival of FIB in water and sediment in aquatic systems, using data from river basins and beaches, in the field and microcosm experiments in the laboratory. Field investigations were carried out with the objective of gaining an insight into the relationship between the presence of FIB in sediments and water in a freshwater stream, and levels of rainfall. The microcosm experiments were intended to represent the behaviour of FIB between water and sediment on a beach, in a laboratory setting.

Weekly sediment and water samples were collected from a tributary of the Bevern stream in East Sussex, which was significantly influenced by faecal contamination from a nearby farm.

Water and sediment samples were also collected from a local bathing beach at Shoreham Harbour, East Sussex. Water and sediment from the beach were also sterilized and placed in sterile containers to form microcosms and inoculated with known concentrations of *Enterococcus*. Viable counts for each of the FIB were performed to ascertain the numbers of organisms present in both the sediments and the corresponding body of water for each sampling occasion. Concentrations of *Enterococcus* were recorded twice a week for the following 10 weeks, and rainfall data were obtained from local meteorological stations. The presumptive *E. coli* and *Enterococcus* from the primary isolation media (mFC agar and mEA agar respectively) were identified to the genus level using

Figure 1. *The Bevern stream, Plumpton, East Sussex*

Figure 2. *Shoreham Harbour beach, East Sussex*

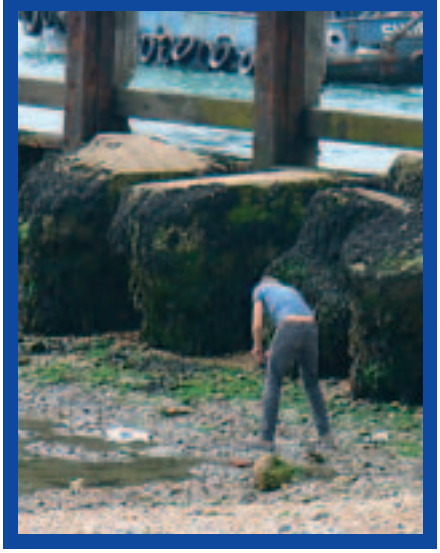
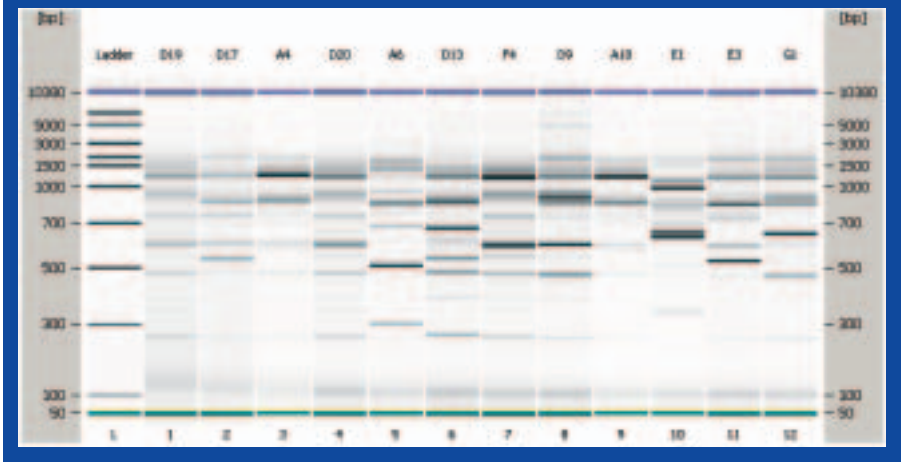


Figure 3. *A typical RAPD gel (Agilent Technologies™) showing a computer-generated image of the banding pattern, as it would be if it had been run on a standard agarose gel (courtesy Dr I. R. Cooper)*



conventional techniques, and any non-FIB were removed from the study. In order to ascertain if certain strains of the FIB were able to survive and persist in the sediments, DNA was extracted from the isolates and subject to RAPD analysis (Williams *et al.*, 1990), using the automated Agilent™ system. RAPD, also known as Arbitrarily Primed PCR (AP-PCR) (Welsh & McClelland, 1990), is an inexpensive yet powerful typing method for many bacterial species and has been used to determine taxonomic identity, and to assess kinship relationships.

Unfortunately, my placement ended before a full analysis could be undertaken, but preliminary results from the field study indicated that both *Enterococcus* and *E. coli* are able to survive in river sediments, and that certain RAPD types were predominant and commonly found in the samples, and some sub-types persisted more than others. There was also a marked increase in bacteriological counts in both sediment and water following heavy rainfall. The microcosm results were inconsistent and showed considerable fluctuations in the bacteriological counts in the sediment. However overall, the microcosm experiments showed how *Enterococcus* survival was higher in marine sediment than in marine waters, and higher than *E. coli* in either matrix.

This study and past studies have clearly demonstrated the ability of FIB to survive in sediment and these results have implications for the accuracy of water quality assessments, microbial source tracking (MST) approaches, and the reliability and robustness of the regulatory standards in place for the protection of water quality.

I would like to thank Dr Jonathan Caplin and Dr Ian Cooper for their support throughout the project. Sampling trips to the Sussex coastline and countryside in the 4x4 were great fun! I would also like to thank the postgraduate members of EPHRU for their company and help in the laboratory. The 12-week secondment has developed my skills in sampling and bacterial identification and analysis, and I have learned much about MST and the analysis of microbial persistence in the environment. These skills should prove useful during my postgraduate studies in Environmental Health at the University of Birmingham in 2012. Finally, I would like to thank the Society for Applied Microbiology for funding the project.

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Does *Clostridium difficile* show a chemotactic response to a variety of chemicals?

During the summer between my second and third year at the University of Northampton, I was lucky enough to be awarded a S*f*AM Students into Work grant. Having never worked on a project of this magnitude I saw it as a valuable opportunity to expand and build upon the skills I had learnt whilst working through my biology degree. My project was based in the microbiology laboratory at the university and appealed to me for several reasons, primarily because throughout my studies I had found chemotaxis interesting, but also due to the originality of the project as there is limited information available regarding the chemotaxis of *Clostridium difficile*.

The aim of this project was to identify the chemical attractants and repellents for the bacterium *Cl. difficile*, with an objective of designing a suitable method for identifying these chemicals.

Clostridium difficile is a motile, anaerobic bacterium which is able to produce endospores and toxins. This bacterium is widely present, particularly in soil and water as well as in the human intestine (Roberts & Mullany, 2010). The presence of this particular bacterium usually goes unnoticed; however, there can be occasional implications such as the development of *Cl. difficile* infection and *Cl. difficile* associated diarrhoea (Norén, 2010). The significance of the problems *Cl. difficile* causes is supported by the fact that between the years of 2006 and 2010, *Cl. difficile* was associated with 1.1% of the deaths in England and Wales (UK Office for National Statistics).

Chemotaxis describes the movement of an organism, such as bacteria, in accordance with the presence of chemicals in the surrounding environment. It is very beneficial, for example, in the case of finding resources e.g., glucose or in the necessity of avoiding a harmful substance. Chemotaxis is overseen by the existence of chemoreceptors which are able to detect specific chemicals without having to metabolize them (Adler, 1969). Applications of identifying the chemoattractants and chemorepellents for *Cl. difficile* include providing insight into conditions within the gut, as well as the potential for developing new methods for drug delivery in treating infections such as pseudomembranous colitis, which is caused by a dramatic rise in *Cl. difficile* within the large intestine.

Before any chemotaxis experiments could be started, it was important to produce growth curves for the strains of *Cl. difficile* that were to be used. This was performed in order to quantify the number of bacteria in a given sample of soft agar after the HAP assays were completed. Growth curves were produced by taking samples of culture, growing in an anaerobic cabinet, at timed intervals. The absorbance of each sample was taken using an optical density meter. At the same time, serial dilutions were prepared in order to estimate the number of CFU/ml at each interval. This was completed for the strains CD630, CD630 CT::*fliC* and R20291. The CD630 CT::*fliC* strain was a gift from the Clostridia Research Group at the University of Nottingham and had been constructed using the ClosTron system (Heap *et al.*, 2010).

Motility assays were also carried out in order to test if the strains used were motile. The strain CD630 CT::*fliC* was used as the non-motile control strain. Completion of the motility assay proved that CD630 and R20291 were motile and could be used in the hard agar plug (HAP) assay. The HAP assay was developed from a variety of existing methods and adapted to suit this anaerobic bacterium. The use of the anaerobic cabinet meant that planning ahead was vital to ensure efficient completion of the HAP assay, something I became accustomed to throughout the duration of this project, as items that were to be used had to be pre-reduced in the cabinet for a minimum of four hours.

To start with, HAPs (containing chemicals) were made with bacteriological agar at 4% w/v with chemicals being added at 0.1% w/v, whereas soft agar (containing bacteria) was made up using bacteriological agar at 0.35% w/v. A bacterial culture was added to the soft agar after being centrifuged and washed several times in phosphate buffer solution (PBS). After pouring, the plates remained in the cabinet for prolonged periods of time and were photographed so that any results were documented.

The HAP assay, used for this experiment, differed from others in terms of the use of the anaerobic cabinet, as well as the addition of the hard agar chemical plugs after the soft agar and bacteria had been poured into the plates. *Clostridium difficile* minimal medium and PBS agar were also used to assess which media produced the greatest result (Cartman & Minton, 2010). A preliminary investigation was also undertaken outside the anaerobic cabinet to assess the effects of aerotaxis.

Although the findings of this project so far are not entirely conclusive, a great effort has gone into determining the best combinations to use for the HAP assay to ensure the greatest chance of obtaining results. In terms of chemical attractants and repellents, further tests will be carried out in order to give greater reliability to the results achieved so far. At present, *Cl. difficile* has lived up to its name, showing itself to be challenging to work with but I feel that because of this I have gained more from the experience. The investigation into the chemical attractants and repellents of *Cl. difficile* will continue. The chemotaxis experiments completed so far will be repeated and it is likely that other chemicals will be tested, as well as the possibility of identifying detection thresholds for such chemotactic responses.

During the time I have spent in the laboratory, I have gained first-hand experience using a range of equipment, as well as acquiring a variety of general and subject-specific skills that I would not have learnt otherwise but will certainly use, not only in the final year of my degree but throughout my career and for that I would like to say thank you to the Society for Applied Microbiology for allowing me to have this opportunity. I would also like to thank my supervisor Dr Gemma Marsden, for enabling me to be part of this project and for her help and guidance throughout. In addition, thanks are due to other members of staff within the laboratory for the support I received.

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Microbial Species: Fact or Fiction?

Systematists like to categorize organisms into discrete groups. The natural world, however, is seldom complicit with their desires. Perhaps the greatest challenge facing systematists today is to define what a prokaryotic species is.

Historical opinions regarding the concept of species have differed greatly and have evolved even faster than the organisms they attempt to categorize. For example, in the mid-nineteenth century Charles Darwin described the species as a grouping "*…arbitrarily given for the sake of convenience to a set of individuals closely resembling each other…*" (Darwin, 1859). Thus, to Darwin, the species concept was little more than a tool for biologists to catalogue similar-looking organisms and contained no special significance as compared with other taxonomical groupings such as the genus or family. Ernst Mayr expressed a very different opinion in his biological species concept in which he defined species as "*… maximally inclusive groupings of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups…*" (Mayr, 1942). Mayr also thought that the species was a fundamental grouping that represented "*the only taxonomic category for which the boundaries between taxa at that level are defined objectively*" and that "*the species is as important a unit of biology as is the cell*" (Mayr, 1942).

Subsequently, several other biologists developed additional species concepts that were more specific to their individual disciplines but were often incompatible with each other; the so-called "species problem". However, most of the definitions carried the central theme that species were metapopulations with the potential to interbreed. While these definitions may be acceptable (or not) for most animals and plants, it has very limited utility for bacteria and Archaea, which by and large do not have traditional sexual reproduction as a part of their life cycles.

So how do we define species for microorganisms? There are two possible answers to this question: the first is that bacteria and Archaea do not form metapopulations and thus, do not form species; the second is that they do form both metapopulations and species, but do so by a different mechanism to eukaryotic organisms. If the former is correct, we would not discard our current taxonomy, but think of it in a more Darwinian fashion, where organisms are grouped into species for convenience; if the latter is correct, what is a prokaryotic species?

In the early half of the twentieth century, bacteria were grouped into species based on phenotypic traits such as their morphology, ability to cause disease and several easily measured biochemical characteristics, such as carbon source utilization or ability to reduce nitrate. Grouping bacteria into species based solely on phenotypic traits was convenient and crucial for the advancement of many disciplines including medicine, food processing and bioremediation. However, this method of grouping does not take into account the evolutionary and ecological relatedness of microbes or the forces that form discrete clusters of phenotypically and genotypically similar groups. Because of these shortcomings, the definition of a bacterial species was altered in 1987 to include genomic data. The resulting polyphasic taxonomy, which is still employed today, groups strains into a species if they share several phenotypic characteristics, at least one diagnostic character and their DNA hybridizes with the species type strain at a level of 70% or greater in standardized DNA-DNA hybridization (DDH) experiments (Wayne *et al.*, 1987).

Despite being the gold standard, DDH experiments are time consuming, difficult to perform, and not suitable for the rapid identification of isolates. Because of these limitations, other molecular techniques have also been routinely employed including 16S rRNA gene sequence analysis, average nucleotide identity among shared orthologous genes and multi-locus sequence analysis. Among these methods, the comparison of 16S rRNA genes has been the most utilized but, due to this gene's highly conserved nature, it is more useful to determine what is not a member of a species (having < 97% similarity), than what is. The incorporation of genetic techniques has resulted in few changes to the established taxonomy, which should not be surprising because the cut-off levels were selected to match the pre-existing phenotype-based groupings.

Aside from being arbitrarily chosen, another potential problem with the cut-off level is that it may be exceedingly low. For example, a comparison of the genomes of different strains of the same species revealed that many differ by as much as 30–35% of their genes; even after hypothetical genes and mobile elements were discarded, they still

differed by >20% (Konstantinidis & Tiedge, 2005). To put this in perspective, *E. coli* strains could differ by up to 1,000 genes, which may be why this species exhibits vastly differing phenotypes, ranging from commensal strains to highly invasive pathogens (Luo *et al.*, 2011). Furthermore, this level of variation in eukaryotic organisms would group all primates into one species (Staley, 1997). This broad definition may partially explain why we have identified millions of animal species, but only thousands of microbes. Regardless of these shortcomings, our current operational definition of a microbial species is adequate for many purposes, such as medicine, agriculture and food production.

Despite the utility of our current functional definition, the question still remains: do microorganisms form true species, defined as a collection of strains that are genomically cohesive and different from others by multiple independent features, or are they a continuum of closely related organisms? To answer this question we must examine how microorganisms evolve to form species.

First, let's consider a group of asexual clones that occupy a specific niche able to support a small population size. As this population multiplies, random mutations occur and, if a fitter mutant arises, it and its descendants will displace the other, less-fit sister cells. This periodic selection event will reduce the continuum of diversity that has accumulated, replacing it with a new ecotype which will remain in this niche until another periodic selection event occurs. This method of speciation is described by Cohan in his ecotype theory (Cohan, 2002). The ecotype that is selected, and its descendants, form a metapopulation with many commonalities attributed to animal species; it is a cohesive group that is co-evolving by selective forces in its environment and is separated from other such groups. While this model is attractive, it also has some shortcomings; most notably its limited observation in "real life" situations and, more importantly, it does not address the effects of horizontal gene transfer (described later).

Another force that drives speciation is homologous recombination (HR). This occurs in all known microorganisms albeit at vastly different frequencies. In some species, such as *Shewanella baltica*, it has been reported that HR is occurring so fast that it is eliminating more base substitutions than those caused by random point mutations (Caro-Quintero *et al.*, 2011). While common, there are barriers to HR such as extreme physical and ecological sequestration, resistance to phage infection or conjugation and the absence of DNA uptake systems. Perhaps the greatest barrier to HR is the log-linear decline in HR frequency with increasing sequence divergence (Vulic *et al.*, 1999). Thus, HR is more frequent among sequences of closely related organisms than distantly related organisms. These facts suggest that Mayr's biological species concept should be very relevant to bacterial speciation by HR, because intra-species (or closely related) gene acquisition should be a cohesive force to maintain species as they are sharing a common gene pool. Likewise, it should also cause them to diverge from other species that do not share this group of genes.

However, like the ecotype theory there are problems with this model as well, most notably the effects of horizontal gene transfer (HGT). HGT is very disruptive to speciation and may invalidate the species concept by confounding the ability to distinguish lineages of organisms. HGT is defined as the non-genealogical exchange of genetic material between organisms, and occurs between all three domains of life, in all possible directions (Boto, 2010). Among microorganisms, HGT has occurred on innumerable occasions, and it is estimated that between 1.6 and 32.6% of the genes within individual genomes are the result of HGT, giving rise to the concepts of closed (evolution driven mostly by mutation) and open (evolution driven by HGT) genomes. In some species, such as *Streptococcus agalactiae*, HGT has resulted in segments of DNA >300kb being introduced (Brochet *et al.*, 2008). This extraordinarily high amount of genetic exchange, from such diverse groups of organisms, may muddy the water to such an extent that tracing the lineage of some organisms is impossible.

Taking all of this into consideration, can we formulate a single, hypothesis-based definition of a microbial species? At present, to the disdain of species monists, we can't. However, perhaps we should be more pluralistic in our views and accept that some microorganisms are more clonal and form species by periodic selection, others by Mayr's biological species concept model, and still others by unknown mechanisms. It is also possible that some microbes do not form species at all, or are so cloaked in HGT events that their phylogeny can never be resolved.

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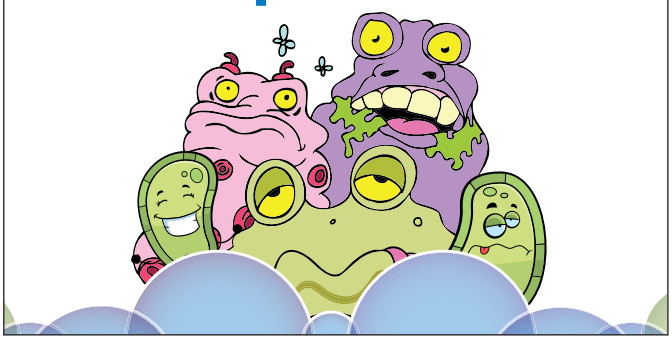

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- All activities are certified to ISO 9001: 2008 with quality control accredited by UKAS to EN ISO/IEC 17025:2005, ensuring consistent high quality products with continual improvement of processes and services in line with customer requirements
- All media production takes place in a clean room environment
- Our poured plate production facility has cleanrooms validated to: ISO 14644-1 (Class 7), EU GMP (Grade C) and all poured plate manufacturing is on fully automated production lines
- GMP and GLP techniques employed throughout all production and laboratory areas
- Rigorous QC examination with certificates available on request

As an independent media producer we have the ability to manufacture in small batches and as such we can supply any commercial or custom-specified formulations to order. So, whether you require a completely new medium, or simply a different pack or fill-size, please call our Customer Services Department.

further information

Visit: www.sglab.co.uk
Tel: +44 (0)1536 403815
Email: info@sglab.co.uk

GDH testing and instrumentation expansion

Laboratory testing for *Clostridium difficile* has received a great deal of attention over the last 12 months with changes to the recommended testing practices, methods and availability of kits and reagents.

Pro-Lab Diagnostics are proud to offer a full range of solutions, including the Prolisa GDH (ELISA), the ProFlow GDH (EIA Lateral flow) and ProFlow C.Diff Tox AB (EIA Lateral Flow) and the Portrait Rapid Molecular Platform. Demonstrations and samples are available on request, as are places at training seminars being held.

Pro-Lab Diagnostics is also expanding its range of innovative laboratory equipment with the additions of the BactiZapper bench top loop sterilizer using infrared heat, and a range of economical hot plates and vortex mixers.

further information

Visit: www.pro-lab.com
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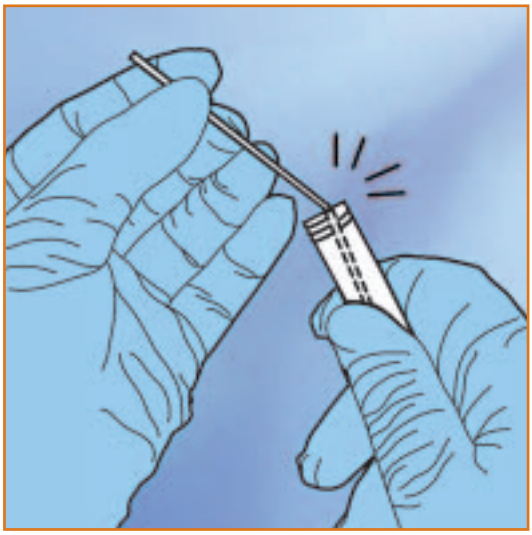

"Snap n' Cap"

All Medical Wire's novel specimen transport devices including Σ-Transwab®, Fecal Transwab®, Σ-Virocult®, and Σ-VCM® feature " Snap n' Cap", the convenient swab capture system. Once the swab specimen is taken from the patient, the swab is simply placed in the transport tube, snapped off, and the screw cap "captures" the swab. This is important for automated de-capping systems, ensuring the swab is lifted out of the way allowing ready access to the various kinds of sampling heads.

The system confers many advantages. Not only is it ready for use on all the automated platforms currently available, it is much more convenient for conventional techniques. The swab can be easily handled for manual plating, or simply removed with the cap, allowing the liquid medium to be sampled before the swab and cap are securely replaced. The tube with captured swab is more compact for transport and is ideal for any future storage and retrieval requirements.

"Snap n' Cap" swab capture is integral to Σ-Transwab®, the specimen collection and transport system for aerobes, anaerobes and fastidious microorganisms, Fecal Transwabs® (enterics), Σ-Virocult® (viruses), and Σ-VCM® (viruses, *chlamydia*, *mycoplasma* and *Neisseria*).

further information

Visit: www.mwe.co.uk
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Email: sales@mwe.co.uk

New rapid detection of verotoxin-producing *E. coli* kit from Mast

New Mast Isoplex®*VTEC* kit is an innovative, easy to use and rapid molecular test for identification of verotoxin-producing *Escherichia coli* (VTEC). Results are produced within 1 hour, direct from the sample without complex extraction methods as required in PCR.

VTEC is an established cause of food-borne infection, causing the onset of bloody diarrhoea which can progress to serious infections such as hemolytic uremic syndrome (HUS). Rapid detection and identification of VTEC is critical to monitor, control and contain infection.

Traditional culture methods such as Sorbitol MacConkey Agar supplemented with cefixime tellurite are employed in the majority of microbiology laboratories as a single screen for *E. coli* O157, although this is the most prevalent other significant serogroups are often overlooked. Collectively with the increase in sorbitol fermenting O157 strains, up to 80% of cases go undetected.

Mast Isoplex®*VTEC* uses novel loop mediated isothermal amplification (LAMP), overcoming the limitations of culture, by detecting all VTEC strains expressing *vtx1* and *vtx2* read either visually or in Real-time.

An evaluation at The Scottish *E.coli* O157/VTEC Reference Laboratory (SERL), UK, revealed that Mast Isoplex®*VTEC* is "a useful adjunct to current VTEC testing at diagnostic laboratories that do not have access to PCR testing."

further information

Visit: www.mastgrp.com
Tel: +44 (0) 151 933 7277
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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.

Hand Sampling Kits reduce risk of skin irritation

Hand Sampling Kits accurately assess hand hygiene without the worry of skin irritation or more serious allergic reactions.

Technical Service Consultants has formulated a Personnel Sampling Solution to provide neutralisation of soap residues, whilst not containing any known sensitising ingredients. Available in two formats, pre-moistened 50cm2 blue sponge sampling kit for whole hand sampling and a transport swab sampling kit with blue breakpoint shaft, viscose tip and 10ml of personnel sampling solution for nails, fingertips and in-between finger analysis.

TSC Hand Sampling Kits offer a quick, cost effective, convenient and safe method for testing of hand hygiene. Popular practice involves hand sampling using environmental surface products, which are not recommended, as these products are likely to contain numerous harsh chemicals in high concentrations.

Hand sampling kits are ideal for safe use in the food and beverage sector, where companies need to be compliant with the 'Hygiene of Foodstuffs' regulations, hospitals and other medical centres, where monitoring is used to detect cross-transmission of MRSA, bacteria and other organisms as well as the pharmaceutical and cosmetics industries, where clean room hygiene is essential and best practice should be followed.

The Personnel Sampling Solution offers sample stability without multiplication during transport or storage for accurate results. Validation results are available for such organisms as *Bacillus* spp., *Staphylococcus* spp., *Pseudomonas* spp. and *E.coli* with common soaps and cleansers like HDS, Virkon and Chlorsan.

further information

Visit: www.tscswabs.co.uk
Tel: +44 (0)1706 620600
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NCIMB and ORA investigate the potential of molecular monitoring methods for optimizing anaerobic digestion

NCIMB are collaborating with the Organic Resource Agency (ORA) in a feasibility study to investigate the potential of transferring molecular monitoring techniques used in the oil industry, to the production of energy from wastes and other renewable sources using anaerobic digestion. The project has received funding from the UK's Technology Strategy Board.

Anaerobic digestion (AD) is a key element in energy generation from biodegradable waste materials and also provides a solution to many waste management issues. The process is dependent on the activity of bacterial populations but current monitoring typically relies on indirect measures such as the products of microbial degradation.

In the oil and gas industry a range of more direct analyses are used to monitor bacterial populations and molecular approaches like qPCR are increasingly being used to determine bacterial biomass in oil field systems.

This new study will investigate the potential of transferring competency and methods developed for the oil industry in combination with ORA's AD expertise to contribute towards development of a new method for optimizing AD processes.

If you are interested in collaborating with NCIMB and/or ORA and would like to discuss a potential project, please contact Dr Carol Phillips email c.phillips@ncimb.com; tel +44 (0) 1224 711100 or Dr Jon Pickering; email jpickering@o-r-a.co.uk, tel+44 (0) 1684 585423.

further Information

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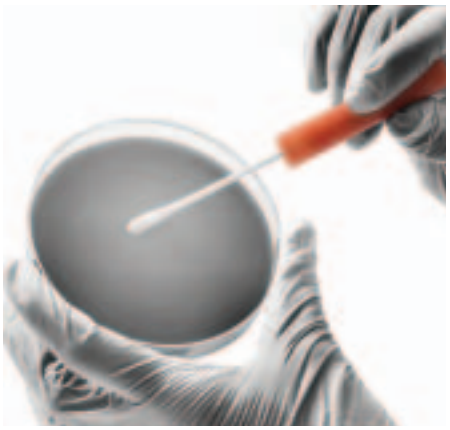

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Microbank™ Bacterial & Fungal Preservation System

Microbank™ is a convenient, ready-to-use system designed to greatly simplify the storage and retrieval of bacterial cultures. It is composed of a unique cryovial system incorporating treated beads and a special cryopreservative solution.

Microbank™ has proven performance and is now the natural choice for microbiologists world-wide and for many specific reference culture collection centres. Microbank™ is a more reliable method for maintaining important cultures than repetitive subculture, which can result in altered characteristics, lost organisms, or contaminated cultures. Microbank™ is much simpler than traditional methods of lyophilization or glycerol broth.

Large 2 ml vials with triple depth external threaded cap which reduces the possibility of contamination. Wider tube diameter provides more room for mixing to ensure beads are properly coated.

Larger writing area allows for complete coding and reference data.

Industry-standard robust cryovial can withstand snap freezing with liquid nitrogen.

Available in five colours to provide laboratories with a system to colour-code different bacterial species.

Specially formulated preservative ensures longer survival of fastidious bacteria and higher quantitative recoveries.

Chemically treated beads improve bacterial adhesion.

Microbank™ World Wide Performance Portfolio

Microbank™ has enjoyed many years of success as the method of choice for storage and retrieval of bacterial and fungal cultures. Extensive reference data are available from customers, centres of excellence, and reference collection sites around the world detailing up to 21 year's successful storage of an extensive range of cultures. Full details can be obtained in the Microbank™ World Wide Performance Portfolio available on the Pro-Lab website.

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