

identification of binding sites of DNA-binding proteins on a genome wide basis (using the ChIP-on-chip technique). DNA microarrays have also been employed for the detection of microorganisms. They have been used to screen environmental samples to obtain a microorganism profile, detect foodborne bacteria to aid food safety, and for the detection and identification of unknown pathogens in clinical samples.

Microarrays can also be employed to investigate the genome of a pathogen, providing genotypic information that can be relevant to clinical decisions. For example, there are now microarrays that allow the subtyping of human papillomavirus (Klaassen, *et al.*, 2004), as only certain strains are strongly associated with the development of cervical cancer.

Antimicrobial resistance is another field where the multiplex capability of microarrays has proven to be very useful. Antimicrobial resistance describes the ability of microorganisms to resist the action of antimicrobial drugs (*e.g.* antibiotics) and has become a major public and veterinary health issue, as it can have a profound effect upon the treatment of infections in man and animals. Determining the antimicrobial susceptibility of bacterial isolates can help inform treatment decisions. The conventional methods for susceptibility testing require that the bacterium is first isolated in culture. The bacterium can then be exposed to a range of antimicrobials in separate assays, and susceptibility assessed by monitoring growth inhibition (Wheat, 2001). Isolated bacteria can also be screened for the presence of antimicrobial-modifying enzymes. These assays all investigate the phenotype of the organism.

Considerable effort has been put into determining the mechanisms of antimicrobial resistance and the underlying genetic components that govern it. This has resulted in the discovery and sequencing of many resistance genes, the detection of which would allow the antimicrobial resistance gene profile of an organism to be described. This information could usefully inform treatment decisions, aid surveillance studies and provide epidemiological information.

Numerous molecular assays, typically PCR, have been developed to detect specific resistance genes or gene

families (reviewed in Sundsfjord, *et al.*, 2004). However, there are many genes that can confer antimicrobial resistance and to test a single bacterial isolate many PCRs would need to be performed. Even when several PCRs are multiplexed into a single reaction, the number of tests required rapidly becomes prohibitively expensive and resource intensive. A genotyping assay that allows rapid and accurate screening of many antimicrobial resistance genes in the same operation would be of considerable benefit. Microarrays allow the detection of a virtually unlimited number of genes in a single experiment and provide a powerful platform to address antimicrobial resistance genotyping needs. A number of microarrays for the detection of antimicrobial resistance genes in Gram-positive and Gram-negative bacteria have been developed, however their widespread adoption has been limited. This is likely to be due to several factors, including a belief that microarray experiments are difficult to undertake successfully and require specialised or expensive equipment and reagents.

Developments in microarray platforms and protocols have addressed many of these practical impediments to widespread use and can assist the transfer of microarrays from the research laboratory to a routine testing environment. An example of an antimicrobial resistance genotyping microarray designed with routine use in mind is the AMR-ve microarray (Identibac, Weybridge, UK) which currently targets 54 resistance genes in Gram-negative bacteria and was originally described by Batchelor, *et al.*,

(2008). It was developed in a collaboration between the Veterinary Laboratories Agency (VLA) and the Health Protection Agency (HPA). This microarray consists of oligonucleotide probes printed on to the base of an ArrayTube™ (a trademark of Clondiag GmbH Jena, Germany — Figure 1).

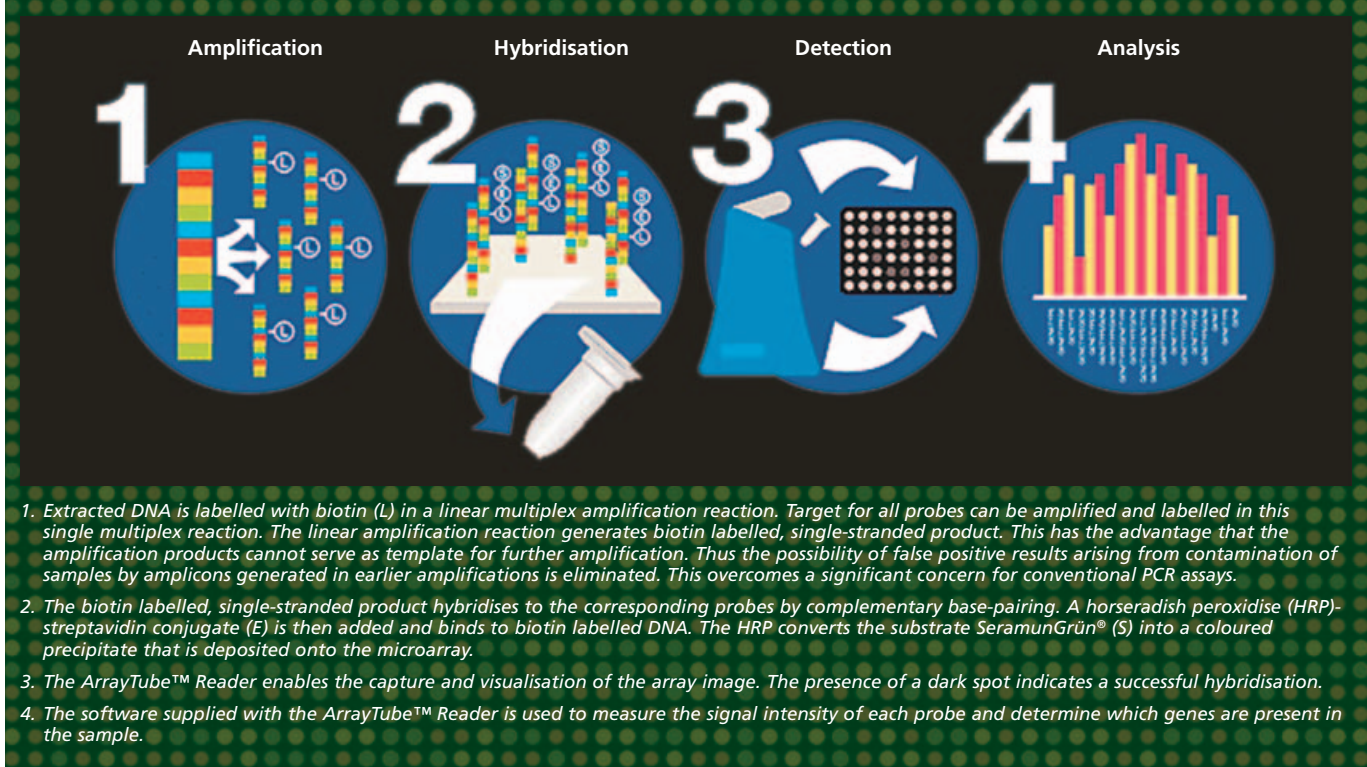
The ArrayTube™ format was developed by Clondiag GmbH (Jena, Germany) and is used by a number of commercially available microarrays. The ArrayTube™ presents a significant advantage as it forms the reaction vessel in which all the steps of the microarray experiment take place, providing convenience and ease-of-use that is not a feature of the glass slide microarrays commonly used in research laboratories. The protocol used with an ArrayTube™ is easy to perform and little expert training is required. An outline of the procedure used by several commercially available microarrays (including the Identibac AMR-ve microarray) is given in Figure 2. The specialised equipment required to use these microarrays is limited to a thermocycler (most standard PCR machines are suitable), a thermomixer (a rotating heating block for hybridisation), and an ArrayTube™ Reader (to capture an image of the microarray). For many laboratories, it is likely that the ArrayTube™ Reader is the only item that would need to be purchased specially. All liquid handling and manipulations employ standard pipettes and equipment and reagents have been selected to be inexpensive and straightforward to use. For example, detection is achieved using an enzymatic reaction which generates a precipitate that is easily distinguished and analysed. This enzymatic system is considerably cheaper than the costly and friable fluorescent dyes employed in many other DNA microarrays. Analysis of the results is easily undertaken using software provided with the ArrayTube™ Reader and the genotype of the sample under study can be quickly determined. Results can be exported to Excel, allowing data to be tabulated and organised as desired. Analysis does not require use of complicated software packages, such as those needed to perform comparative gene expression studies, significantly reducing staff training and time costs.

ArrayTube™ microarrays have been developed to allow rapid testing and results can usually be produced within



Figure 1. An ArrayTube™

**Figure 2.** Overview of the ArrayTube™ microarray procedure



six to seven hours, from extraction of genomic DNA to final analysis. This speed of analysis is quicker than many of the conventional methods microarrays are designed to replace or complement. For example, determining the antimicrobial resistance gene profile of a bacterium with the Identibac AMR-ve microarray can be accomplished more rapidly than many conventional methods for antimicrobial susceptibility testing.

The ability of a microarray to deliver accurate results in a dependable and consistent manner is critical to its move out of the research laboratory and into a routine testing environment.

User-friendly protocols and simple analysis contribute to reliable microarray performance by reducing variation arising from the procedure. However, two additional requirements for broader acceptance of microarrays are manufacturing systems that control microarray quality and a robust validation of microarray performance.

Quality control systems should be used to demonstrate the fidelity of microarray manufacture. Accurate and precise deposition of the probes onto the microarray is critical to performance. Each production run of a microarray should be quality controlled

to ensure that all probes are present, in the correct location and operate properly. Any reagents supplied with the microarray should be functionally tested on the microarray to ensure that performance meets defined standards.

Extensive testing and validation should be performed on a microarray to assess its performance. An important part of this process is the testing of a panel of samples previously characterised by a definitive benchmark test where microarray results are compared to the results obtained with the benchmark test, and the sensitivity and specificity of the microarray determined. The benchmark test can be a conventional and well established phenotypic test (*e.g.* for an antimicrobial resistance microarray this benchmark test could be conventional antimicrobial susceptibility testing). This analysis therefore compares the genotype determined by the microarray with the phenotype determined by the benchmark test, and sometimes disagreement between the two methods is seen for several reasons. For example, the phenotype could be conferred by a gene that is not detected by the microarray (either a gene with no corresponding probe or a variant gene that is not detected by the existing

probes). Alternatively a gene may be present but not expressed (and thereby conferring no corresponding phenotype). These discrepancies can be investigated further by employing a different genotyping test, such as PCR or sequencing. When the data from this second genotyping test are compared to the microarray results the agreement is frequently found to be very high (see, for example, Batchelor, *et al.*, 2008). An important consideration with microarrays is that they can detect only those genes that are targeted by the probes and allow only a prediction of the phenotype to be made. However, microarrays can be expanded by the inclusion of additional probes that target newly identified genes or gene variants. When introducing new probes it is important to consider the need for additional validation, to ensure that the microarray acquires the new detection capabilities without compromising its original performance.

Despite often rigorous validation and strict quality control systems, many microarrays are designated as 'for research use only'. They cannot, therefore, be used for clinical diagnosis of samples from humans, but their utility in the veterinary field, for surveillance and epidemiology remains. For use in

clinical diagnosis in the European Union a microarray requires a CE mark, indicating that it meets all the essential requirements of the relevant European Directives, including that on *in vitro* diagnostic medical devices (98/79/EC). The assurance that a CE mark gives is widely recognised and microarrays are being developed with this in mind. An example of a CE marked microarray is Prove-it™ Sepsis (Mobidiag Ltd, Helsinki, Finland), which can identify 23 bacterial species and the *mecA* methicillin resistance marker in DNA extracted from positive blood culture.

The full advantage of the powerful multiplex capability of microarrays is realised when many different gene targets need to be detected in the same operation. Therefore, microarrays are best employed to address questions that involve many possible genotypes, such as the generation of an antimicrobial resistance gene profile. There are many other applications where the strengths of a microarray can be brought to bear, particularly for bacterial genotyping applications.

The application of microarrays are typified in relation to *Salmonella*. Infection with *Salmonella* species constitutes one of the most common causes of food poisoning in man and disease in animals. Two species of *Salmonella* are recognised: *Salmonella enterica* (which is divided into six subspecies) and *Salmonella bongori*. The determination of the *Salmonella* serovar is important for several reasons including epidemiology, surveillance, disease control, food safety and animal husbandry.

A microarray product that has been designed to detect all salmonellae and identify 64 common and important *Salmonella* serovars is named Premi®Test Salmonella (DSM Nutritional Products, Geleen, The Netherlands). This microarray uses the ArrayTube™ platform and has been tested with a large panel of samples (Wattiau, *et al.*, 2008). The microarray uses a novel approach that targets 13 DNA markers which are not directly associated with the genes that govern the antigenic formulae. Instead, the specific marker profile obtained with each sample tested is compared to a database of known profiles and the serovar thereby determined. Recent improvements to this microarray have increased the number of DNA markers used and the

number of serovars that can be identified.

Another area where the multiplex capability of microarrays can be exploited is the pathotyping of *Escherichia coli* strains. Pathogenic *E. coli* strains present a considerable public health problem worldwide and cause disease by means of virulence factors that are encoded by a number of different genes. A microarray that can detect many of these virulence genes has been described (Anjum, *et al.*, 2007), and is able to identify virulence genes present in *E. coli* isolates of both human and animal origin that are associated with a range of pathotypes (including pathogenic extraintestinal, enterotoxigenic, and enteroinvasive *E. coli*).

A final example of bacterial genotyping microarrays is provided by those designed to detect genes or gene groups associated with antimicrobial resistance and virulence factors in *Staphylococcus aureus* (Moneke & Ehricht, 2005). Such microarrays can detect genes associated with resistance to a range of antibiotics, including methicillin and vancomycin. Given the importance of detecting and characterising methicillin-resistant *S. aureus* (MRSA), these microarrays have considerable utility.

## Conclusion

Since the introduction of microarrays over fifteen years ago their powerful multiplexing capability has proven useful in a diverse range of applications. These applications include the genotyping of bacteria to generate, for example, antimicrobial resistance or virulence gene profiles. The information revealed by a single microarray experiment significantly exceeds that obtained from many PCRs and can greatly assist treatment decisions, surveillance and epidemiology.

With platforms such as the ArrayTube™, microarray techniques and data analysis have been improved and simplified. These developments have delivered microarrays with considerable user-friendliness and with relatively inexpensive set-up and running costs. This has facilitated their introduction into laboratories with minimal additional equipment or training costs. The availability of quality controlled and validated microarrays that use these simplified platforms will enable a wider

range of laboratories to undertake microarray experiments with confidence. Microarrays possess an enormous potential for the rapid screening of bacteria for important genes, and they are becoming sufficiently developed to move successfully from the research laboratory into a routine testing environment.

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## acknowledgement

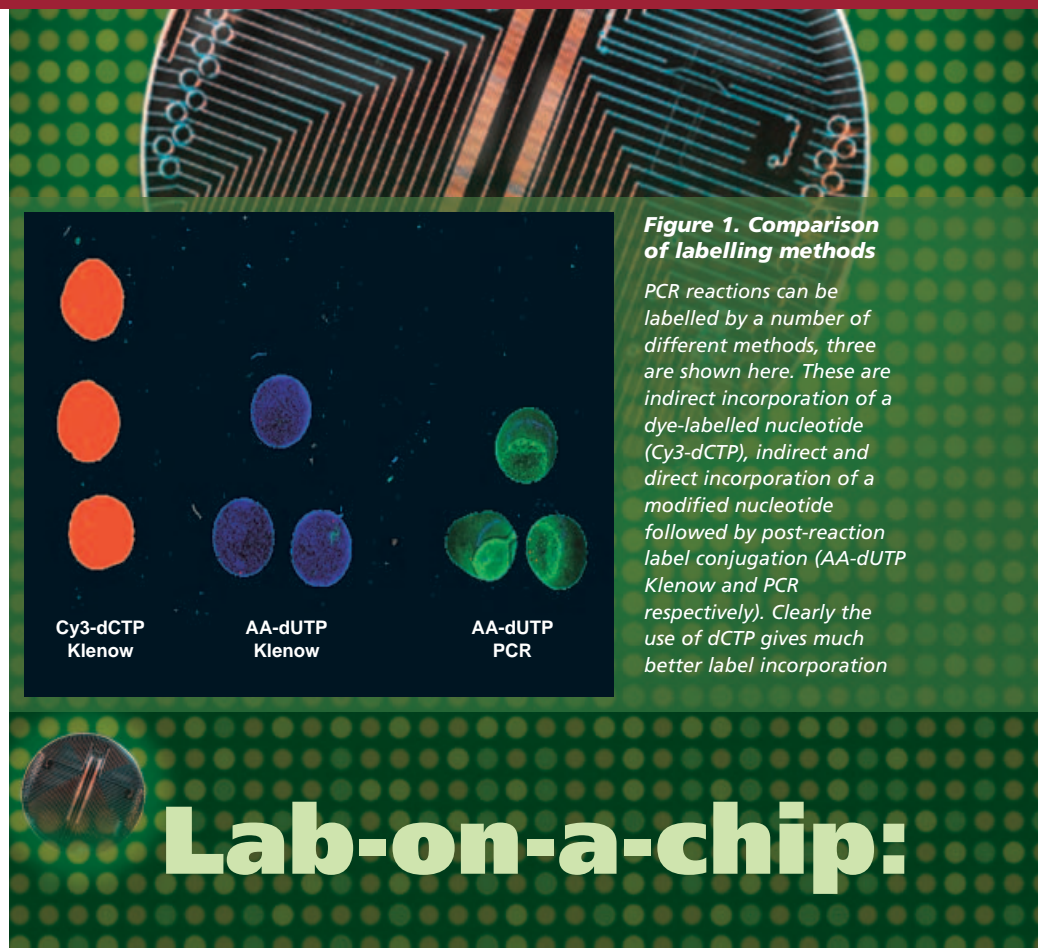
- ArrayTube™ is a trademark of Clondiag GmbH (Jena, Germany).

**Rod Card**  
Veterinary Laboratories Agency



The prospect of a lab-on-a-chip is one that is incredibly exciting for microbiology. Wouldn't it be a fantastic tool to have all of the diagnostic assays that you need to run in one format without having to make decisions about the assays that need to be performed? The idea conjured up by the name is of a small size assay system which is capable of running a number of tests in parallel for biochemistry, molecular biology, immunoassay *etc.*, but how real is this actual concept? At the Health Protection Agency's Porton Down laboratories we have invested considerable time and effort into exploring the potential offered by DNA microarray technology for the molecular diagnosis of infectious diseases.

Our rationale was to develop an assay system where, irrespective of the sample type and the pathogen present, we could use a consistent "front-end" approach to extract and amplify the pathogen nucleic acids irrespective of whether it had an RNA or DNA genome. It was also important that we should have an assay capable of being run to examine samples where other specific assays had yielded negative results; thus we would have no *a priori* knowledge of the pathogen present. DNA microarrays were chosen because they offered great potential to meet the above two criteria, although not without their own inherent disadvantages. The main problems were that they were not rapid assays, requiring a minimum of 16h incubation as well as the fact that as they had never been used for these type of clinical assays, we felt that the reproducibility was likely to be very low. Additionally, the only reported use for DNA microarrays at this time (2001) was to analyse changes in gene expression. The rationale behind the use of DNA microarrays for diagnostic purposes was that a single channel assay would be run; expression microarrays utilise two channels, one of which is based upon the extracted mRNA the other on cDNA. Comparison of the former against the latter gives the measure of gene expression relative to the number of gene copies within the genome. For diagnostic use, nucleic acids extracted from a sample would be amplified (this also allows conversion of RNA genomes into cDNA for hybridisation), labelled and hybridised, with the readout being a plus/minus hybridisation rather than a relative value. In this hypothetical



**Figure 1. Comparison of labelling methods**

PCR reactions can be labelled by a number of different methods, three are shown here. These are indirect incorporation of a dye-labelled nucleotide (Cy3-dCTP), indirect and direct incorporation of a modified nucleotide followed by post-reaction label conjugation (AA-dUTP Klenow and PCR respectively). Clearly the use of dCTP gives much better label incorporation

## Lab-on-a-chip:

system, it is of course possible to use the second channel as a "control", however, since the system was envisaged as a diagnostic where no preconceived decision was required, what would constitute a suitable control?

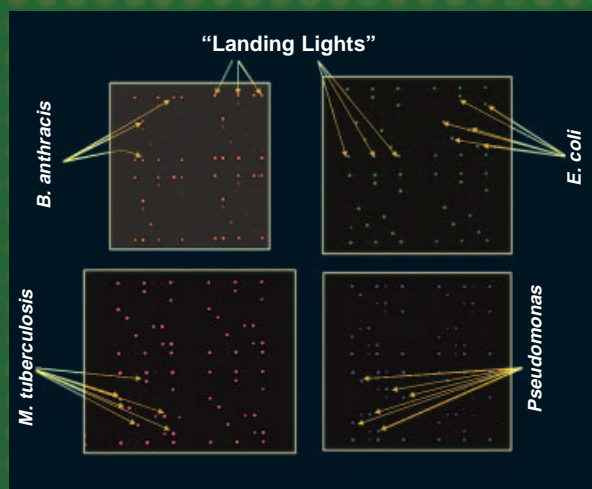
In order to develop the microarray assay, a number of key decisions needed to be made. Firstly, how would the microarray physically be constructed? Amino-silane coated glass slides (and subsequently Epoxy-coated slides) were chosen for construction of the initial arrays which would be spotted using a direct contact printer. Microarrays could be fabricated using cDNA clones, PCR fragments or long oligonucleotide probes (it should be noted that nomenclature for these assays can be somewhat different to conventional hybridisation assays; here the term probe applies to the nucleic acid fragment bound to the solid matrix). Oligonucleotides were chosen as the other two alternatives were felt to offer less control over the probe design and were more expensive to produce (Tomiuk & Hofmann, 2001). Initially the technology was evaluated using a relatively small number of different probes, all of which were based on detection of variable regions within the 16S rRNA genes of six key bacterial species (*Bacillus*, *Escherichia*, *Streptococcus*, *Mycobacterium*,

*Pseudomonas* and *Neisseria*). Two 50-mer oligonucleotide probes were designed for each of the six species; these were spotted in triplicate into 16 replicate sub-arrays on the amino-silane glass slide matrices. Another key decision to be made surrounded the choice of hybridisation conditions, under which the assay would be run. The choice of 50-mer oligonucleotide probes allowed absolute control, during the design phase, of parameters such as the probe melting temperature ( $T_m$ ) and secondary structure. Numerous protocols were available for the hybridisation of expression arrays; these had little comparability with each other and conditions were therefore chosen which offered medium stringency conditions for the hybridisation and wash steps of the assay. A concise review was published by Hegde *et al.* in 2000 and covered many of the available decisions and options that were then currently available.

Test hybridisation assays were performed using generic primers to PCR amplify an approximately 800bp fragment (including both of the designed probe sequences in each case) from the 5' end of the 16S rRNA gene. The resulting PCR products also needed to be labelled post-amplification in order to visualise the hybridisation reaction. Once again there were numerous

**Figure 2. Panel of 4 microarray assays**

Identical arrays are shown here, but incubated with DNA from four different bacterial pathogens. The spot location is indicative of the pathogen identity as the images are false-coloured for clarity. Landing light spots are indicated, these are used to locate the grids used in the analysis software



## the trials and tribulations of diagnostic DNA microarray technology

methods available to label these fragments — many of which have a long provenance for use in Southern & Northern blotting techniques. Initially, we used amino-allyl dUTP in the PCR mix in order to incorporate a nucleotide which could then easily be labelled post-amplification; the fluorescent dye, Cyanine 3 (Cy3) was used for these labelling reactions. Incorporation of amino-allyl dUTP is relatively inefficient using *Taq* polymerases, as evidenced by the poor levels of dye incorporation when fragment and dye concentrations were determined prior to hybridisation. An alternative method was also investigated which used the Klenow fragment of DNA polymerase I for incorporation of these modified nucleotides employing a random priming reaction which used random nonomers to prime the second strand synthesis. This method resulted in better dye incorporation, but post-amplification coupling of dye to modified nucleotides never yielded particularly highly labelled fragments. The best method, in our hands, for ensuring that fragments were labelled with high dye incorporation ratios, was to incorporate Cy3 or Cy5 labelled dCTP directly at the PCR amplification stage. An example of the differences in dye incorporation can be seen in the figure which was produced by drying a small

spot of the reaction (after removing any incorporated dye) on to a slide and scanning in the usual way.

This method of dye incorporation finally yielded our first signals with the microarray assay; every hybridisation up to this stage had resulted in a blank picture. Actually we felt that we had done quite well, as in the space of four months, we had learnt to use the microarray spotter (these are not for the faint hearted!), spotted numerous arrays, been through three different incarnations of labelling reactions and finally got a result. When we talked about our trials and tribulations with the wider microarray community, this was felt to be rather fast progress as one colleague told us, “it took us 18 months to get to the same stage”. So we had the bones of an assay and it was actually giving really good, clean hybridisation patterns, albeit with probes for only six different bacterial pathogens. The next step was to try and move away from using specific PCR amplification of genes, to a generic amplification method. We knew that this would result in a decreased signal and probably higher background, however we decided that this step was critical for the development of this assay. Throwing caution to the wind, we also decided to increase the number of probes within the assay on a dramatic scale at the

same time. Firstly the amplification reaction; this was a difficult decision to make (whether an amplification step should be used) as ideally one would not wish to skew results by introducing an amplification step. Neil Boonham and colleagues at the Defra Central Science Laboratories managed to achieve this with their plant pathogen array (Boonham *et al.*, 2003), however, the concentration of pathogen nucleic acids present in clinical samples is often extremely low and thus we felt that in order to achieve our aim of a true multiplexed microarray assay, that some amplification was needed. This would also allow RNA genomes to be reverse-transcribed (into cDNA), amplified and labelled in the same way as DNA.

A method based upon random amplification was chosen to avoid having to make prior decisions regarding primers sets to be used. A number of published methods were available in the literature and on microarray group websites. The method that we used was modified from that described by David Wang in his seminal paper from 2002, which was itself derived from an earlier described method (Bohlander *et al.*, 1992). This method is based upon a three-step amplification, using random short oligonucleotides (nonomers) tagged with site-specific tails in the first round, a specific PCR primer which is complementary to the site-specific tail in the second and incorporating the dye in the third. Wang and colleagues modified this method by using a reverse transcriptase in the first step instead of Klenow. We further modified both published methods by using a mixture of Klenow and a reverse transcriptase in the first step, thus allowing the amplification of both DNA and RNA genomes and then using the second round (PCR amplification step) to incorporate the dye.

The second aspect was to increase the number of probes to include known virulence genes, genotyping markers and probes for a wide range of viral pathogens. Coincident, was the publication of David Wang’s viral probes set — 600 oligonucleotides covering approximately 140 viral pathogens. These probes were also synthesised and incorporated directly into our microarray assay which now comprised 2200 unique probes and covered around 150 different bacterial and viral pathogens.



The proof is in the pudding, as the saying goes, and we were not disappointed, even though there were obvious problems with the assay. One of the most obvious issues with the assay was that having vastly increased the number of probes on the array, we now had a number which showed significant cross-reactivity; this was despite all of the probes having been designed to have minimal secondary structure, equivalent  $T_m$  values and being checked by Basic Local Alignment Search Tool (BLAST) analysis against the GenBank database. To cut a very long story and considerable effort short, removal of some probes and development of the assay conditions allowed us to get the assay working well again.

Several different parameters of the assay have been evaluated. Sensitivity was felt to be one of the biggest potential issues and we published some of these data in 2005 (Burton *et al.*, 2005); direct PCR amplification of a specific gene allowed detection of femtogram quantities of pathogen nucleic acids, with the random amplification method, the limit of detection was in the low pictogram range and in the nanogram range with no amplification. These limits of detection correspond at best to approximately  $10^2$  organisms per mL of sample. Similarly, another potential issue was the time taken to perform the assay. Most interestingly, time course analysis of the hybridisation kinetics showed us that the favoured 16h overnight incubation was much longer than was needed and actually gave poorer results in terms of signal output. For conventional hybridisations, 2h was found to give optimal signal. Subsequently, moving the assay to an automated platform, where the hybridisation is not static improved the kinetics enormously. Using this technology, hybridisation results can now be obtained in approximately 20min.

In summary, what are the real prospects for the future of “lab-on-a-chip” assays? Hopefully what we have shown is that there are some really good prospects for this type of technology. Some of the original fears turned out to be ill-founded, as the assay can be made to be sensitive, rapid and highly reproducible, although the largest errors in the assay are unsurprisingly due to operator variation. The current choice of

matrix (glass slides) are not suitable for any sort of robust assay due to their inherent fragility, but the assay could theoretically be transferred onto a bead-based system or similar, which would render it a lot more user-friendly. The biggest hurdle which still needs to be overcome is the problem associated with “contaminating” DNA present in clinical samples. This is, of course, amplified with the pathogen nucleic acid and really decreases the apparent assay sensitivity. Numerous groups have now reported such problems and until a work-around is devised, the utility of the assay remains rather limited. At the beginning I posed the question: how real was the concept of a small size assay capable of running numerous tests in parallel? Clearly there has been considerable progress towards this desire, in fact it can and has been partially achieved and I think that it certainly has a huge future potential.

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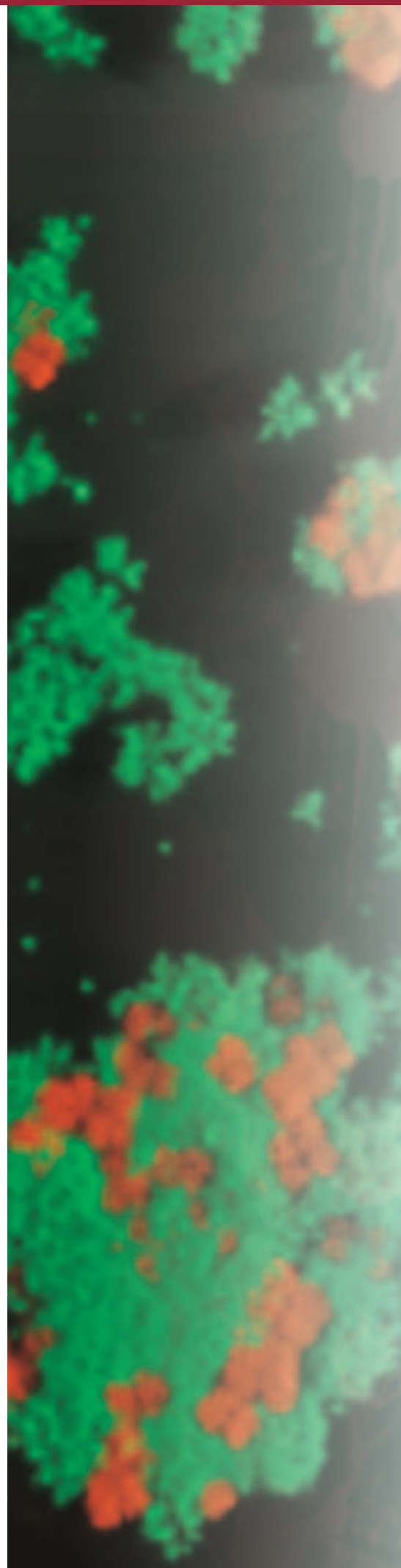
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## acknowledgements

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**Nigel Silman**

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# Bacterial sociology in a biofilm world

**Alex Rickard**, a recipient of a SfAM New Lecturer Research Grant, is an assistant professor at Binghamton University and is studying inter-species bacterial interactions. **Kyung Min** is a PhD student at Binghamton University and is studying coaggregation between freshwater bacteria

The words *sociology* and *biofilm* are seldom used in the same sentence, let alone together in a title. The word *sociology* is typically used as a term to describe human interaction and community composition but in general relates to social organisation, collective behavior and interaction between individuals. This word is also used to relate to developing an understanding of individuals' relationship to a group. The word *biofilm* is often used to describe a thin but robust layer of mucilage adhering to a solid surface which contains bacteria and other microorganisms that generated it. How can these two words be linked? They can be linked by the word *community*.

Most biofilms consist of multiple species of bacteria. For example there can be as many as 500 species of bacteria within dental plaque biofilms. Each species within this community has a unique repertoire of abilities. Species can coordinate these abilities with other species, under specific environmental conditions, to maintain a niche or even expand within a multi-species biofilm. For instance, the human oral bacterium *Veillonella atypica* is unable to ferment carbohydrates found in normal saliva and instead the species metabolises organic acids such as lactate. Lactate is a waste product of carbohydrate fermentation by oral streptococci, which also happen to be the numerically dominant species in healthy dental plaque (50-80% of the species). *V. atypica* cells recognise and specifically bind to the surface of streptococcal cells and use the waste lactic acid to promote its own growth. Without lactic acid, *V. atypica* cells would not grow and would be unlikely to be able to maintain a niche within dental plaque. But the story does not end there.

Early evidence also suggests that *V. atypica* may, by the act of attachment or by the production of chemical signals, upregulate *S. gordonii*  $\alpha$ -amylase-gene-expression which is hypothesised to provide more sugar to be converted to lactic acid (England *et al.*, 2004). This interaction could be considered to constitute a form of inter-bacterial signaling by which *V. atypica* cells can enhance their ability

to grow. Furthermore, this signaling phenomenon highlights a property that many bacteria possess – the ability to communicate with other bacterial species. Coupled with the increasing acceptance that most bacteria exist in biofilms (Costerton *et al.*, 1987), some researchers now believe that inter-species bacterial communication facilitates highly reproducible biofilm community development (Irie & Parsek, 2008; Kolenbrander *et al.*, 2006; Parsek & Greenberg, 2005).

How do bacterial species communicate with one another and become fit for *existence* in a multi-species biofilm? Simply put, there are many ways a species can communicate and potentially “socialise” — these are too numerous for this short article. However, inter-species bacterial communication can be broken down into two broad categories: physical communication strategies and chemical communication strategies. Through the use of one or both of these forms of communication,

the potential for a species to maintain a niche in a biofilm community can be enhanced. It should also be noted that increasing evidence suggests that, as with any social gathering and associated forms of communication, there can be cheater species which manipulate communication mechanisms to integrate and potentially dominate within a community (Hochberg *et al.*, 2008). Thus, it is the aim of this short opinion/review to give a brief overview of inter-species physical and chemical communication, including a description of how species may ingratiate themselves or cheat their way into biofilm communities through masquerading or manipulating communication mechanisms. Oddly enough, many of these cheaters are also thought of as problematic or pathogenic species.

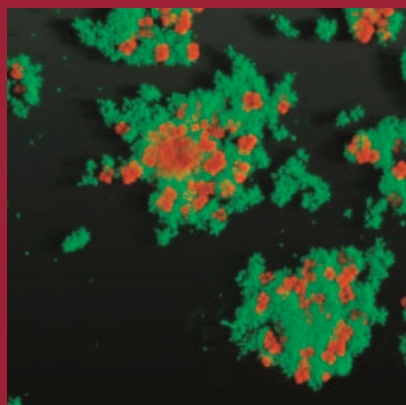
## Physical communication

Do biofilm communities consist of bacterial species that randomly clump and grow together or is there evidence that specific species exhibit a *preference* to clump with other specific species? As with most living systems, organisation can enhance functionality. Functionality improves success and success leads to self perpetuation and an improved chance of survival. Thus, if specific species could recognise and positively interact with one another, it is conceivable that their co-localisation will improve the pair's survival and maybe a form of mutualism or even altruism will develop. Indeed, since early studies of oral bacteria by Gibbons and Nygaard (1970), it has become increasingly clear that there is potential for different species to specifically recognise and adhere to one-another. This process is called coaggregation. Using visual aggregation assays, it has now also become evident that coaggregation is not just a phenomenon that occurs between oral bacteria. Coaggregation is a phenomenon that occurs between bacteria in many biofilm communities, including those from freshwater, gut, urogenital tract and wastewater ecosystems (Rickard *et al.*, 2003).

In order for coaggregation to occur, lectin-like adhesins must be expressed on the cell

**Figure 1.**

Confocal Scanning Laser Micrograph showing the freshwater bacteria *Sphingomonas natatoria* 2.1 (green cells) and *Micrococcus luteus* 2.13 (red cells) coaggregating together and settling onto a glass-microscope slide. *S. natatoria* 2.1 forms small clusters of rosettes and *M. luteus* 2.13 forms groups of tetrads. When mixed together, the two coaggregate to form macroscopic flocs that are easily seen by eye in a test-tube.



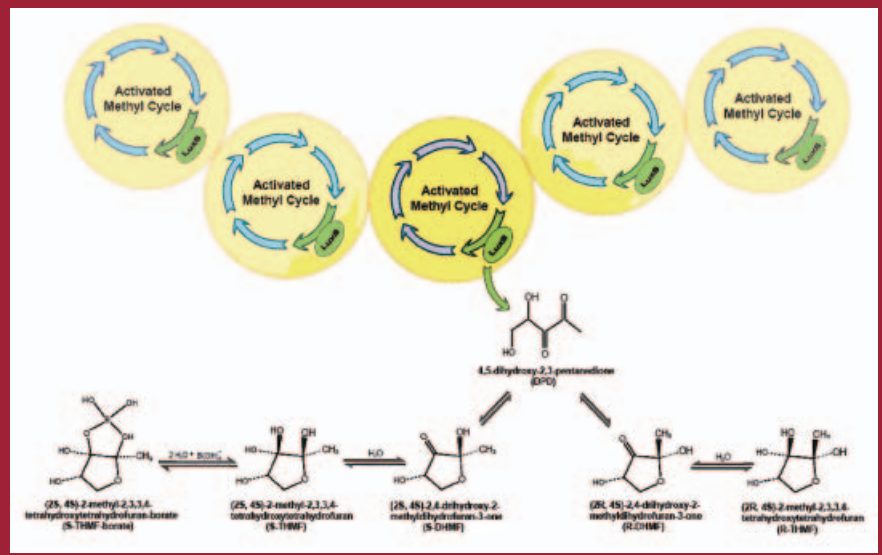


surface of one species and complementary polysaccharide receptors must be expressed on the partner species' cell-surface (Kolenbrander *et al.*, 2006). While this seems simple enough and studies have shown that coaggregation between oral bacteria is due to constitutively expressed adhesin and receptors, coaggregation between freshwater bacteria is growth-phase dependent (Rickard *et al.*, 2003). Furthermore, expression of coaggregation by freshwater bacteria can be asynchronous, with one species only expressing adhesins when in a physiological state that is akin to stationary phase, and the other species only expressing receptors when actively growing, much like when in exponential-phase. But when time and conditions are suitable (which raises the interesting question which will not be addressed here: Why *certain* conditions?), freshwater species coaggregate (Figure 1). The question then arises, "So what? Is there a role for coaggregation other than bringing species together?" Early evidence indicates that there are certainly at least two good reasons to coaggregate. First is the obvious reason: fluid shear. In flowing environments such as the oral cavity or in a flowing stream, surfaces can be exposed to high fluid shear. Shear can prevent cells from attaching to surfaces, adhering to surface bound bacteria, or integrating into pre-existing biofilms. Because the polymers that mediate coaggregation often facilitate strong, localised physico-chemical interactions between cells (often through interactions that are similar to those mediated by lectin-saccharide interactions), the ability to coaggregate will likely enhance the potential for a species to colonise surfaces in flowing environments (Rickard *et al.*, 2003). The second reason why the ability to coaggregate may be favorable for a species, relates to the importance of *juxtaposition*. Cells of different species that are in close proximity are more likely to detect and communicate with one another through chemical cell-cell signal molecules than those cells that are distantly located.

**Chemical communication**

Chemical inter-bacterial communication is at present receiving much attention from microbiologists. This form of inter-bacterial communication includes the production and use of metabolites, or the production and detection of low molecular weight extra-cellular cell-cell signal molecules (Kolenbrander *et al.*, 2002). A variety of forms of extra-cellular cell-cell signal molecules are being studied with great interest and many mediate communication between cells belonging to the same strain or species. These signal molecules include competence stimulating peptides and

Figure 2.



Autoinducer-2 (AI-2) is an inter-species signal molecule. AI-2 is a family of inter-convertible molecules formed by the rearrangement of DPD. In the diagram, a metabolically active streptococcal cell (bright yellow cell) is producing AI-2 by virtue of activity of the activated methyl cycle (specifically the LuxS enzyme). The far left form of AI-2 (S-THMF-borate) is recognized by *Vibrio harveyi* while the far right form (R-THMF) is recognised by *Salmonella Typhimurium*. The form(s) of AI-2 that streptococci recognise and use to chemically communicate is unknown.

acyl-homoserine-lactones (for review see Irie & Parsek, 2008). However, when considering that biofilms are typically composed of multiple species, candidate inter-species cell-cell signal molecules should also be investigated. This area of study has been spearheaded by the recent discovery of 4,5-dihydroxy-2,3-pentanedione (DPD), also called autoinducer-2 (AI-2). AI-2 is produced by LuxS, an enzyme that is part of the activated methyl cycle in many bacterial species. Chemical analysis and synthesis of AI-2 has led to the realisation that AI-2 is able to spontaneously generate a variety of inter-convertible structural forms that are in equilibrium. Different environmental conditions likely favour different forms (Figure 2). Thus AI-2 is an umbrella term, and increasing evidence suggests that many bacterial species produce AI-2 and/or can recognise different forms of the molecule.

**“Loneliness is never more cruel than when it is felt in close propinquity with someone who has ceased to communicate”.**

*Germaine Greer, The Female Eunuch (1971).*

Of recent interest is the possibility that AI-2 mediates communication between oral bacteria. This is, in part, because most human oral AI-2 producing species are also known to coaggregate and consequently juxtapose in

multi-species biofilms. Summed concentrations of AI-2, when cells are in close proximity, could drive a “chemical discourse” (Bassler, 2004). Such a hypothesis, while being hotly debated, is being investigated and appears to be a valid and intriguing avenue of oral microbiology research (Kolenbrander *et al.*, 2006; McNab & Lamont, 2003; Williams *et al.*, 2007). Furthermore, because different oral species seemingly produce different quantities of AI-2 and also modify their local environment, different concentrations of specific forms of AI-2 may elicit different responses from a particular organism. For example, AI-2 has been demonstrated to mediate mutualism (also described as luxuriant interdigitated growth, Figure 3) between the commensal coaggregating oral bacteria *Streptococcus oralis* 34 and *Actinomyces naestlundii* T14V, when grown together in a biofilm that is fed human saliva (Rickard *et al.*, 2006). Both species produce AI-2, but when *S. oralis* 34 is replaced with an isogenic (i.e., genetically identical) *luxS* mutant that is unable to produce AI-2, mutualism is abolished. Mutualism can be restored by the addition of high picomolar to low nanomolar concentrations of chemically synthesised AI-2 (Rickard *et al.*, 2006). The potential for other streptococci/oral bacteria to respond to nanomolar concentrations of chemically synthesized AI-2 has also been demonstrated in *Streptococcus intermedius* (Pecharki *et al.*, 2008) and *Streptococcus mutans* (Sztajer *et*



al., 2008). Of particular interest is the work by Yoshida *et al* (2005) that elegantly demonstrated that AI-2 producing oral streptococci and AI-2 producing oral pathogens *Porphyromonas gingivalis* and *Actinobacillus (Aggregatibacter) actinomycetemcomitans* restore the biofilm-forming ability of a *luxS* mutant of *S. mutans* to that of the wild-type strain. To add an extra dimension and even more intrigue to this story, very recent work by Maeda *et al.*, (2008) has indicated that *P. gingivalis* cells may moderate AI-2 production during initial colonisation of oral biofilm communities. This could possibly enhance integration of *P. gingivalis* into the community.

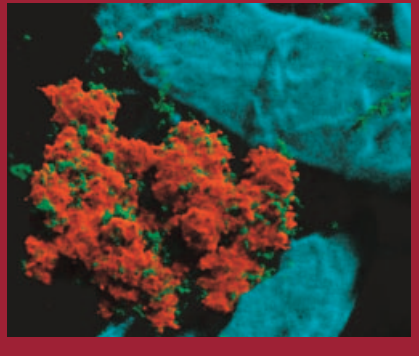
### Social dynamics and an ever-developing community.

Multi-species biofilms originate from the aggregation of cells with unique properties (i.e. species). Evidence indicates that coaggregation can bring specific partner cells into close proximity and cell-cell signaling molecules mediate signaling among juxtaposed cells. In oral communities, streptococcal species predominate and this is in part due to their ability to coaggregate with one another (e.g. *S. oralis* coaggregates with *S. gordonii* which coaggregates with *S. sanguinis* and *S. mitis*). This numerical dominance may also relate to the production and detection of cell-cell signal molecules as well as being metabolically adept at growth in saliva.

Within any community there is potential for exploitation by cheaters. These include organisms that can manipulate communication mechanisms to integrate themselves into a community usually at the detriment of the other species within the invaded community. Such a possibility is now recognised to occur between many multi-cellular organisms. For example, staphylinid beetles can chemically and physically mimic activities of ants to gain access to the highly protected colonies and consume stored food and destroy ant larvae (Hollдобler & Wilson, 1990; Witte *et al.*, 2008). This is only wild conjecture, but a similar phenomenon may occur in oral bacterial communities, where coaggregating and signal producing pathogens attempt to integrate into biofilms that are numerically dominated by commensal *Streptococcus* species. Studies have already indicated that many oral pathogenic species such as *P. gingivalis* and *Actinobacillus (Aggregatibacter) actinomycetemcomitans* are able to coaggregate as well as produce and respond to AI-2 (James *et al.*, 2006; McNab *et al.*, 2003; Shao *et al.*, 2007). If these pathogenic species can gain acceptance into a commensal oral biofilm and then grow and divide at an enhanced rate (possibly at the detriment of the

**Figure 3.**

Confocal Scanning Laser Micrograph showing luxuriant inter-digitated growth (mutualism) between the coaggregating oral bacteria *Streptococcus oralis* 34 (green cells) and *Actinomyces naeslundii* T14V (red cells) on nitrocellulose acetate filters (blue fibres) within a sorbarod biofilm system (Rickard *et al.*, 2008). Luxuriant inter-digitated growth is mediated by the production of AI-2 by *S. oralis* 34.



streptococcal species), then the commensal community will reduce in size and the pathogenic community will dominate. Thus, it is possible that these pathogenic species integrate into commensal oral biofilms by utilising physical and/or chemical communication mechanisms and, much like the staphylinid beetle, destroy the community from within.

The importance of physical and chemical communication in the development of bacterial biofilm communities outside the human oral cavity is also receiving increasing attention. The role and function of AI-2 in other communities is less clear and evidence indicates that some species neither produce nor respond to AI-2 and do not coaggregate. These bacterial species may use other physical and chemical communication mechanisms. Regardless, it is now becoming increasingly clear that bacteria are not solitary units of life and favour seemingly social biofilm gatherings as a pretext to forming complex multi-species communities.

### acknowledgements

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

## Comparison of regression lines

# Statnote 18

The relationship between two variables may have been studied at various times or in different laboratories giving rise to two or more independent estimates of the relationship between Y and X. In these circumstances, it may be of interest to discover whether the different regression lines are the same, and if they are, an investigator may wish to combine the data from the different studies and fit a single regression line to the whole of the data. In addition, an investigator may wish to determine the relationship between Y and X given one set of conditions compared to the relationship under a different set of conditions. Hence, regression lines can be compared not only with the intent of combining data but to reveal that they are in fact different, potentially as a consequence of the different conditions. This Statnote describes the statistical methods associated with comparing two or more regression lines.

### Scenario

To illustrate the method, we return to the scenario first described in Statnote 14 (Hilton & Armstrong, 2008). Essentially, adequate skin antisepsis prior to invasive procedures is important in preventing infections. Nevertheless, skin antiseptics permeate poorly into the deeper layers of the skin and into hair follicles, which may harbour microorganisms and cause infection when the protective skin barrier is broken. One potential mechanism of delivering antiseptics deeper into the skin is to co-administer a 'carrier' compound to facilitate movement of the biocide through the various skin layers. Hence, the aim of the study was to evaluate the permeation of a commonly used biocide into the full thickness of human skin when applied alone, or in combination with a 'carrier' compound.

Full thickness human skin samples were obtained from patients undergoing breast reduction surgery. The skin permeation studies were performed using vertical diffusion cells with the stratum corneum of the skin sample uppermost. One millilitre of antiseptic solution in the presence or absence of the carrier compound was aliquoted onto the skin, and

incubated for two minutes, 30 minutes, or 24 hours. The assay was performed in triplicate. Following the exposure to the antiseptic solution (+/- carrier) the skin was washed with PBS and three 7mm punch biopsies taken from each sample. The biopsies were cut with a microtome into 20 $\mu$ m slices from the skin surface to a depth of 600 $\mu$ m and 30 $\mu$ m slices from 600 $\mu$ m to 1500 $\mu$ m. The weight of the skin samples was determined and each sample was analysed by HPLC to determine the concentration of antiseptic present as  $\mu$ g antiseptic per mg of tissue.

A number of mathematical models might describe the pattern of penetration of the antiseptic into the skin. In the correlation analysis described in Statnote 14, the data suggested that a model based on passive diffusion poorly described the penetration profile observed and further models were then tested and specifically, whether the antiseptic alone would have penetrated the skin according to a 'power-law' model similar to the inverse square law. A variable Y is distributed as a power-law function of X if the dependent variable has an exponent 'a', i.e., a function of the form  $Y = CX^a$ . If penetration of the antiseptic does follow such a law, then a log-log plot of the data should result in a linear relationship between Y and X. In Statnote 16 (Hilton & Armstrong, 2009), this model was fitted to a single set of data, i.e., without carrier. In the present Statnote, we extend this analysis to test whether the presence of the carrier 'EO' affected the degree of penetration through the skin.

### Data

The data comprise several pairs of measurements of two variables, *viz.*, the concentration of antiseptic (Y) and skin depth (X) with  $(Y_1, X_1)$  under two conditions, *viz.*, with and without the carrier 'EO'  $(Y_2, X_2)$  and the data are illustrated in Figure 1.

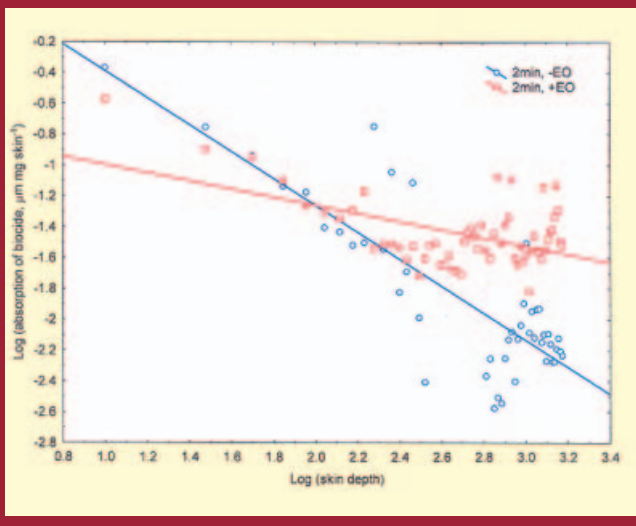
### Analysis

#### *How are the test statistics calculated?*

Regression lines may differ in three properties. First, they



**Figure 1.** Relationship between the degree of penetration of the biocide with (+EO) and without (-EO) carrier and depth of skin (Pearson's correlation coefficient -EO  $r = -0.84$ ,  $r^2 = 0.71$ ; +EO  $r = 0.53$ ,  $r^2 = 0.28$ ).



may differ in residual variance, i.e., in how good a fit the lines are to the data and therefore, one line may fit the data better than the other. Second, they may differ in slope and hence one line may exhibit a greater change in  $Y$  per unit of  $X$  than the other. Third, the lines may differ in elevation, i.e., if the two lines have similar slopes and are therefore parallel they will intersect the  $Y$  axis at different points. Whether the lines differ in 'goodness of fit' can be tested by comparing their residual variances using a two-tail 'F' test (see Statnote 5, Hilton & Armstrong, 2006). Whether the two lines differ in slope or elevation can be tested using an analysis based on analysis of covariance (an extension to analysis of variance in which a covariate  $Y$  is included in the analysis) (Snedecor & Cochran, 1980). Essentially, individual regression lines are fitted to each set of data separately and a further common line is then fitted to the data pooled from both sets of data; the analysis essentially comparing the individual fits with that of the pooled regression. Differences in the slopes and elevations of the lines can then be tested. If there is a significant difference between the slopes of the regression

**Table 1.** Analysis of covariance comparing the slopes and elevations of two regression lines

Variation	Sums of squares (SS)	DF	Mean square (MS)	'F'
Between slopes	2.2375	1	2.2375	39.63***
Error	5.7596	102	$5.65 \times 10^{-2}$	
Between elevations	4.4217	1	4.4217	
Error	7.9971	103	$7.7642 \times 10^{-2}$	

lines, then it is not necessary to test the elevations as these will vary at each value of  $X$ . If the lines have similar slopes, then their elevations can also be tested. If two or more regression lines are shown to have the same slopes and elevations, then they are statistically similar and the investigator may wish to combine the data in a single regression analysis.

### Interpretation

The first stage in the analysis is to fit a linear regression to each set of data separately and the lines of best fit are shown in Figure 1. Examination of these lines suggests that they differ in slope. The values of ' $r$ ' and ' $r^2$ ' indicate that both sets of data can be fitted adequately by straight lines, although the linear regression is a better fit to the data without the carrier 'EO'. A regression line is then fitted to the data pooled from both data sets and this enables the slopes and elevations of the lines to be compared. The analysis of covariance table is shown in Table 1. There is a highly significant difference between the slopes of the two regression lines ( $F = 39.63$ ,  $P < 0.001$ ). The comparison between heights or elevations of the two regressions is also included here but would not be relevant in this example as the lines have different slopes. Note that if the slopes of the two lines were not significantly different, i.e., the lines were parallel, then it would be important to test the difference in elevation.

Hence, the regression of log absorption of biocide declines less rapidly with log skin depth when the carrier compound 'EO' is present. Note that the  $Y$  axis represents a negative logarithmic scale, and hence the lower negative numbers represent a greater degree of absorption. Hence, we can conclude from this experiment that the presence of the carrier 'EO' does alter the kinetics of penetration of the antiseptic into the skin and increases the amount of antiseptic reaching the deeper levels of the skin.

### Conclusion

In many circumstances, it may be of interest to discover whether two or more regression lines are the same. Regression lines may differ in three properties, *viz.*, in residual variance, in slope, and in elevation; all of which can be tested using analysis of covariance. If there are no significant differences between regression lines, an investigator may wish to combine the data from different studies and fit a single regression line to the whole of the data.

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## careers



## Science Communication

Editor of *Microbiologist* and Communications Manager of SfAM, **Lucy Harper** explains how and why she came to be in such a role and discusses the key skills and qualities required

I began my career taking the traditional science route — I studied my undergraduate degree in Medical Biochemistry at the University of Birmingham and remained in Birmingham for many years taking a number of short-term research posts. The last of these posts generated sufficient results for publication and a PhD, and I was awarded my PhD from Aston University in 2002.

I've always loved writing and was one of the few bench scientists I knew at the time who actually preferred writing up results to generating them. During my PhD and subsequent post-doctoral positions in microbiology, I wrote a number of non-peer-review articles for various publications, including the then 'SfAM News' and later, '*Microbiologist*'. I loved the creativity of writing and the way I could essentially paint a picture with words. I also relished the opportunity to communicate complex scientific principles to a non-specialist audience enabling all to understand the principles behind the science I was explaining.

During what was to become my final post-doctoral position, I was given the opportunity to apply for the position of Honorary Editor of *Microbiologist* and I must admit I didn't immediately jump at the chance. It's a big responsibility editing a magazine which will reach thousands of people worldwide. I was very wary of potentially making a mistake when producing such a visible 'product' as this could reflect badly on the Society. However, despite my initial hesitancy, I decided to bite the bullet and was successful.

To say I enjoy working on the *Microbiologist* is an understatement and probably reflected in the fact that I'm still doing it five years on. I love the creativity of the job and the fact that I am able to commission articles from such a wide variety of scientists who are experts in a diverse range of areas. Tying different areas of microbiology together with a common theme is sometimes a challenge, but it's always very satisfying to see the finished product lying on someone's coffee table (and that of the SfAM office of course).

So, I was working as a post-doctoral researcher and editing *Microbiologist* in my spare time and, with only six months left on my contract, I was contemplating my next career move. Then I heard about a new workshop on communicating science through the media, run by the then newly-established organisation called Sense About Science. The workshop was called 'Standing up for Science' and those who wanted to attend were asked to apply for a place by sending in a CV, so I applied straight away keeping my fingers crossed (see page 18 for a write-up of

the most recent of these workshops which I'm glad to say are still going strong). I was lucky enough to be afforded a place at the workshop and found the whole experience enlightening and inspiring. I was determined from then on to make a career of communicating science to the general public. I wasn't entirely sure what type of job would enable me to do this, but I knew from then on that science communication was where I wanted to be.

My experience as Editor enabled me to apply for editing or writing roles, but in addition I applied for general communications roles in an attempt to expand my communications repertoire. I left the laboratory to become the Communications Officer for Med-Vet-Net, the EU framework programme 6 network of excellence for zoonoses research (see page 20). As SfAM are the partner institute for dissemination within Med-Vet-Net, this position enabled me to continue in my editorial role (albeit in my spare time) and to expand my communication skills through training. Whilst in this position I received training in a wide range of communications skills from how to write a press release, to using desktop-publishing software. I learnt about the communication of science at science centres, crisis management in the corporate world, science policy and the less tangible aspects of communication such as influence and the public perception of science.

Whilst working for Med-Vet-Net it became clear that SfAM needed somebody in a communications role in addition to the role of the Editor of *Microbiologist*. Med-Vet-Net also required additional communications support as the network delivered its objectives, so Med-Vet-Net employed a Senior Communications Officer and I became the Communications Officer for SfAM — a role which would enable me to put all my new-found skills into practice.

If I were to describe the work of a Communications Manager\* in one word I would use the word "diverse". Every day is different and often the most stringent, carefully thought-out plans go to waste in the time it takes to receive a phonecall.

My role at SfAM involves most aspects of science communication and can be split into six general categories:

- Editor — *Microbiologist*
- Website and new technologies (e.g. podcasting/Facebook/Twitter)
- Media — both media enquiries and proactive media work (press releases/press briefings)
- Policy — responding to relevant government consultations



- Public engagement/outreach
- Internal communication

I don't have the space to go into every aspect of this role: a whole article could be devoted to careers in each of these areas of science communication. But many organisations have individuals or even departments dedicated to each of these areas of communication, so it's not difficult to imagine the diversity of the skills set required of a Communications Manager.

### Skills and qualities

The ability to write is a fundamental skill required of a Communications Manager. Not only should one be able to write, but the writing style must be adapted depending upon the nature of the written piece. Before beginning a piece of writing it is vital that the writer *understand* (not just know) who their audience is going to be — this could be school children, journalists or scientists and the level at which the work is pitched will be very different for each audience. Before writing, it can be useful for a writer to spend some time researching the audience's demographics, their training, and their likely level of scientific understanding. This will ensure the finished product has the correct pitch so the majority of readers will benefit from a greater understanding of the subject.

It is imperative that a Communications Manager remains up to date with the development of all kinds of new technologies. The tools people use in order to communicate are changing rapidly. Keeping up with the development of new technologies is essential to science communicators — for example, so many people rely on Twitter as a means of communication and information dissemination, that to ignore this communication tool could mean that you are ignoring a large proportion of a potential audience. It is also important to consider the effect of such developments on those in associated professions. For example, the role of science journalists is changing as a result of new technologies which enable news to be 'reported' in real time through Twitter, Facebook and blogs. So the approaches science journalists are taking to report science are also changing. As a press officer, a Communications Manager is a potential supply of information to a science journalist through press releases or briefings. If a journalist needs a certain depth of information, or information presented in a certain way that's different to the way the information has been supplied in the past, it's the Communications Manager's job to ensure current information is supplied in a usable format.

Time management and versatility are inherent in the role of a Communications Manager. For example I could be engrossed in proof-reading an article on influenza for the magazine when the phone rings and suddenly I'm thrust into the role of press officer and I have to drop everything to find an expert for a journalist who's doing a piece on antibiotic resistance. Equally, I could be assisting in the organisation of a public engagement event where I'm required to find venues for the event, produce costings and report my findings to various different subcommittees, whilst at the same time trying to get to the bottom of a technical problem with the website. So it is important that Communications Managers are comfortable with switching from one type of task to another quickly and seamlessly to get results.

### Training and related organisations

Briefly, it is possible to go into science communication without any formal qualification in the subject, but it is advisable to seek training where possible. This can take the form of a MA or MSc course in science communication, many of which are run by UK universities including Imperial College London and the University of Bath. In addition there are many accredited courses which can be undertaken alongside full time employment.

Some of the career paths taken by those in science communication can include:

- Communications Officer/Manager
- Press Officer — for university, research council, or government science organisation (see *Microbiologist* vol. 10., no.2, pp12-13)
- Public Relations Officer/Manager
- Public Outreach/Engagement Officer
- Editor
- Science Writer (see *Microbiologist*, vol. 9., no. 1., pp40-41)
- Medical Writer for a medical communications agency (see *Microbiologist*, vol. 9., no. 4., pp44-45)
- Science Journalist (see *Microbiologist*, vol. 9., no. 1., pp40-41)
- Publications Manager (see *Microbiologist*, vol. 8., no. 2, pp42-43)
- Guide at hands-on science centre

There are many organisations which provide support, guidance, advice and career development for science communicators, including:

- Science Technology Engineering and Medicine Public Relations Association (STEMPRA) <http://www.stempira.org/>
- Science Media Centre (SMC) <http://www.sciencemediacentre.org/pages/index.html>
- Sense About Science <http://www.senseaboutscience.org.uk/>
- American Association for the Advancement of Science (AAAS) <http://www.aaas.org/>
- British Science Association (formerly British Association) <http://www.britishtscienceassociation.org/web/>
- Association of British Science Writers (ABSW) <http://www.absw.org.uk/>
- World Federation of Science Journalists (WFSJ) <http://www.wfsj.org/>
- Wellcome Trust <http://www.wellcome.ac.uk/>
- Royal Society (report) <http://royalsociety.org/downloaddoc.asp?id=3052>
- Beacons of Public Engagement <http://www.rcuk.ac.uk/sis/beacons.htm>
- Chartered Institute of Public Relations (CIPR) <http://www.cipr.co.uk/>

NB: This is a selection of the organisations available and should not be considered a comprehensive list.

If you're interested in science communication but you don't want to leave the laboratory, there are numerous ways in which active scientists can participate in science communication activities. Providing expertise to journalists and press officers, participating in science festivals and other public engagement activities, and visiting schools to talk about science, are just a few of the ways scientists can play a part. In fact this is fast becoming an integral aspect of a scientist's career, with research councils and funding bodies recognising the dissemination of scientists work outside of peer review as a metric for evaluation.

I find the diversity, variety and subject of my job fascinating. I'm constantly being stretched and finding new ways of tackling sometimes difficult tasks, situations and (rarely) people. I love the fact that my job requires me to meet so many interesting experts, and keep up-to-date with changing technology (this feeds my inner geek). I would recommend a career in science communication to anyone who values diversity, complexity and who relishes a new challenge every day.

\*As I am now fortunate to be the Communications Manager for *SfAM* the article is written using this job title but the reader should note this information also applies to the Communication Officer role

**Lucy Harper**  
Communications Manager



News from the SfAM Post-Graduate and Early-Career Scientist Committee

## PECS NEWS

George Aboagye and Jess Rollason have stepped down from their committee positions and we would like to thank them for their dedicated work with PECS. We are pleased to announce that, Simon Gould (Chair) and Amara Anyogu (Secretary) have been duly elected into these positions, welcome to you both.

The PECS committee invites nominations for events officer, events team and communications officer. The posts are available from October 2009 (for one year's term) and are open to all student or early career scientist members of SfAM.

## Congratulations!

Congratulations go to Jo Heaton a SfAM early career scientist who was awarded the SGM outreach award for 2009 in recognition of her dedicated work promoting microbiology to school children.

If you know a SfAM student or early career scientist who has been awarded a PhD/ prize/award then get in touch, email: v.l.mccune@newcastle.ac.uk.



**Vicki McCune**

PECS Communications Officer, Newcastle University



## Summer conference 2009 student session — Networking

So many postgraduate and early career scientists find the prospect of networking daunting, therefore this year's summer conference students session organised by the PECS events team, was an interactive workshop on how to make the most of a networking situation. The hosts of the session were Beverley Gartside and David Harrison from Fairplace. Some of the skills taught included how to enhance your ability to network successfully, what to say and what to ask, what you can observe about others and how to feel more confident. The workshop highlighted why networking is so important for scientists at the beginning of their careers, especially as it enables them to build relationships that could lead to collaborative work or even getting a new job at the end of their PhD.

Beverley summarised what networking is by quoting Steven D'Souza; *"networking is the art of building reciprocal relationships that help individuals and the community as a whole to achieve their goals"*. One of the most interesting aspects of the session focused on "your personal brand". Successful commercial brands were discussed and then participants had to think about their sixty second story; what is it that others think about them during an initial meeting and what makes them stand out? According to Mehrabian's rule of communication only 7% of good

communication skills are associated with spoken words, 38% is associated with the tone of voice and 55% with body language. If you think about a person you know who is good at networking, what do you notice about them? They usually have good posture giving the appearance of confidence, a smile and are interested in what others are saying.

The following top tips were also given for breaking in and out of groups.

Breaking in: aim for groups of three as this will not be a closed conversation, look for an opening in the group and walk towards it with confidence, then mirror the body language of the group. When the alpha finishes speaking be the first to ask a question or give a compliment. Breaking away: connect the person you are speaking with to someone else, give a plausible reason to leave, i.e., getting a drink or using the facilities and always leave a positive impression.

By the end of the hour those attending were certainly more confident and prepared to test out their newly acquired networking skills at the summer conference trade show!



**Katie Fisher**

PECS Events Officer  
University of Northampton



# Students into Work Grant reports

information

## 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 in each issue of *Microbiologist*.

For further information visit:  
[www.sfam.org.uk/grants.php](http://www.sfam.org.uk/grants.php)



## Resistance of strains of *Pseudomonas aeruginosa* to photodynamic antimicrobial chemotherapy

During the summer before my final year, I decided to undertake a research studentship within the School of Pharmacy at Queens University, Belfast (QUB). I believed that this work would provide me with an insight into the industry and research side of pharmacy, as well as giving me some indication of what a PhD might involve. It would also help prepare me for my final year project for my MPharm degree.

The main aim of my research was to investigate the resistance of various strains of *Pseudomonas aeruginosa* to photodynamic antimicrobial chemotherapy (PACT). Resistance of bacteria to PACT is an area in which very little research had previously been carried out and experts believed that resistance was limited.

PACT is the combination of a photosensitiser, alongside light and *in situ* oxygen. In this case the photosensitisers meso-tetra (N-methyl-4-pyridyl) porphine tetra tosylate (TMP) and methylene blue (MB) were chosen. The photosensitisers accumulate both rapidly and selectively in the cell membranes of the bacteria. When light of a suitable wavelength is irradiated on the cell this causes the production of a reactive oxygen species which leads to the destruction of microbial cells but leaves the host tissue unharmed.

The original aim of the project was to choose a bacterial species which was resistant to antibiotics and to determine if combining sub-lethal concentrations of PACT with the antibiotic would lead to cell death. However, some problems were encountered over the ten weeks and the aim of the research moved in a different direction. The bacterium chosen to be the main focus of testing was *P. aeruginosa* strain DF1. This was known to be resistant to, among others, ceftazidime and meropenem. After initial testing the strain was exhibiting low percentage kill with MB and therefore a decision was made to change the photosensitiser to the stronger toluidine blue O (TBO). However, after many attempts this also

was showing a low percentage kill. As this particular strain was more resistant than originally expected, the irradiation time was increased from five to ten minutes. This proved to be beneficial as a kill of 99.3% was achieved.

Due to this strain displaying greater resistance than other bacteria previously tested, the aim of the research was changed to investigate if other *P. aeruginosa* strains, grown planktonically, displayed the same degree of resistance. Further problems were also encountered with the light source used, as it had to be returned to the manufacturers. During this period, testing was carried out using a red LED light source. However this produced results which were insignificant as 100% kill was achieved both with and without the presence of light.

Over the course of the ten weeks, twelve different strains of *P. aeruginosa* were tested and they generated a wide range of results, with very little trend displayed between the different strains. On various occasions some of the strains which were proven to be resistant to antibiotics also displayed a higher degree of resistance to PACT; however, this was not observed in every case. In order to achieve an acceptable level of kill, each strain required either five or ten minutes of irradiation and different TBO concentrations. These ranged from a minimum of 25µg/ml for more susceptible bacteria to 500µg/ml for the more resistant bacteria. For each of the strains tested, the method was carried out a minimum of two times. The effect of another photosensitiser, TMP, was tested by another summer student and was found to exhibit the same variation in results as was demonstrated with TBO. A possible explanation for the variation observed between the strains could be the presence of efflux pumps in the bacterial cell. It is believed that these pump the photosensitiser out of the cell membrane and inhibit its effects.

Overall, although the results were not what we originally expected, I believe that those generated are significant to

the field being researched due to the fact that they show that bacteria, especially *P. aeruginosa*, do display a certain level of resistance to PACT. It would be both beneficial and interesting to further investigate this using the antibiotics as originally planned. This would help to determine if there is any similarity between bacterial resistance to antibiotics and resistance to PACT. Further research could also be carried out to see if isolates growing in biofilms produce different results to those grown planktonically. I hope that my work will be continued within the School of Pharmacy and that one day this field of research will prove to be clinically useful.

Finally, I would like to thank Ryan Donnelly and the School of Pharmacy, QUB for allowing me to carry out this research and giving me the opportunity to extend my knowledge in the field of microbiology.

Kathryn Sally

## Identification and antibiotic treatment of bacteria isolated in sputum from patients with Cystic Fibrosis

**Cystic fibrosis (CF) has been** identified as one of the most common genetic disorders of the Caucasian population ultimately leading to death. It is known that CF is the result of a defect in the CF transmembrane conductance regulator gene. One of the outcomes of this mutation is that the CF patient produces thick mucous secretions in their lungs that they are unable to clear which results in frequent and chronic airway infections. Fortunately, the prognosis of CF has improved dramatically with the introduction of antibiotics.

One of the principal pathogens causing acute pulmonary exacerbations is *Pseudomonas aeruginosa*. (Tunney *et al.*, 2008). Thus, during the first few weeks of this summer placement selected *P. aeruginosa* isolates, cultured from sputum samples from CF patients before and after treatment of an acute exacerbation, were tested for their susceptibility to ceftazidime, meropenem, tobramycin and

piperacillin/tazobactam. These antimicrobial agents are commonly used in the management of pulmonary infection in CF. Therefore, the aim of this initial investigation was to establish if the susceptibility of the bacteria to these antibiotics differed before and after an acute exacerbation. The susceptibility was determined using E-test strips (Bio-Stat, Stockport, UK) according to the manufacturer's instructions. The desired isolate was cultured on MHA (Mueller-Hinton Agar) at 37°C for 24 hours and then the minimum inhibitory concentrations (MICs) were read. The findings of this study were that, in general, the susceptibility of each isolate to the various antimicrobial agents did not change. For example, those *P. aeruginosa* isolates which were initially susceptible to the antibiotics also remained susceptible after an acute exacerbation.

The second aim of the placement was to identify aerobic isolates cultured from CF sputum. Genomic DNA was first extracted from the isolates and screened for *P. aeruginosa* using the polymerase chain reaction (PCR). To identify an unknown isolate as *P. aeruginosa*, one of the published gene sequences, OprL, was targeted for PCR amplification using a previously described method (Xu *et al.*, 2004). The PCR products were then visualised by gel electrophoresis. Subsequently, PCR amplification of the 16S ribosomal RNA gene was performed for those isolates which were negative for the OprL gene (LiPuma *et al.*, 1999). The PCR amplicons were then sequenced with nucleotide sequences compared with previously published sequences using a basic local alignment sequence tool (BLAST).

Surprisingly, after plating out one of the selected isolates on agar, two different colonies could clearly be viewed indicating that two potential pathogens were growing on the solid medium. One colony of each variant was selected separately, streaked out on fresh agar and incubated aerobically before PCR amplification of the OprL gene. Results revealed that the first colony was positive for *P. aeruginosa* whilst the second colony was negative. These colonies were different in appearance to those previously inspected and a Gram stain also revealed that the microorganisms cultured were small Gram-negative rods.

Morphological and Gram stain appearance suggested that this isolate may have been *Haemophilus influenzae*. This bacterium possesses complicated nutritional requirements and, therefore, to optimise growth the isolate was cultured on supplemented Brucella blood agar in 5% CO<sub>2</sub>. The growth of this suspected *H. influenzae* isolate resulted in the project taking a new direction. To confirm the identity of this isolate as *H. influenzae*, PCR was required. However, there was no established protocol for identifying *H. influenzae* using PCR amplification. Therefore, during the latter half of my placement the aim was to develop a specific PCR procedure to detect this pathogen.

Firstly, oligonucleotide primers were designed by Dr. Deirdre Gilpin, which were complementary to unique sequences next to the gene segment (gyrase B gene) to be amplified. The predicted amplicon size was 225bp which is ideal. In order to ensure specificity, the conditions for the PCR reaction were determined by trial and error. Two variables which can easily be altered are the annealing temperature and the concentration of magnesium chloride (MgCl<sub>2</sub>) present in the mastermix. The MgCl<sub>2</sub> concentration was increased in increments of 0.5mM from 2.0 to 5.0mM. Low annealing temperatures of 50°C and 55°C were also investigated. A bright band was observed on the agarose gel when 2mM MgCl<sub>2</sub> was present in the mastermix and the PCR reaction was carried out at 55°C. Hence, these proved to be the optimum reaction conditions. Furthermore, to ensure that the PCR method only amplified the gyrase B gene in *H. influenzae*, other isolates (*Escherichia coli*, *P. aeruginosa* and *Staphylococcus aureus*) were screened with no bands apparent for any of these isolates.

In addition to aerobic bacteria, anaerobic bacteria such as *Prevotella* and *Actinomyces* species, have been isolated from the sputum samples of CF patients (Tunney *et al.*, 2008). Hence, several selected anaerobic isolates were cultured under strict anaerobic conditions. As previously described, the susceptibility of these isolates to various antimicrobial agents (clindamycin, meropenem, metronidazole and piperacillin/tazobactam) were determined using E-test strips.



Resistance was apparent to both clindamycin and metronidazole.

Towards the end of the placement, ten anaerobic isolates were selected at random and cultured on milk agar. It was proposed that if the isolate produced proteases, which contribute to the poor lung function in the CF patient, then these enzymes would degrade the protein present in the milk. After incubation at 37°C for one week in the anaerobic cabinet, white colonies could be observed on 10/10 plates. However, the nutrient medium for 2/10 isolates had turned completely translucent whereas the remaining 8/10 remained opaque. Therefore, those isolates, whose growth resulted in the medium undergoing the transition from opaque to translucent, were believed to produce proteases.

This placement introduced me to various microbiological techniques such as culturing bacteria under aseptic conditions and also molecular methods including PCR. I have also gained invaluable experience of working in a laboratory and how to organise my time appropriately to ensure that I work as efficiently as possible. This has allowed me to gain confidence to work on my own in unfamiliar situations and shown me how challenging and rewarding a career in research could be.

I would like to thank my supervisor, Dr. Michael Tunney for giving me the opportunity to study this project, as well as all the staff of the School of Pharmacy, in particular Dr. Deirdre Gilpin, for their assistance, guidance and patience during my summer placement. Finally I would like to thank SfAM for making this placement possible.

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#### Laura Gillanders

School of Pharmacy, Queen's University Belfast



## Evaluating and improving the performance of footbath product for preventing bacterial growth on the feet of farmyard animals

**For eight weeks I have been** working in collaboration with Provita — a company in Northern Ireland specialising in the manufacture of animal healthcare products. Nearing completion of year three of a four year MPharm course I applied for a grant in order to enable me to gain experience of laboratory research. I felt that the project would give me an insight into the work that may be involved in a PhD and industrial pharmacy and would be of great help in undertaking my final year project at university.

The focus of the project was to investigate the antibacterial properties of a footbath preparation and to compare it with other available products.

Footbath preparations are used in farmyard footbaths primarily for dairy cattle to prevent the growth of bacteria such as *Escherichia coli* on the feet of animals. Damp conditions and slurry promote this growth and footbathing is the choice action to controlling infection. If the animal is not managed correctly and incurs large amounts of bacterial growth around the hooves, it will develop painful sores and may

become lame. This can predispose cattle to other diseases thus lowering production and fertility at the expense of the dairy farm business. The cost of lameness is estimated by Esslemont and Kossaibi to be in the region of £178 per affected cow.

Lameness continues to be a serious problem on many farms despite the fact that it can be relatively easily prevented through careful consideration of animal husbandry factors, such as nutrition and environmental conditions. Despite advances there has been little improvement in lameness over past years. It is clear to see why footbath products are important in agriculture not only to maintain profit and good supply but more importantly to safeguard animal welfare.

I began my investigations by establishing the minimum inhibitory concentration (MIC) for a range of antibacterial footbath products. Various concentrations of the market dilution were prepared. *E.coli* was cultured overnight and centrifuged to a pellet followed by dilution to optical density 0.3 followed by further dilution for addition to the test tubes. A sample of dung was collected and autoclaved. It was then added to the tubes in suitable portions to mimic environmental conditions. After running the experiment, test solutions were plated and incubated overnight to observe any bacterial growth. The desired outcome was to discover the lowest concentration of antibacterial required to inhibit bacterial growth.

The rate of kill was later investigated. The products were prepared to market dilution and two repeats run for each. Recordings were taken at five different time points in clean and dirty conditions. This determined the time taken for a given footbath product to achieve a bacterial load of zero.

A formulation study was also carried out by isolating constituents of a given footbath preparation and investigating their rate of kill individually. Different concentrations were investigated in order to seek the optimum formulation. My overall experience of the Students into Work scheme has been very positive. It was flexible and enjoyable and has taught me a volume of skills, both specialist skills such as operational techniques and experimental procedures and general skills such as organisation, team work and time management.

As a result, my confidence in the laboratory has greatly improved.

An added benefit to this particular project is the involvement it has presented with an industrial company. This has provided me with experience in a field of pharmacy that not many students get the chance to obtain.

I would definitely recommend a summer research post to anyone with a keen interest in microbiology or those considering a PhD or seeking an alternative to community pharmacy. I believe it best to explore all possible options before choosing a career pathway and a research project will certainly help with those decisions, not to mention make a great addition to your CV.

Finally I wish to express my gratitude toward all those involved in the project: Cecilia Brennan and Dr Teresa Allen, both representing Provita, who supervised and guided my work, Dr Brendan Gilmore and Professor David Jones of Queen's University Belfast who oversaw and directed the project and the funding body of SfAM who supplied the grant for the eight weeks.

Jessica Edwards

## President's Fund reports

### information

#### am I eligible - can I apply?

If you often find difficulty in funding attendance at meetings.

It is not only our student members who require our help. Senior microbiologists often find difficulty in funding attendance at meetings. If you are in this position you are eligible for this fund.

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## Characterisation of actinomycetes from Helmcken Falls volcanic cave and their antimicrobial activity against the honey bee pathogen *Paenibacillus larvae*

**American foulbrood disease (AFB)** is one of the most important bacterial diseases of honey bees around the world (Neuendorf *et al.*, 2004). The cost in lost income, due to destruction of bees and equipment associated with infected hives and the possibility of antibiotic residues left in the hives is estimated in millions of dollars (Clay 2000). The responsible agent is the Gram-positive, spore-forming bacterium, *Paenibacillus larvae* (Allipi *et al.*, 2002). This pathogen causes infection of the gut of honeybees larvae (*Apis mellifera* L.) leading to death in the larval stage. Death occurs when the bacterium breaks down the epithelium of the mid-gut region of the gastrointestinal tract. The bacterial spores are resistant to a variety of adverse environmental factors such as heat, cold, desiccation and chemical disinfectants and may remain viable for 35 years or more (Chantawannakul & Dancer, 2001). In Canada, McRory (2004) reported that 7% of 719 Ontario bee yards showed signs of AFB infection. Currently, oxytetracycline (OTC) is the only approved antibiotic for treatment of AFB worldwide. However, in recent years, OTC resistant strains have emerged in the United States of America, Canada and Argentina (Miyagi *et al.*, 2000).

It has been shown that actinomycetes, filamentous Gram-positive bacteria with a high G+C content, are a prolific source of new bioactive compounds (Lazzarini *et al.*, 2000). Approximately two-thirds of naturally occurring antimicrobial products are produced from actinomycetes (Okami & Hotta, 1988). Large numbers of novel actinomycetes were reported to be present in unexplored habitats worldwide including cave environments (Groth & Saiz-Jimenez, 1999; Jones, 2001; Northup & Lavoie, 2001). Several novel actinomycetes were isolated from caves



as exemplified by the description of *Beutenbergia cavernae*, a new genus of L-lysine-containing actinomycete (Groth *et al.*, 1999) and *Agromyces subbeticus* being isolated from a cave in southern Spain (Jurado *et al.*, 2005).

The significance of microbial diversity in the search and discovery of novel metabolites has been underlined in a recent article (Keller & Zengler, 2004). Consequently, the main focus of this study was to search in previously uncharacterised habitats to uncover novel actinomycetes that may produce antimicrobial agents against *P. larvae*, the causative agent of AFB. A volcanic cave habitat was chosen as a potential source of antimicrobial producing actinomycete strains because of their unique characteristics and the lack of study in such habitats that may lead to higher probability of finding such novel strains.

This report represented some preliminary work on the isolation of cave actinomycetes and screening for antimicrobial agent(s) that is a part of a larger study on microbial diversity in caves of Canada.

One hundred and nineteen strains of actinomycetes were isolated by dilution plating from sixteen soil samples collected from Helmcken Falls, a volcanic cave in Wells Gray Provincial Park, British Columbia and screened for their antimicrobial activity against *P. larvae* using a paper disc diffusion method. The antimicrobial activity was carried out in three fermentation broths namely YMG, AMHU-4, and V8 juice, at 28°C. Among these, strain E9 grown in V-8 juice medium showed significant antibacterial activity against *P. larvae*, and produced a zone of inhibition equivalent to that of a 30µg tetracycline control disc. E9 began to produce antimicrobial activity against *P. larvae* after 2 days of incubation and continued up to day 8. The antimicrobial activity peaked on day 4 of incubation with an inhibitory zone of about 10mm. During fermentation, the pH of the media varied from 7 to 9 with an optimal antimicrobial activity at pH 7. The growth pattern of E9 during antimicrobial agent(s) production was observed and presented as the average percentage of packed cell volume (%PCV). Strain E9 showed a typical bacterial growth curve during the 10-day fermentation in V8 juice medium. This information could be very useful

for both optimisation studies and large-scale production of antimicrobial agent(s).

Strain E9 was identified using a combination of genotypic and phenotypic characterisations. Strain E9 morphology and chemotaxonomic markers matched with those of *Streptomyces* species. Phylogenetic analysis based on almost complete 16S rRNA gene sequence revealed that strain E9 is a member of the genus *Streptomyces* and closely related to

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*Staphylococcus aureus*. In addition, strain E9 showed distinct differences to other validly described *Streptomyces* species, there is a possibility that it may represent a new species. However, this requires further study, in particular DNA:DNA hybridisation to establish its taxonomic status.

This study suggests a high possibility of finding new antimicrobial agent(s) from less-studied actinomycetes in volcanic cave habitats. There is no previous record of any antimicrobial production in *S. aureus*. This is the first report on antimicrobial activity against *P. larvae* from a volcanic cave actinomycete isolated in Canada.

This article is based on a poster presentation made at the 14th International Symposium on Biology of Actinomycetes in Newcastle upon Tyne, UK in August 2007. I would like to thank SfAM for giving me the opportunity to attend and present this work at this meeting.

#### Wasu Pathom-aree

## Essential oils for improved skin antiseptics: the way forward?

Advances in modern biomaterial science have led to an increase in the diversity, availability and therefore use of a variety of medical devices, for example intravascular and peripheral catheters. Hospitalisation, especially in in-patients receiving intense management, is often accompanied by the use of such devices; however surgical insertion carries a risk of infection as the barrier properties of the skin become breached. Infections associated with intravascular catheters can vary in severity from local infections around the site of insertion, to systemic infections of the bloodstream, which may in turn lead to further complications (Worthington & Elliott, 2005). They are thus associated with significant patient morbidity and mortality, as well as increasing costs to the health service. The main bacteria responsible for causing intravascular device related infections are the skin microorganisms, in particular *Staphylococcus epidermidis*, although

*Staphylococcus aureus*, Gram-negative bacilli and *Candida* species are also often associated with infection. Many of these microorganisms are capable of producing biofilms that allow them to become irreversibly attached to surfaces such as catheters, as well as protecting the microorganism, reducing their susceptibility to the effects of antimicrobial agents and host defence mechanisms. Prevention of infection associated with surgical incision of the skin is governed by adequate skin antisepsis prior to breach.

Several UK hospitals use 0.5% (w/v) chlorhexidine in 70% (v/v) alcohol for skin antisepsis, however, recent recommendations in the Evaluation of Processes and Indicators in Infection Control (EPIC) guidelines list that 2% chlorhexidine gluconate in 70% isopropyl alcohol (IPA) should be used (Pratt *et al.*, 2007). Chlorhexidine is one of the most widely used antimicrobials within clinical practice, yet its efficacy is significantly reduced by pH and organic matter (McDonnell & Russell, 1999). For these reasons, along with the increasing prevalence of resistant microorganisms, it is becoming more important to investigate novel strategies for improving skin antisepsis.

Many antimicrobial agents have been tested for use as preventative measures against bacteria. These include a range of chemicals such as aqueous chlorhexidine, as well as many complementary and alternative medicines including essential oils, which are regarded as mixtures of compounds that produce a flavour or aroma, and are from natural sources such as flowers, spices and aromatic herbs. Phenolic compounds are responsible for most of the antimicrobial activity of essential oils derived from herbs and spices, but secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids found in plants also possess antimicrobial properties.

The medicinal properties of tea tree oil were first reported in the 1920s in a series of papers published by Penfold, however, Aborigines were the first people documented to have benefited from the plant, by using crushed leaves to heal wounds and making broths with soaked leaves to heal sore throats. Like many other natural remedies, the use and popularity of tea tree oil has increased over recent years and is sometimes used in low concentrations to

treat insect bites and cuts. The oil is well reported to have many assets including broad-spectrum antibacterial, antifungal, antiviral, and anti-inflammatory properties and could therefore be beneficial in the treatment of a wide range of infections. There are a number of compounds within tea tree oil that possess antibacterial activity including  $\alpha$ -pinene,  $\beta$ -pinene and linalool, but the principal antibacterial constituent is terpinen-4-ol. The active components of tea tree oil cause loss of membrane function and integrity. Some bacteria lose intracellular material; some lose the ability to maintain homeostasis while others are inhibited from respiring.

Studies into the clinical efficacy of tea tree oil have shown it to be effective in the treatment of acne as well as the eradication of MRSA carriage when given as a nasal ointment (Bassett *et al.*, 1990; Anderson & Fennessy, 2000). At present, it is used as an antiseptic in a range of skincare products and some cosmetics, in concentrations of between 2 and 5%, and interest is being shown into the possibility of adding it to hand-wash solutions within clinical and care settings.

Recent research at Aston University, UK, has confirmed the antimicrobial activity of a wide range of essential oils that may potentially be incorporated into a skin antisepsis regime, in particular eucalyptus oil (Karpanen *et al.*, 2006). Eucalyptus oil is also plant-derived and demonstrates antimicrobial properties when applied directly to the skin as a balm, used in mouthwashes or inhaled as a spray. Around 70% of the oil is eucalyptol (or cineole) and about 14% is  $\alpha$ -pinene, though the amount of oil and its contents varies between eucalyptus species. Eucalyptus oil contains volatile components such as alcohols, ketones and aldehydes, and like tea tree oil, eucalyptus contains monoterpene hydrocarbons and terpenoids of which the main constituents are terpinen-4-ol,  $\alpha$ -terpineol and 1,8-cineole. Research at Aston has demonstrated that while eucalyptus oil alone possesses excellent antimicrobial activity, it exhibits synergistic activity against biofilms of *S. epidermidis* when in combination with 2% chlorhexidine digluconate. Furthermore, the lipophilic nature of eucalyptus has been known to facilitate the permeation of chlorhexidine into the

deeper layers of the skin, where many microorganisms exist as biofilm-like micro-colonies, surrounded by sebaceous secretions.

The antimicrobial properties of many other oils are also currently being investigated and include cinnamon, lemon and rosemary oils. However, there are at present limitations that need to be overcome before essential oils can be routinely used in skin antisepsis. Research is needed into the safety of oils as both tea tree and eucalyptus oils are known to be toxic if ingested and can cause irritation and occasional allergic reactions when applied to the skin, although these depend in part upon the concentration used and sensitivity of the person exposed to the oil. Also, the quality of the final product would need to be assessed as quantities of antimicrobial compounds within all essential oils may vary with season or strain of plant. When this is combined with the knowledge that essential oils are volatile and have poor solubility, common use of them in the healthcare profession will require further research to enable restrictions in clinical application to be overcome.

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Emma Hendry



## Control of *Aspergillus flavus* in corn using natural materials

I would like to thank SfAM for awarding me a President's Fund grant to attend the 9th European Conference on Fungal Genetics held in Edinburgh, UK on 5-8 April 2008.

I presented my paper on the organisms that produce the mycotoxin, aflatoxin, especially *Aspergillus flavus*. This can infect important food and feed crops, including corn, before, during and after harvest. These fungi are normal soil-borne inhabitants in our environment, growing on both living and decaying plant matter. Mycotoxins include metabolic by-products produced by a number of different fungi.

The importance of this study comes from the prevalence of *Aspergillus flavus* producing aflatoxin in corn, wheat and barley crops. It is possible to control contamination by preventing the presence of the fungus (*Aspergillus flavus*) and removing or affecting its capability to produce the toxin. This could be done by using natural materials and may lead to the production of toxin-free food and feed and increased safety from a health point of view whilst preserving grain food wealth.

I studied the control of *Aspergillus flavus* using natural materials namely, the essential oil marjoram (*Origanum majorana* L.) and salt (sodium chloride) and their effect on fungal growth and the quantity of aflatoxin production.

### Effect of different concentrations of marjoram (*Origanum majorana* L.) essential oil and salt (sodium chloride) on *Aspergillus flavus*:

One millilitre of *Aspergillus flavus* containing  $5.0 \times 10^4$  cfu/ml was added to 100 grams of corn (sterilized in a 250 ml conical flask) and distributed without the addition of marjoram or salt (control sample). Also, one millilitre of the same *Aspergillus flavus* with the addition of different volumes of marjoram essential oil (0.05, 0.1, 0.2, 0.3, 0.5 and 0.7 ml) and one ml of the same *Aspergillus flavus* with the addition of different weight of salt (0.2, 0.4, 0.6, 1.0, 1.5 and 2.0 grams) were each added to 100 grams of sterilized corn (placed in a 250 ml sterilized conical flask) and mixed.

Sterilised distilled water was added to raise the moisture content to 18% and the mixture was shaken to make it homogenous. These were stored at 25°C for 21 days. Sampling to determine *Aspergillus flavus* count and aflatoxin concentration was made at intervals of 7 days until 21 days.

Adding 0.05 ml of marjoram essential oil did not stop the growth of *Aspergillus flavus* during storage and up to 21 days, in inoculated corn. At the beginning of storage *Aspergillus flavus* count was  $4.0 \times 10^2$  cfu/g and gradually increased on day 21 to reach  $1.5 \times 10^3$  cfu/g.

The corresponding values in the control sample were  $4 \times 10^2$  *Aspergillus flavus* count and 0 ppb aflatoxin production at the beginning of storage and  $3.2 \times 10^3$  *Aspergillus flavus* and 150 ppb aflatoxin production at the end of storage (21 days). This indicates that the addition of 0.05 ml marjoram caused a reduction of *Aspergillus flavus* count of 62.5% at 21 days of storage. Raising the level of marjoram to 0.1 ml inhibited the growth of *Aspergillus flavus* and aflatoxin production completely at 7 days until the end of storage. These results are in agreement with those of Paster *et al.*, (1995) who reported that the essential oil of marjoram has been shown to inhibit mycelial growth and aflatoxin production in *Aspergillus parasites* and *Aspergillus flavus* — both fungi which grow on stored grains. This observation was noted clearly with other higher marjoram levels.

It can be observed from the effect of different salt concentrations on *A. flavus* count during storage of inoculated corn up to 21 days that the control sample gave a gradual increase in *A. flavus* number during storage from 0 to 21 days. It increased from  $6 \times 10^2$  cfu/g to  $5 \times 10^3$  cfu/g at 0 and 21 days storage period respectively. The corresponding values, in inoculated corn sample treated with 0.2 grams of salt were  $6 \times 10^2$  cfu/g to  $9 \times 10^2$  cfu/g at the same periods of storage. Although the salt concentration (0.2 gram) was quite low it still produced a slight effect towards reducing the rate of growth of *A. flavus* during storage. This trend increased during storage, and was more obvious when 0.4 grams of salt was added. At this concentration the number of *A. flavus* at 0, 7, 14 and 21 days storage period became  $6 \times 10^2$ ,  $6.3 \times 10^2$ ,  $6.6 \times 10^2$  and  $7 \times 10^2$  cfu/g, respectively. At

0.6% salt concentration growth inhibition reached 83.3% and aflatoxin production reached 50 ppb between 0 and 21 days while the control sample reached 180 ppb during the same period. Hence this concentration is not suitable for controlling *A. flavus* growth during storage.

Raising salt concentration to 1 gram caused a complete inhibition of *A. flavus* growth and aflatoxin production during the whole experimented storage periods (7, 14, and 21 days). The same observation was noted when 1.5 and 2 grams of salt were used. Accordingly the minimum inhibitory concentration to prevent *A. flavus* growth in corn is 1 gram salt/100 gram corn and can be successfully used in the storage of corn up to 21 days. It is also suitable to prevent aflatoxin production. Moreover salt is traditionally used as a food, feed ingredient and food preservative, so the hazard would appear negligible.

It is possible to conclude that using marjoram at concentration of 0.1 ml and above per 100g corn at 18% moisture inhibited the growth of *Aspergillus flavus* completely at 7 days and up to the end of storage also prevented aflatoxin production.

Also it can be concluded that the minimum inhibitory concentration to prevent *A. flavus* growth in corn is 1 gram salt/100 gram corn. This concentration can be successfully used in the storage of corn up to 21 days. It is also suitable to prevent aflatoxin production.

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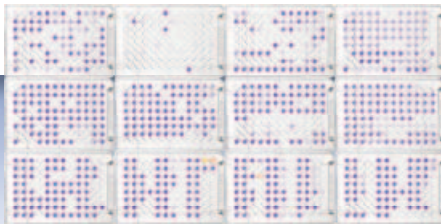
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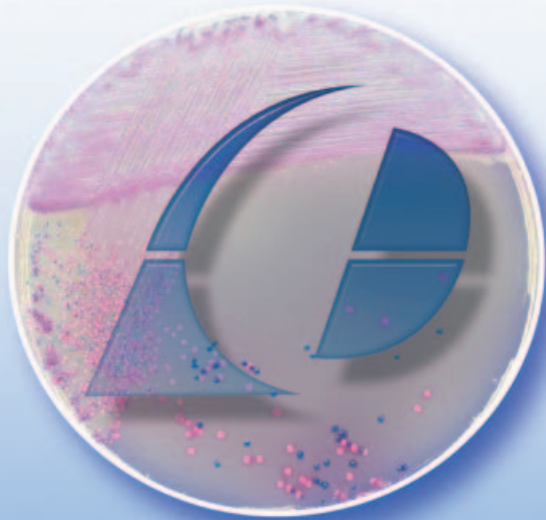


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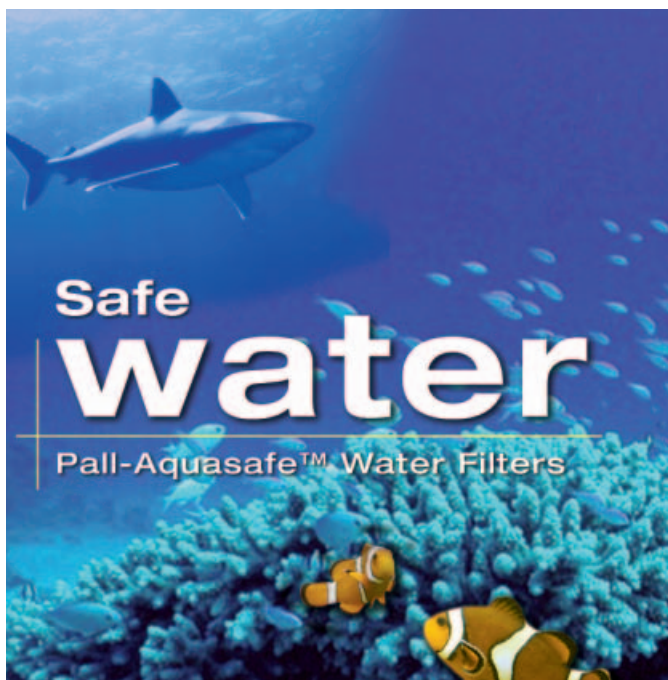
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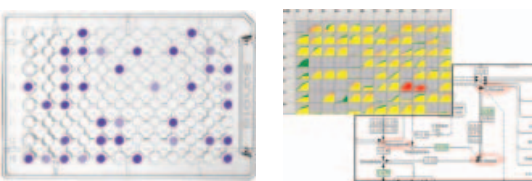


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*Methods* (Review 1). Using simple tables for ease of cross-referencing, it lists over 400 kits from around 50 kit manufacturers. It covers pathogens of interest to the agri-food chain, spoilage organisms (e.g. yeasts) and hygiene testing. In addition to the name and principle for each method, it gives the time taken for the test, the name of the manufacturer along with contact details, comments on validation status of the method, and references for further reading.

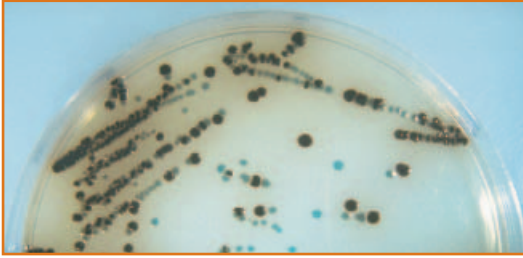
As well as these methods publications, *A code of practice for microbiology laboratories handling food, drink and associated samples* (Guideline No. 9), now in its third edition, will help food microbiologists to run their laboratories safely and efficiently and help ensure that they generate results that are valid and meaningful.

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## Fungal Toxin Mystery Solved Using Biolog's Phenotype MicroArray™ Technology

An important breakthrough in fungal toxin biology has been made possible through the use of Biolog's Phenotype MicroArray technology. This major advance is described in two recent publications from a group at CSIRO in Queensland, Australia. The work by Donald Gardiner and his collaborators has recently been published in online editions of the journals *Fungal Genetics and Biology* and *Microbiology*.

The fungus *Fusarium graminearum* is a major pathogen of wheat. It is the causative agent of head blight and results in substantial worldwide crop losses. Central to the infection process, the fungus produces a trichothecene toxin called

deoxynivalenol. Although the toxin is produced at high levels during wheat infection, researchers have never been able to induce *Fusarium* to produce significant levels under laboratory culture conditions. This inability has hampered study of head blight disease and approaches to disease prevention.

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The GLP compliant ProtoCOL 2 software can be integrated into a 21 CFR Part 11 environment and is modular so users can cost-effectively customise their system to include the colony counting or zone sizing analysis programmes which suit them.

### Further information

Visit: [synbiosis.com](http://www.synbiosis.com)

Tel: +44 (0)1223 727125

Email: [sales@synbiosis.com](mailto:sales@synbiosis.com)

## Microbiology at the Speed of Light. Neogen's rapid Soleris test for total yeast and mould receives AOAC approval

Neogen Europe has received approval from the AOAC Research Institute for its rapid and accurate test for yeast and mould.

Neogen's newly approved Soleris™ test (AOAC-RI No. 040901) detects yeast and mould in 72 hours or less—conventional methods can take up to 5 days. Neogen's Soleris technology is now used by approximately 400 of the world's largest food, nutraceutical, and personal care product manufacturers to detect indicator and spoilage microbes, including yeast and mould, in a fraction of the time needed for traditional methods.

The Soleris system is a rapid optical method for the detection of microbial contamination based on an innovative application of classic microbiology. The optical assay measures microbial growth by

monitoring gas production, pH, and other biochemical reactions that generate a colour change as microorganisms grow and metabolise. In addition to the yeast and mould test, Soleris tests provide:

- Total viable count (TVC) results in as little as 6-8 hours
- Coliform results in 9-10 hours
- *E. coli* results in 7-10 hours
- Lactic bacteria results in 30-35 hours

### Further Information

Visit: [www.neogen.com](http://www.neogen.com)

Tel: +44 (0) 1292 525275

Email: [info@neogeneurope.com](mailto:info@neogeneurope.com)



## Pharmaceutical Microbiology Seminar

Technopath will be holding a free to attend, one day Microbiology Seminar in Birmingham, UK, on Thursday the 8th of October 2009.

The comprehensive program will be given by end users and experts in their respective fields.

Covering a broad range of topics and issues affecting the QC microbiology lab there is sure to be something of interest to most QC microbiologists.

- Topics include:
  - formulating and validating a cleaning and disinfection program, a user evaluation of the new environmental monitoring application & real time sterility test using the ChemScan, end user evaluations of the new Biolog identification system, phenotype or genotype identification — what's best for you, maintaining an in-house culture collection, and helping to meet the auditors requirements with training and competency.

### further information

visit: [www.techno-path.com](http://www.techno-path.com)

Tel: +44 (0)283 0833 808

Email: [info@techno-path.com](mailto:info@techno-path.com)

## information

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

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

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