microbiologist

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The magazine of the
Society for
Applied Microbiology

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Fermented foods

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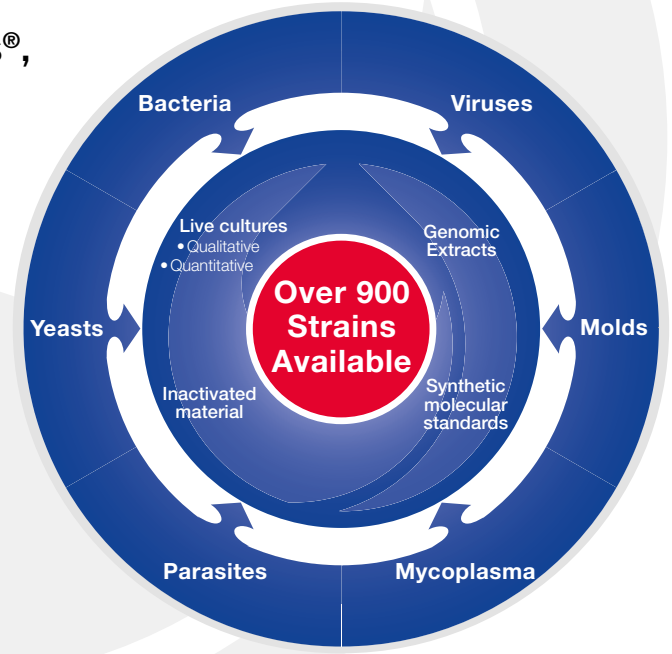

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Dr Paul Sainsbury reviews the content of this issue

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Current applications of tools and technologies

As I join *Microbiologist*, the magazine has never been in a stronger position due to the combined efforts of a strong Editorial Board with a range of specialist expertise in all areas of applied microbiology. I am especially delighted to be taking over the helm as Editor from Nancy Mendoza who has done a great job in the last two years of updating *Microbiologist* and reaching out to our readership.

Continuity will remain the theme of my Editorial and we shall continue to provide the articles you ask for across the many topics of applied microbiology. The first two features on NGS and MALDI-TOF reflect this as Lori Snyder from Kingston University and Haroun Shaw from Public Health England show us how some of the most widely used technologies in microbiology are currently being applied.

It will soon be time for the Society for Applied Microbiology's Summer Conference, which this year will be held at the Intercontinental Dublin and we hope to see many of you there. The theme this year is '*Fermented Foods and Beverages*' and this issue of *Microbiologist* includes a number of articles on the subject to trailblaze the conference.

Staying on the topic of food, World Health Day was celebrated on 7 April, with the World Health Organisation highlighting the challenges and opportunities associated with food safety. This indicated the start of a number of initiatives to increase access to adequate, safe, nutritious food for everyone, and will culminate in the release of a detailed report on the global burden of foodborne diseases expected in October 2015.

Campylobacter jejuni, the main culprit and biggest cause of food poisoning remains omnipresent in the popular news with a report by the UK's FSA indicating a much higher rate of contamination than previously thought of supermarket chickens – up to 70%. A recent paper in our own *Journal of Applied Microbiology* highlighted that almost 99% of cases of *C. jejuni* infection can be prevented using disinfectant wipes on surfaces, reminding us the importance of thorough cooking and that it may be time we ditched the popular notion of a reusable dishcloth.

NEWS IN BRIEF

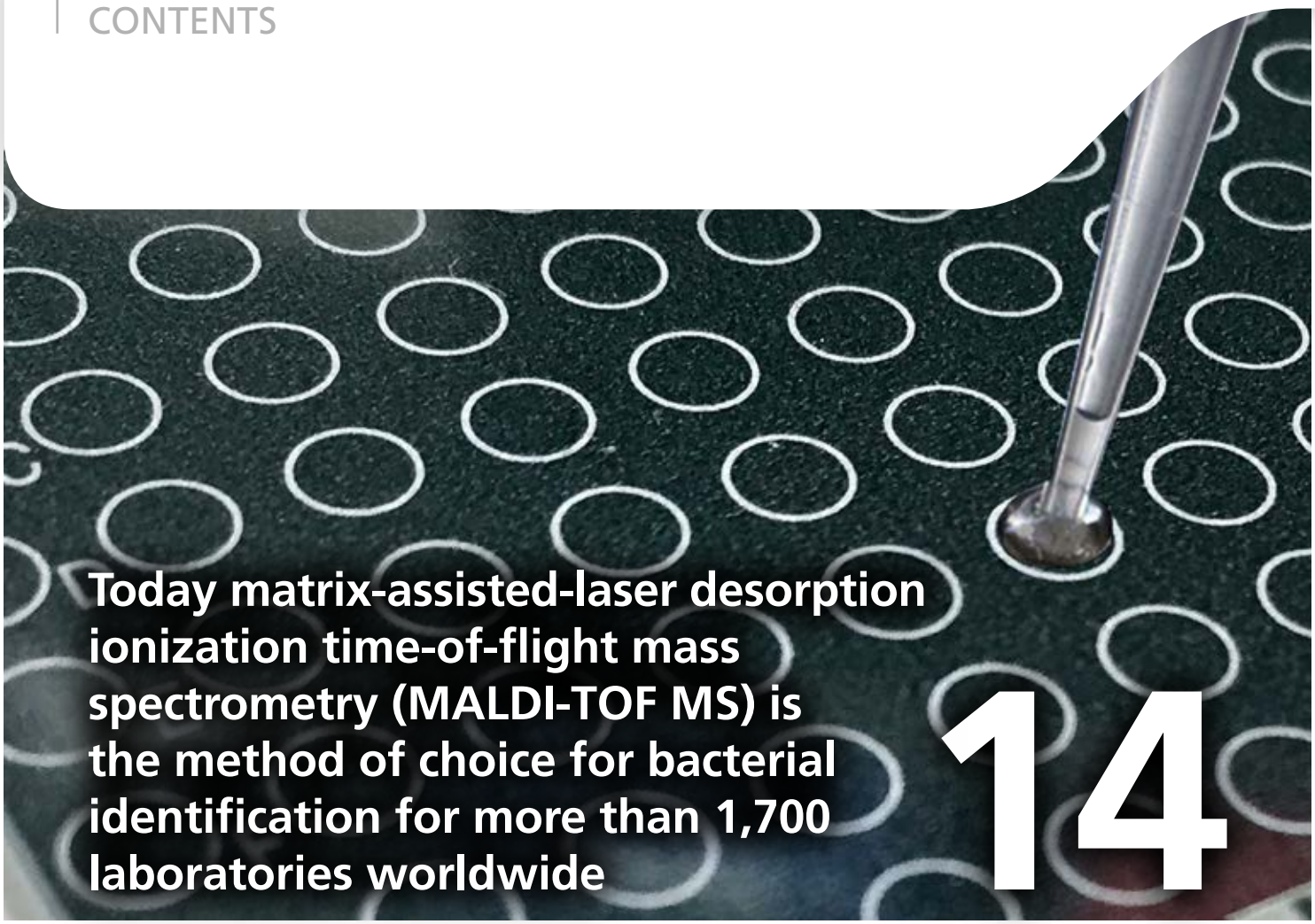
Stay healthy!
World Health Day 2015: From farm to plate, make food safe.
http://bit.ly/1IfMGQ6

Cook the chicken
More than 70% of fresh chickens being sold in the UK are contaminated with *Campylobacter*, the Food Standards Agency (FSA) has revealed.
http://bbc.in/1FP69oN

Ditch the dishcloth
Use wipes in the kitchen to reduce the risk of food poisoning by 99%.
http://bit.ly/1GYK068

Artemisinin-resistant malaria in Africa
Finally, worrying news of parasite resistance to Artemisinin – the most effective malaria drug we have.
http://bbc.in/1KOoL8k

Dr Paul Sainsbury, *Editor*



Today matrix-assisted-laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is the method of choice for bacterial identification for more than 1,700 laboratories worldwide

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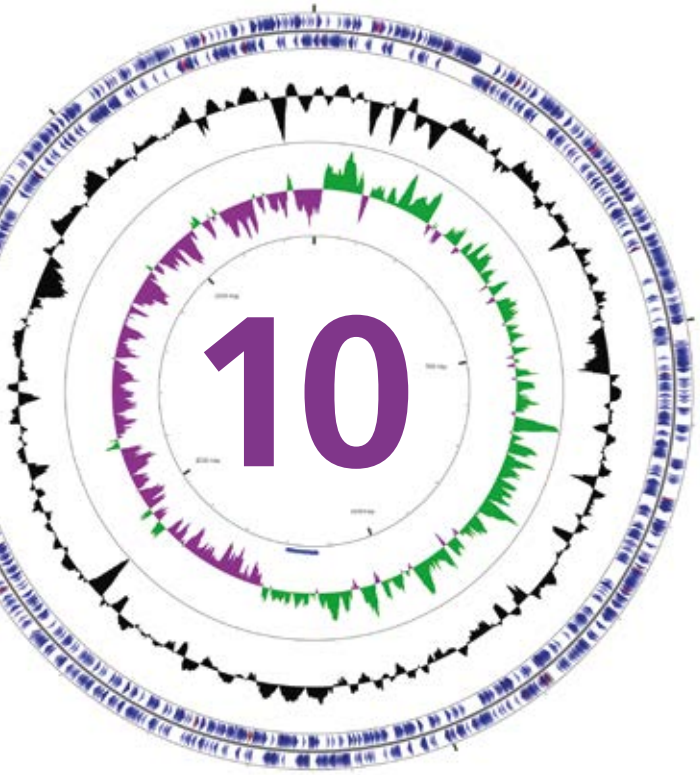
FEATURES



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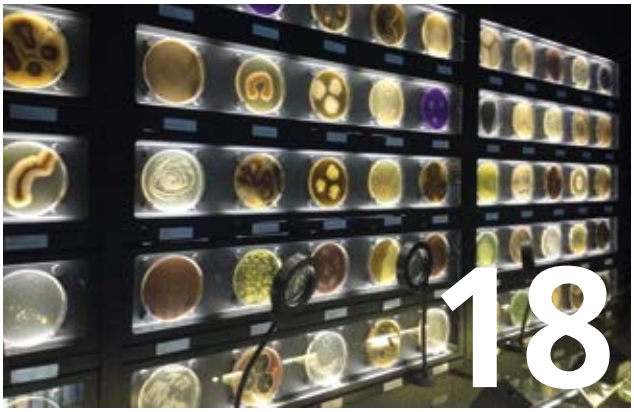

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President's column

Looking at titles to set for a student dissertation recently I realized it was 10 years ago that I first choose the title *Bacterial probiotics – benefit or con?* At the time, the work done on gastrointestinal (GI) microbial composition was mostly based on carrying out bacterial counts of some key indicator GI bacterial groups: coliforms, lactobacilli, bifidobacteria. Other GI groups were not really considered as they were difficult/impossible to culture routinely and were only being looked at in a few specialist laboratories. What a difference a decade makes! Now such studies are more likely to be based on looking at the diversity changes of the whole GI microbiome. Analysis of the uncultured microbiota using next generation sequencing (NGS) analysis that requires a high level of sophisticated bioinformatics is now becoming routine. This is a classic example of a new technique producing a step-change in the way we can study microorganisms and revolutionizing our understanding of their associated ecosystems.

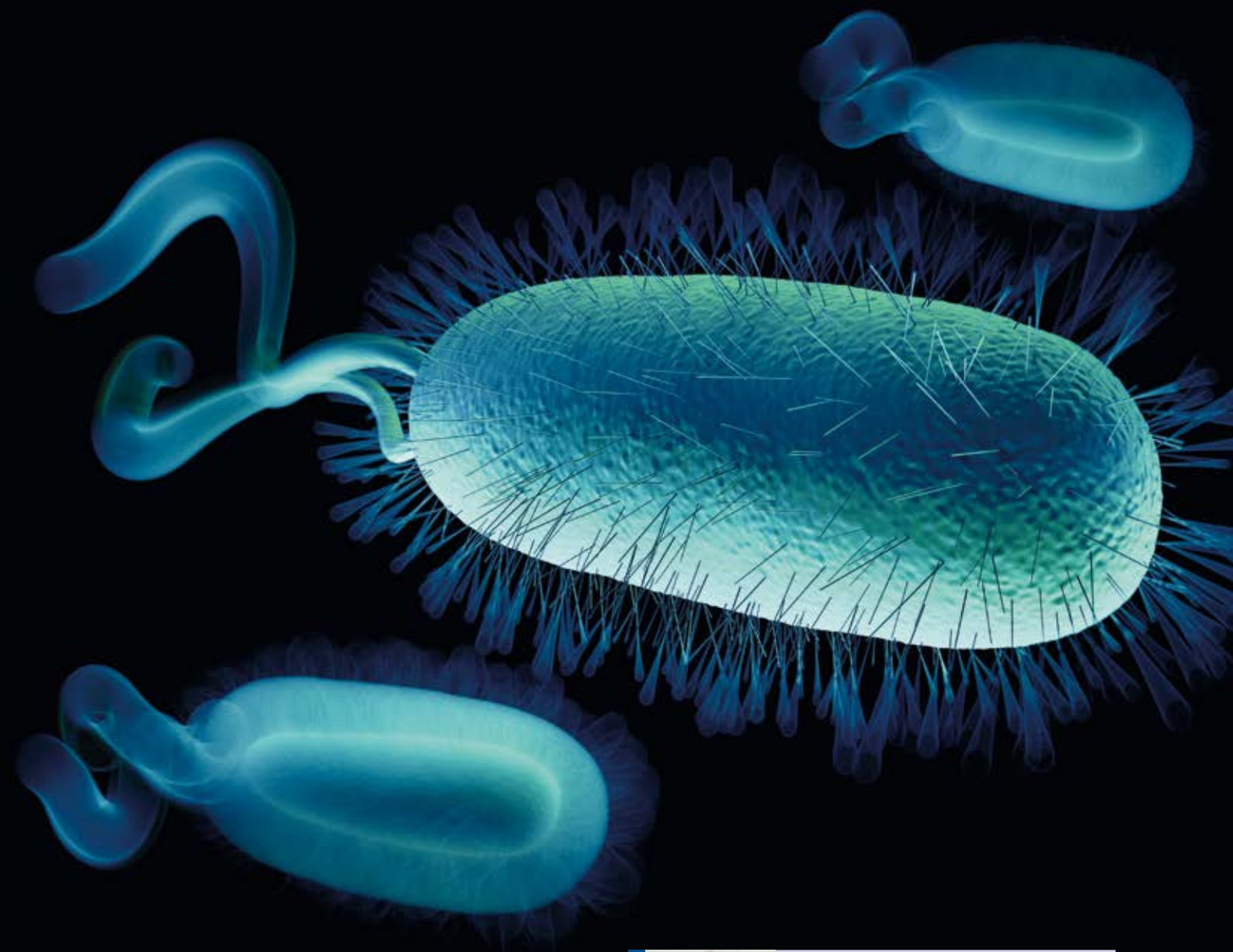
Whilst NGS has been used to examine a whole range of microbial ecosystems to understand their diversity and function (and those of you attending the Summer Conference will hear more on this in the context of food fermentations), it is its application to look at the human microbiome and understanding its function that attracts a great deal of fascination and could revolutionize health treatment. Whilst 10 years ago it was recognized that our gut microflora was important, the progress in understanding its contribution to our overall health and well-being, and the causes and impact of dysbiosis are considerable and often surprising. A testament to the need for a balanced healthy flora is the advent of faecal microbiota transplantation treatments of patients whose modified intestinal microbiota compositions have led to chronic GI disease. Increasingly, the metabolic role of the intestinal microbiota in influencing other aspects of our health is also emerging. Obesity, type 2 diabetes and even neurobehaviour are all examples of medical conditions being examined in the context of gut microbiota composition: and because this in turn is influenced by our diet (amongst other factors) the old adage '*you are what you eat*' takes on a whole new significance. That microbial treatments may be the way that such conditions could be addressed in the future is truly astonishing.

Not only is it our gut microflora that is under scrutiny, but the variations in our skin, vaginal, oral and faecal communities facilitates another completely enthralling discussion. If you have a spare 17 minutes I can highly recommend you watch Rob Knight talking about '***How our microbes make us who we are***' (http://youtu.be/i-icXZ2tMRM). That our later health is influenced by the method of delivery at birth and the consequentially acquired neonatal microflora makes a captivating consideration.

Surprisingly another impact of NGS for me has been on my teaching; even five years ago I would not have considered discussing bacterial taxonomy in detail at a level above genus; there was the odd mention of family nomenclature, e.g., *Enterobacteriaceae* but class and order were esoteric ideas. This year I started teaching higher order taxonomy as without this the average NGS ecosystem paper is unapproachable. As a former taxonomist that truly is astonishing!!

A testament to the need for a balanced healthy flora is the advent of faecal microbiota transplantation treatments

Those of you attending the Summer Conference will hear more on NGS in the context of food fermentations



Christine Dodd
President of the Society

Harper's Postulates:

Notes from the Chief Executive

Applied microbiology as culture (pardon the pun)

What do your friends and family think of when they think of microbiology? I know that many consider microbes the enemy, causing untold ill health the world over. It seems that never before in recent history has microbiology been the subject of so much headline news, from the Ebola outbreak in West Africa (Members can read a blog from S*f*AM Member and Editor Louise Hill-King who is currently in Sierra Leone providing much needed laboratory support), to food poisoning cases and the reporting of disease outbreaks such as measles: a disease which is making a resurgence in some parts of the world because not enough people in a population are vaccinating their children to enable herd immunity to take effect.

It's true that microbes do cause infectious disease and that control of infection spread is essential for good health, hygiene and survival. The fact that this headline news is more often than not, negative, doesn't help to promote the incredibly wide-ranging positive side of applied microbiology.

How many of your friends and family realize that microbes are essential, that we are literally 'crawling' with bugs that cover our skin surface and mucous membranes, and that without them we wouldn't survive? When I tell people that we have 10 times more microbial cells within our bodies than human cells, their reactions vary from the mildly surprised – "*Oh, really?*" – to the visibly shocked – "*ewwww, yuck*" – the latter normally accompanied by a pained facial expression.

How many of your friends and family know how vital microbes are to the manufacture of some of life's great pleasures? I'm referring to cheese and wine in particular, but I expect you can think of countless foods (bread, yoghurt) and drinks (beer, spirits) that wouldn't exist without microbial intervention. As for the incredibly positive environmental impact microbes can have, the bioremediation of pollutants is impossible without the application of microbiology.

The SCI-ART movement has begun to blur the boundaries between art and science, promoting cross-disciplinary projects enabling artists and scientists to work together – there are some beautiful examples of microbiologists working with artists to create some visually stunning pieces of art (http://bit.ly/1IQluqa). To my mind, a great example of applied microbiology taking on a new form and becoming part of cultural life as well as scientific progress.

Finally, from a Learned Society perspective, S*f*AM practices and supports public engagement, talking about applied microbiology and engaging with new audiences to emphasize how vital applied microbiology is to our lives every day. So next time somebody you know turns up their nose when you mention microbes, it's worth reminding them that they're not all bad.

We are literally 'crawling' with bugs

Lucy Harper
S*f*AM *Chief Executive*

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Finding sunken treasure in the **genome sequencing** data flood

In the era of fast, inexpensive, high-throughput sequencing, we are flooded with vast quantities of data being generated each day. In the last few years we have seen the contents of GenBank swell and the availability of benchtop genome sequencing machines will just serve to raise the floodwaters even higher. It is quite natural to want to get above the deluge and assess the situation from higher ground. In genomics, this sort of approach has certainly yielded some important insights made possible through bioinformatics and computational analyses. Yet, there are times when diving into the data, facing the floodwaters head on, can yield sunken treasures.

Sequence duplications and chromosome rearrangements

Before the introduction of disruptive next generation sequencing (NGS) in 2006, published bacterial genome sequences were complete, fully assembled and annotated by a person. During this process, discoveries were made. TIGR were able to identify a 32 kb tandem duplication in *Neisseria meningitidis* strain MC58, which was only evident when looking at sequence coverage of the assembled genome (Figure 1). During assembly, identical regions such as this will be assembled into one contig with greater coverage of the rest of the genome, as will the multiple, near identical copies of the rRNA loci. The Sanger Institute was able to propose a never before described inversion-mediated phase variable capsule expression system in *Bacteroides fragilis*, identified when attempting to complete the final assembly of the genome. Today, with the vast majority of genome sequence data in an incomplete, permanent draft state, discoveries such as these may not be made. Currently, NGS is dominated by short-read technologies, which are best suited for human-centric sequencing applications. As a result, bacterial genome sequencing projects too frequently use technologies that are available or are popular for eukaryotic applications, rather than those that are best suited to the experimental design for their organism and

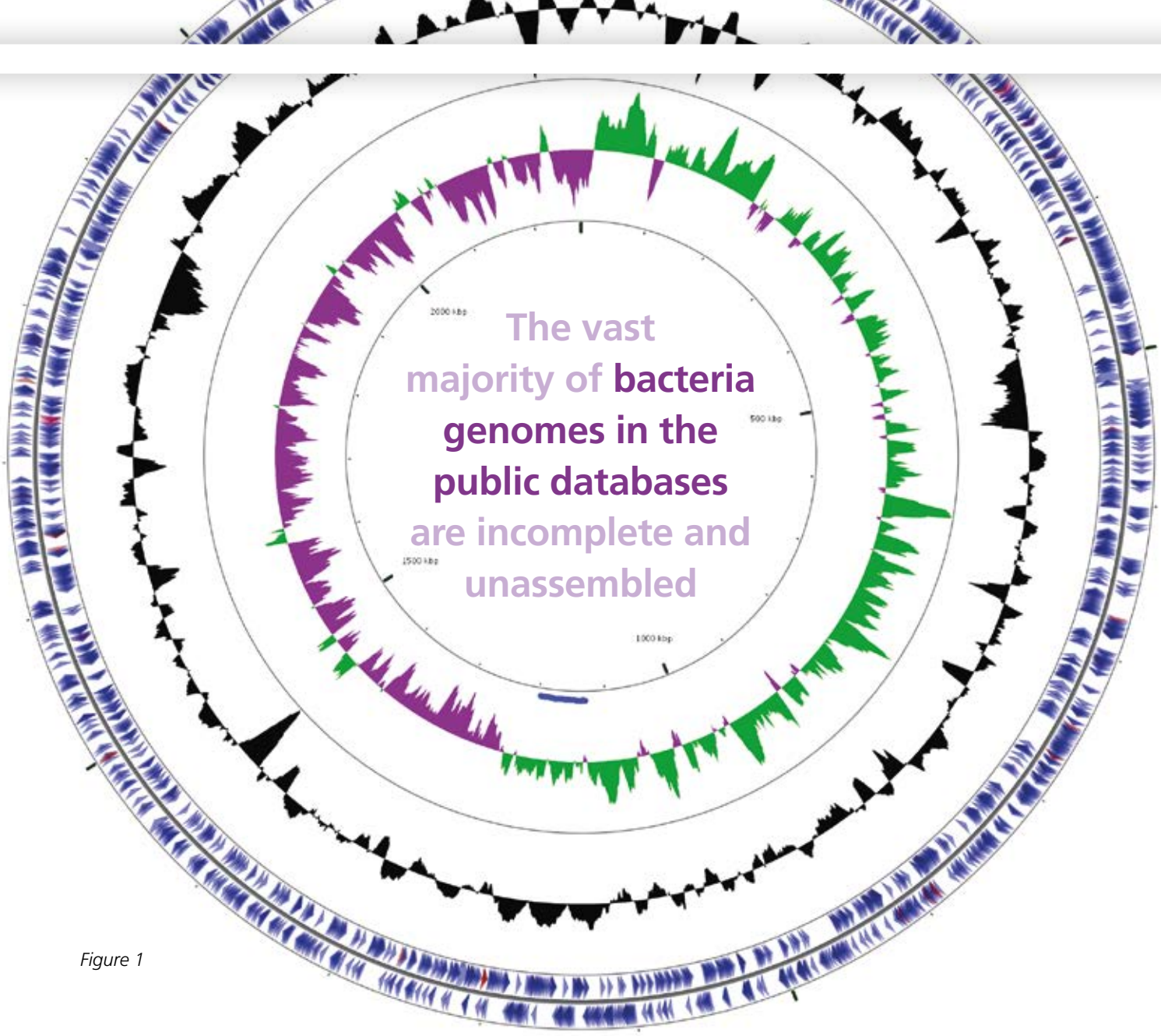

Figure 1

sequencing across the repeat profiles within its prokaryotic genome. In addition, other insights into the bacteria are waiting to be discovered – sunken treasures in the genomic data flood.

Comparative analyses of published complete genome sequences have provided insight into the features contributing to chromosomal rearrangement in *N. gonorrhoeae*. From this, a model could be proposed where ISNgo2-mediated excision and reintegration contributed to the chromosomal structure of *N. gonorrhoeae* strain NCCP11945, while homologous recombination between repetitive sequences are proposed to have caused rearrangements in strain FA1090. Investigations into chromosomal structural changes and rearrangements can only be investigated with complete, correctly assembled genome sequences. The *N. gonorrhoeae* strain FA1090 assembly was aided by a physical map. Before we conducted our detailed comparative analysis of the *N. gonorrhoeae* strain NCCP11945 genome sequence against strain FA1090, we performed PFGE to verify the assembly. Yet, the vast majority of bacteria genomes in the public databases are incomplete and unassembled.

Current status of sequencing projects

As of 18 March 2015 there are 39,257 bacterial genome sequencing projects in the Genomes OnLine Database (GOLD; Figure 2). Of these, 22,017 are permanent drafts (56.1%) and 13,034 are incomplete (33.2%). Even amongst the complete and published (3,057) and complete (608) genome sequencing projects, only a few of these will be closed chromosomes and not in contigs. Using hard work in the laboratory, a bit of clever bioinformatics, and some time and patience, some of

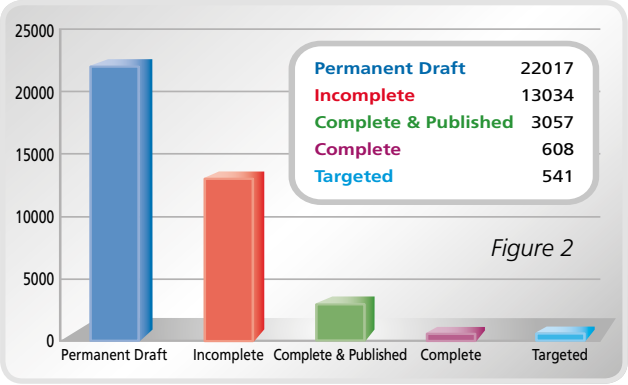

Figure 2

NGS METHOD	READ LENGTH*	MANUFACTURER WEBSITE
Illumina	2 x 300 bp on MiSeq 2 x 250 bp on HiSeq 2500 RapidRun	http://www.illumina.com/systems/sequencing.html
Roche (454)	up to 1 kb on GS FLX+ ~700 based on GS Junior Plus	http://www.454.com/
Ion Torrent PGM	400 bp mate-pair libraries, 2-8 kb inserts	http://www.lifetechnologies.com/uk/en/home/brands/ion-torrent.html
PacBio RS II	half of the reads are >14,000 bp	http://www.pacificbiosciences.com/
Oxford Nanopore	"tens of kb"	https://nanoporetech.com/

Table 1. Next generation sequencing methods for bacterial genomics.

** source of information is the manufacturer website listed*

these bacterial genomes could be closed. However, the investigators involved with these projects have likely already obtained the information they required from these projects; closing the genome requires additional funding and staff time in the lab.

Sequencing technology

One of the contributing factors to the abundance of incomplete genome sequencing projects is the technology itself. A vast amount of sequencing can be obtained from the Illumina sequencing machines, but only very short reads, particularly on the most popular models in use for most eukaryotic applications, the HighSeq (Table 1). In bacterial genome sequences, which can be riddled with repetitive sequences that confound assembly, this can be counterproductive to understanding the bacterial genome, or transcriptome for that matter. Other technologies have sought to overcome this issue. The Ion Torrent PGM system is particularly well suited to the prokaryotic genome and not only has 400 bp read lengths, but has also launched a TrueMate kit system that is specifically designed for closing bacterial chromosomes (Table 1). In the final stages of assembly of a bacterial genome sequencing project, the contigs are often divided by the ribosomal RNA loci, which are present multiple times on the chromosome and are approximately 6 kb. The Pacific Biosystems sequencing system has the capability to sequence through a region of this size, and therefore enable an assembly of a bacterial genome, regardless of homologous regions of the genome, even those as large as the rRNA loci (Table 1). However, issues with the cost per genome and the initial outlay cost for the machine have not made this a technology that is widely used. Likewise, the read-length potential of Oxford Nanopore technology could overcome issues for assembly (Table 1), but this technology is not yet available to everyone and there are concerns about its issues with accuracy that need to be resolved.

Trawling the sea of data

Even though the vast majority of bacterial genomic data is fragmented and destined not to be assembled into complete, circular genomes (or linear

TOOL	DESCRIPTION	URL
Gold	Genomes OnLine Database.	https://gold.jgi-psf.org/
Mauve Multiple Genome Alignment	Multiple bacterial genome alignments of complete or incomplete genomes.	http://asap.genetics.wisc.edu/software/mauve/
xBASE	Database of bacterial genome sequence data, including an annotation service and next generation pipeline.	http://www.xbase.ac.uk/
PubMLST	Public databases for molecular typing and microbial genome diversity.	http://pubmlst.org/
The Sequence Manipulation Suite	A collection of web-based programmes for analysing and formatting DNA and protein sequences.	http://www.bioinformatics.org/sms/index.html
CGView Server	A comparative genomics tool for circular genomes.	http://stothard.afns.ualberta.ca/cgview_server/
Center for Genomic Epidemiology	Web-based tools for analyses and extraction of information from genome sequence data.	http://www.genomicepidemiology.org/
SEQanswers Software wiki	A listing of software tools for analysis of sequencing data.	http://seqanswers.com/wiki/software
Galaxy	An open, web-based platform for data intensive biomedical research.	http://galaxyproject.org/
Wellcome Trust Sanger Institute	Collection of software tools from the Wellcome Trust Sanger Institute.	https://www.sanger.ac.uk/resources/software/
Broad Institute Software	Collection of software tools from the Broad Institute.	http://www.broadinstitute.org/scientific-community/software
J. Craig Venter Institute	Collection of software tools from the J. Craig Venter Institute.	http://www.jcvi.org/cms/research/software/
EBI: Next Generation Sequencing Practical Course	On-line training course including a collection of video lectures providing an introduction to Next Generation Sequencing.	http://www.ebi.ac.uk/training/online/course/ebi-next-generation-sequencing-practical-course

Table 2. Resources for bacterial sequences and analyses.

BACTERIAL SPECIES	FINDINGS	REFERENCE
Pseudomonas aeruginosa	Sequencing of isolates from a six-year-long hospital outbreak, identifying changes in the genome over time.	Snyder, *et al*., *Euro Surveill*. 2013
Acinetobacter baumannii	Sequencing of isolates from a hospital outbreak, resolving transmission events.	Lewis, *et al*., *J Hosp Infect*. 2010
E. coli	Rapid sequencing confirmed an outbreak in a neonatal unit was due to a unique clone.	Sherry, *et al*., *J Clin Microbiol*. 2013
Mycobacterium tuberculosis	TB isolates from two students attending the same school were identified only by sequencing to be identical.	Török, *et al*., *J Clin Microbiol*. 2013
E. coli	Identification of the isolate causing an outbreak, contributing to its control and identification.	Rohde, *et al*., *N Engl J Med*. 2011 Rasko, *et al*., *N Engl J Med*. 2011 Mellmann, *et al*., *PLoS One*. 2011
Neisseria meningitidis	Sequencing of outbreak cases revealed that isolates within the same ST were genomically distinct.	Lavezzo, *et al*., *BMC Infect Dis*. 2013

Table 3. Examples of next generation sequencing applied to infectious diseases.

chromosomes, as appropriate), this genomic flood of data is still full of sunken treasure. To exploit this vast resource of data, we need only form hypotheses and look for evidence. We may find that our hypotheses are incorrect or need to be revised once new perspectives have been gained, but the availability of genome sequence data gives us the freedom to make these discoveries. It is time to dive in and explore, not only the complete and well-annotated sequence data, but also the incomplete, draft sequence data, which may contain N's and may not be annotated. It is important to be cautious in our use of annotations; annotators are only human and can make mistakes, or as is more frequently the case, annotators are computers making best hit matches and not investigating the biology of the organism or the published literature. Also, keep in mind that for some hypotheses, the evidence may only be in the raw sequence data that comes straight off the sequencing machines themselves, not in the assembled contigs and scaffolds that are submitted to the public databases. Evidence of inversions of sequences or changes in simple sequence repeats in phase variation will be lost during the creation of contigs. Most importantly, don't be intimidated by the volume of data and remember that we are fortunate as bacteriologists that bacterial genomes are relatively small; it could be worse!

Even without complete genomes, there are important insights that can be gained, which is why we continue to sequence genomes and leave them in an incomplete state. A great deal can be learned about the evolutionary relationship between strains by extracting out from a fragmented genome the scattered pieces of information such as the rRNA loci sequences, Ribosomal Multilocus Sequence Typing (Rmlst) sequences, MLST sequences, antibiotic resistance marker sequences and comparing these between isolates (Table 2). Indeed, genome-wide single nucleotide polymorphism analyses, even on incomplete genome sequences, can help identify if isolates are related and, therefore, if disease cases are due to clones, and thus a potential outbreak, or are from distinct bacterial isolates (Table 3). Sequencing is also enabling us to understand the rate in which bacteria accumulate mutations, through genome sequencing of isolates known to have been transmitted between patients and isolates from the same patient over time (Table 3).

There is already a great wealth of sequence data available and undoubtedly more to come, which may, as the technologies develop and improve, one day generate a completely assembled sequence of the bacterial chromosome.

FURTHER READING

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Lori A. S. Snyder
Kingston University

MALDI-TOF: A laboratory's journey

The principal challenge of microbial pathogenicity is to reveal the mechanisms and inner workings of the infecting cell. These include factors that enable them to adapt to the host environment, survive its defences, up-regulate a plethora of virulence factors (including toxins) against the host and, of particular importance today, acquire antibiotic resistance. All cellular processes involve proteins and various techniques have been used over the years to gain insight into their expression and characterization. In recent years, these tools have been replaced almost entirely by new, advanced forms of mass spectrometry (MS).

Haroun Shah from Public Health England (PHE) describes the PHE's journey with MALDI-TOF from the arrival of their first benchtop MALDI through to it becoming the principal diagnostic tool for the Proteomics Research Unit at PHE, and why coupling mass spectrometers is still their method of choice for whole genome sequencing (WGS).

On 22 August 2014, Emeritus Professor Franz Hillenkamp (1936–2014), renowned scientist and pioneer in the field of mass spectrometry passed away. His legacy is immense but microbiologists will remember him largely for his work on MALDI-TOF mass spectrometry, the name he coined to describe the technique he developed in the 1980s and which is now an integral part of applied microbiology. When invited to PHE (then Health Protection Agency, London) to deliver a Plenary Lecture on genomics and proteomics of human pathogens in 2008, he expressed his nervousness in speaking to a microbiological audience for the first time. Prior to the conference, he spent two days in a genomics and proteomics laboratory and commented that as a physicist, he was astonished that his work would have such far-reaching implications across the field of microbiology.

The major impetus for change in laboratories came with the arrival of the first benchtop MALDI-TOF mass spectrometer which, unlike its forerunners, was small, compact and relatively simple to use. Built by Kratos Analytical, Manchester, their Kompact Alpha MS showed potential use for microbiology as reported by Claydon *et al*. in 1996. There was general scepticism in the microbiology community as earlier attempts to introduce various forms of mass spectrometry such as Pyrrolysis MS, Electron Impact MS and Fast Atomic Bombardment MS had failed (largely because of their complexity and lack of microbial reference databases). It was against this backdrop that PHE set out to systematically develop the diagnostic applications of MALDI-TOF MS over a decade of dogged persistence. With Kratos Analytical and Manchester Metropolitan University, PHE co-organized the first conference on 27 October 1998 entitled "Intact Cell MALDI-TOF MS" to explore its potential use for microbiology. It was unlikely that any of the 150 participants of that conference could have foreseen the impact of this technology on life sciences or the numerous hurdles it would need to overcome to eventually meet the approval of applied microbiologists.

For PHE, this journey began just after its conference in 1998 and required redesigning an instrument specifically for microbiological applications. Built by Micromass, this 2nd generation instrument (designated M@LDI) contained most of the automated features that are present in current instruments and was used intensively by PHE for eight years to establish parameters for assembling its first database in 2004. The first commercial instrument (Bruker's Microflex) was not installed in PHE for diagnostics until December 2011, just ahead of the London 2012 Olympics. Today, this technology, known as 'matrix-assisted-laser desorption ionization time-of-flight mass spectrometry' (MALDI-TOF MS) is the method of choice for bacterial identification for more than 1,700 laboratories worldwide, while some 2,000 publications have appeared in a diverse range of journals.

In brief, MS typically consists of 3 main modules:

An ionizer which converts a portion of the sample (e.g., bacterial, viral or fungal proteins on the target into an ionized sample preparation).

A mass analyser which sorts the ions according to their inherent mass. This is often done by 'time-of-flight' (TOF) or 'Quadrupole' in other instruments.

A detector measures the quantity of ions arriving from the sample and provides the data for calculating the abundance of each ion present to produce the characteristic mass spectrum.

The two primary methods used for the ionization of whole proteins are MALDI-TOF MS and electrospray ionization (ESI). The MALDI part of the acronym refers to the ionization technique in which larger organic samples are mixed with a suitable matrix and applied to targets on a stainless steel plate (Figure 1). The samples are then subjected to a laser-initiated process that results in the ionization of the analyte molecules. The TOF part of the acronym refers to the use of an electric field to accelerate the ions and the time they take to reach the detector. If the ions all have the same charge then velocity is dependent on mass which can be accurately measured.

In applied microbiology, the application of this technique has been used to generate characteristic MALDI-TOF mass spectra for numerous bacterial species. Thus, by comparing an unknown spectrum to one present in a database, the isolate can be assigned a specific designation. MALDI-TOF MS analyses abundant proteins (i.e., the predominantly stable ribosomal proteins) and therefore masks signals from less abundant proteins, such as toxins and virulence factors. The latter cannot be identified by a single linear MALDI-TOF MS and tandem mass spectrometry approaches need to be carried out.

Widespread global acceptance of this technology is a testament to the continuous research and development programmes in industry, research centres and clinical laboratories. The primary reason for adopting this technology in applied microbiology is the improvement in species identification, compared with the gold standard, 16S rRNA sequencing. However, MALDI-TOF MS also significantly reduces costs and the time taken for identification whilst technically remaining a relatively simple procedure. The list of pathogens being analysed by MALDI-TOF MS is extending to the point that this is now the primary diagnostic tool for bacterial identification in many organizations.

The basic configuration of MALDI-TOF MS continues to change and more recent models have incorporated automated laser settings, improved target plates and optimized matrices. One example that has proved particularly valuable for microbiology is 'surface-enhanced laser desorption ionization time-of-flight mass spectrometry' (SELDI-TOF MS).

SELDI-TOF MS enables rapid detection and simultaneous screening of a wide range of intracellular and membrane proteins. Its main advantage is the use of ProteinChips which act as a miniature chromatographic step. Each chip contains a specific affinity capture surface

Figure 1. Selective capture of various classes of proteins using SELDI ProteinChip technology

MALDI-TOF MS also significantly reduces costs and the time taken for identification whilst technically remaining a relatively simple procedure

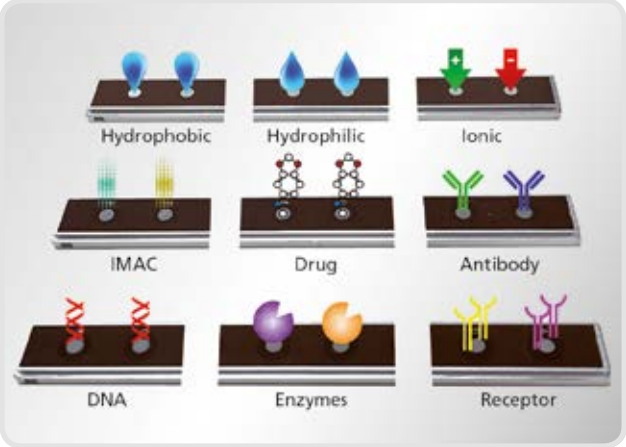

Figure 2. Fragmentation of the precursor ions to generate sequence-specific MS/MS data

(e.g., ionic, hydrophobic, hydrophilic, etc.) designed to capture proteins of interest (Figure 2). SELDI-TOF MS analysis of the bound protein molecules enables direct profiling of proteins from complex biological samples and bypasses complicated purification steps. The selectivity of the protein capture can be increased by using different buffers, thereby altering pH and washing conditions. The technique has been successfully applied to the direct bacterial profiling of numerous pathogens including *Clostridium botulinum*, *Legionella* spp., *Neisseria gonorrhoeae*, *Cl. innocuum*, *Cl. difficile* and *Staphylococcus aureus* (MRSA and MSSA) (Lancashire *et al*., 2005).

The technique is not without its limitations and although the linear model MALDI-TOF MS produces highly reproducible diagnostic mass spectral profiles for microbial identification, it lacks the resolution to deduce the nature of the proteins in the mass spectrum.

To achieve the latter, various forms of tandem mass spectrometers (designated MS/MS) coupled to high performance liquid chromatographs (HPLC, together: LC-MS/MS) are now commonly used.

As early as 1981, McLafferty showed that coupling mass spectrometers in series provided a new method to analyse complex mixtures of organic compounds. The targeted compound was selectively ionized and its characteristic ions were separated from others in the first MS. The selected primary ions were then decomposed by collision and from the resulting products the final mass analyser selected the secondary ions, characteristic of the targeted compound to yield its identity. Five years later, Hunt *et al*. (1986) reported the use of tandem mass spectrometry for protein sequencing using enzymatic and/or chemical degradation of the protein to yield peptides, which were then separated by liquid chromatography (LC) and sequenced using tandem mass spectrometry.

Unlike MALDI-TOF MS, ESI may produce multiple charged ions, effectively extending the mass range of the analyser to accommodate the KDa-MDa orders of magnitude observed in proteins and their associated polypeptide fragments. The sample is first dispersed by electrospray into a fine aerosol from an LC prior to tandem MS (designated LC-ESI-MS/MS).

A mass analyser is then required to separate/resolve the ions formed in the ionization source according to their mass-to-charge (*m/z*) ratios. In PHE Colindale, an LTQ Orbitrap MS has been in operation since 2005 which incorporates an Orbitrap system for high mass accuracy and resolution, and a linear trap quadrupole (LTQ) for mass filtration and molecular fragmentation. This combination allows large-scale comprehensive studies of a specific proteome.

The data generated using the LTQ Orbitrap MS may be typically derived from the cell extract of a particular pathogen and the fragment data then used to deduce the sequence of a peptide. The identification of unique peptides representative of a given protein enables identification of proteins in the cell lysate. The MS/MS spectrum interpretation is based upon fragmentation from both the N and C termini. The N-terminus contains the fragments designated "a" and "b" ions, and the C-terminus containing the fragment "y" ions as shown in Figure 3.

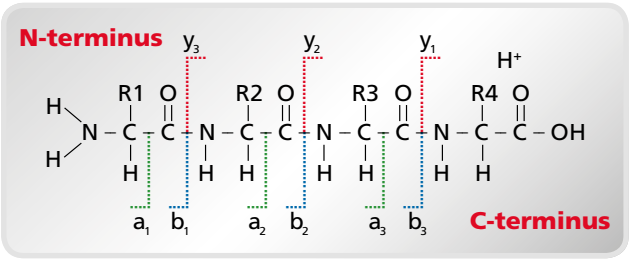

Figure 3. The MS/MS spectrum interpretation is based on fragmentation from both the N and C termini of a peptide. Definition of sequence-specific ions used for MS/MS spectrum interpretation, e.g. the commonly used "b" ions and "y" ions of a peptide

Each amino acid yields characteristic "b" and "y" ions which can be used to derive the amino acid sequence of a peptide. This approach, in which the identities of the proteins are calculated from piecing together these peptide fragments is referred to as "bottom-up", as the protein profile is generated in a hierarchy upwards of peptide sequences.

This method differs from the more recent approach referred to as "top-down" which can be achieved with instruments such as ThermoScientific's "Q-Exactive". Top-down proteomics involves direct dissociation of intact proteins without enzymatic digestion. Fragmentation is accomplished by electron-capture dissociation or electron-transfer dissociation along the amide backbone of the protein. (Note: there is a

"middle-down" proteomics approach in which analysis of longer peptides are generated by restricted enzymatic proteolysis using enzymes such as Lys-C, Lys-N, OmpT prior to MS/MS analysis.) The main benefits of top-down analysis of complex mixtures are its capacity to detect degradation products, sequence variants (proteoforms) and combinations of post-translational modifications (PTMs). The potential use of top-down was elegantly demonstrated by Julia Chamot-Rooke and her team to decipher detailed attachment mechanisms involved in the pathogenicity of *Neisseria meningitidis* to the nasopharynx via its major pilin protein PilE. It was shown that its initial attachment and its subsequent release to re-colonize a new site was modulated through changes in PTMs on PilE. Such mechanisms would otherwise be impossible to deduce from genome sequencing because PTMs are not encoded in gene sequences but occur during/post protein synthesis.

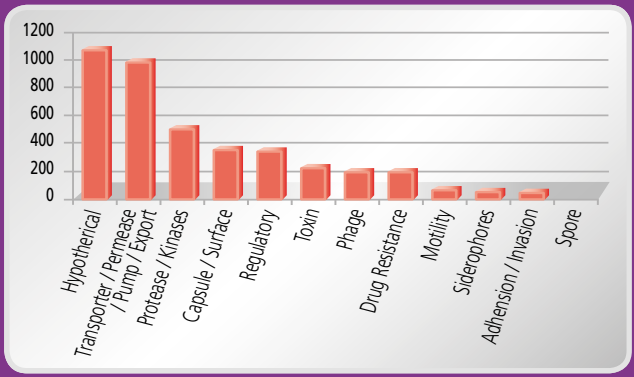

Figure 4. Comparative proteome analysis of Clostridium difficile. The figure shows the large number of hypothetical proteins

WGS of large numbers of species reveals that between 40–60% of the proteome are designated hypothetical proteins (Figure 4). To ascertain the function of such genes, accurate assessment of protein expression will be required through painstaking, in-depth proteome analysis by tandem MS of large numbers of species; a monumental task since expression is not readily predictable. For example, some 30 different growth conditions were used to deduce just 35% of the theoretical proteome of *Halobacterium salinarum*. Nevertheless, with the rapid advances being made in tandem MS, aspects of the biology of microbes that were hitherto obscure will be illuminated. Evidence has already shown that the detection of new mechanisms of antibiotic resistance may be revealed through proteomics, particularly where WGS provides no clues. Similarly, new potential vaccine targets are being elucidated through proteomics for vaccines such as BCG, whooping cough and others, where the efficacy of current preparations is failing. Studies on the secretome or architecture of cellular components such as the bacterial membranes using novel capture methods prior to MS are developing areas where tandem MS will be indispensable.

There is considerable debate about the potential of tandem MS to supersede MALDI-TOF MS as a rapid approach to microbial identification. However, with the recent approval of MALDI-TOF MS for the clinical identification of microorganisms by the U.S. Food and Drug Administration and the European Union "CE" Mark Commission, the author's view is that MALDI-TOF MS will retain its role as a primary diagnostic tool for the foreseeable future.

Applications of mass spectrometry are rapidly changing the landscape of microbiology; spanning direct applications in diagnostics, metabolism and structural chemistry, to decipher detailed mechanisms of pathogenicity.

Today, protein databases are populated with deduced sequences derived from whole genome analysis. With the huge amount of data becoming available daily, the field of proteogenomics will continue to expand enormously in the foreseeable future with instruments providing detailed analysis of the proteome, against a background of the genome, in real time.

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Micropia

A magical museum of microbes

Leeuwenhoek's drawings adorn the entrance to the museum and I'm already transfixed. I crouch down and read the description of each of the images, which show his drawings from one angle and a real micrograph of the microbes from another.

Micropia is the newest addition to Amsterdam's Artis Zoo and the first of its kind in the world – a microbial museum. The creators had big plans when they came up with the idea; it's not just about showing visitors what microorganisms look like, but about bringing science closer to its stakeholders.

"*Lucy! Lucy!*" I turn around and my family is standing there looking impatient. "*Do you want to go into the museum?*" asks the volunteer. She's holding a round card that she explains is for collecting microbes on our Micropia journey. She demonstrates on a stamp next to one of Leeuwenhoek's drawings and the kids grapple to go first. We're all set and the lift doors open to welcome us up.

As soon as the doors close, the lift springs into life. Light directs our gaze to the ceiling and we get a beautiful introduction to the world of microbes. When we step out, we're guided to a huge wall that displays the tree of life. It's interactive and pushing buttons lights up sections of the tree, telling stories about its inhabitants.

A spectacular display of bubbling water in a huge tank focuses on oxygen and there's a microscope set up to look at real-life samples. After searching for the first stamp, the boys start to play with the microscope, instinctively knowing how to focus it and use the touchscreen display to get information about what they see.

We then look at algae and diatoms – three small round tanks have an oxygen supply you can switch on and off with a button. Another live microscope and a static version with images of the microbes to flick through keep us occupied until a big round table catches our attention. By spinning the disc you can find out about microbial reproduction; the boys are fascinated by cell division, and love the idea of a T4 bacteriophage recreating the moon landing, but are quite horrified by conjugation.

And it doesn't end there. Behind an interactive video display, where you can use your hands to point to

Micropia is the first museum of its kind, displaying the invisible world of microorganisms. It is located in the centre of Amsterdam.

For more information visit: http://www.micropia.nl/en/

different body parts for information on what's living there, is a kissing booth. A great way to embarrass boys, you can stand on a podium and kiss; the screen will tell you how 'hot' your kiss was and how many bacteria you shared.

As they make a run for it, the boys discover the real lab at the back of the museum. Behind glass, two scientists are working in white lab coats. We're all totally absorbed by what they're doing and notice some 'fresh' samples to the left of the glass. This is really a live museum and the microbes you see are changed regularly.

We move through exhibits showing symbioses (an enormous, real-life ant farm on an island!) and bioluminescence, and travel to the world's harshest environments to look at extremophiles. There's also a visual library of microbes: a wall of agar plates with a variety of bacteria and fungi growing on them. An interactive video screen shows some of the plates growing in time-lapse, and we watch shapes emerging like the keypad of a mobile phone and a hand.

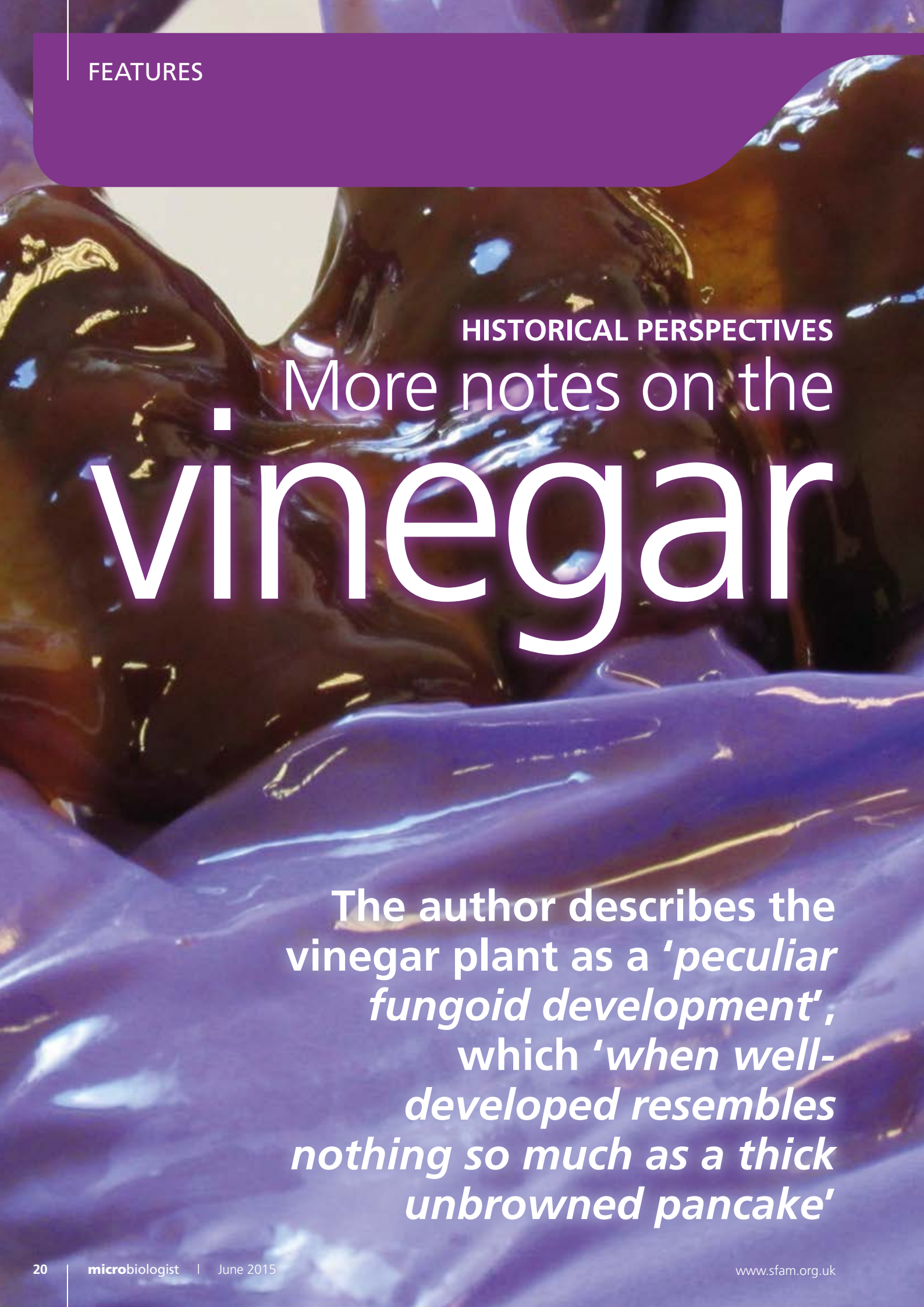
Downstairs, big cases with doors prop up beautiful glass models of viruses and the displays inside put them in societal context. Industry features heavily, with a large section of the museum dedicated to the use of microbes in manufacturing, food and beverages. We peer into glass cases to find out which products we consume are made by bacteria, spray antibacterial soap to find out why it can be counterproductive and discover how microbes can be used in fuel production. There's even a display you can smell (if you dare).

A huge screen, spanning the height of both floors and extending up into the gallery, is the centrepiece. The cards we've stamped fit under a giant microscope, prompting the display to show details about the microbes you have collected.

Eagerly anticipating an exciting shop, I'm disappointed; the souvenirs consist of a few notebooks, a 'grow-your-own' (which I'm slightly concerned about) and a couple of books. Despite walking away empty-handed, this has been the single best museum experience I've had in years. It's engaging, fascinating and beautiful, and it kept us all entertained for hours.

I take a moment to look again at Leeuwenhoek's drawings. I imagine him sitting 40 miles away from here, 300 years ago, gazing at his animalcules and piecing together his own microbial display. I wonder what he would have thought of Micropia?

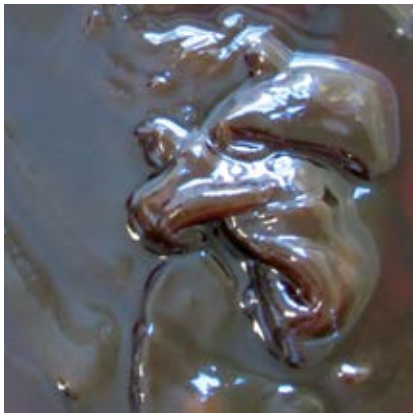
Lucy Goodchild van Hilten

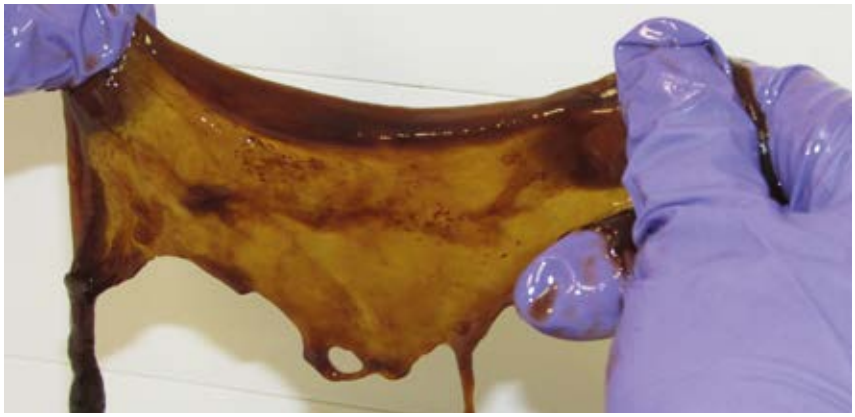


HISTORICAL PERSPECTIVES

More notes on the vinegar

The author describes the vinegar plant as a '*peculiar fungoid development*', which '*when well-developed resembles nothing so much as a thick unbrowned pancake*'





The vinegar plant (far left and above)

plant

In the autumn of 2014, I was contacted by Mr Welbourne from Surrey, who was looking for a new home for a vinegar "plant" that he had been looking after for 40 years. As curator at NCIMB, a national repository for genetic material, I was delighted to accept this microbial growth, which has an intriguing history.

Mr Welbourne had received the vinegar plant from an elderly lady who lived in Woodbridge in Suffolk. She was keen for it to live on because it had managed to survive sugar rationing during World War II. Moreover, he told us that the plant was sometimes displayed at fetes as a novelty, with people paying to see it.

The plant is easy to maintain. It looks like a cross between a wash-leather and a jellyfish and, like its new guardian, loves sugar. The plant is placed in a tub of sugar solution, the top of which is covered in brown paper, and it is kept in a cupboard to protect it from the light.

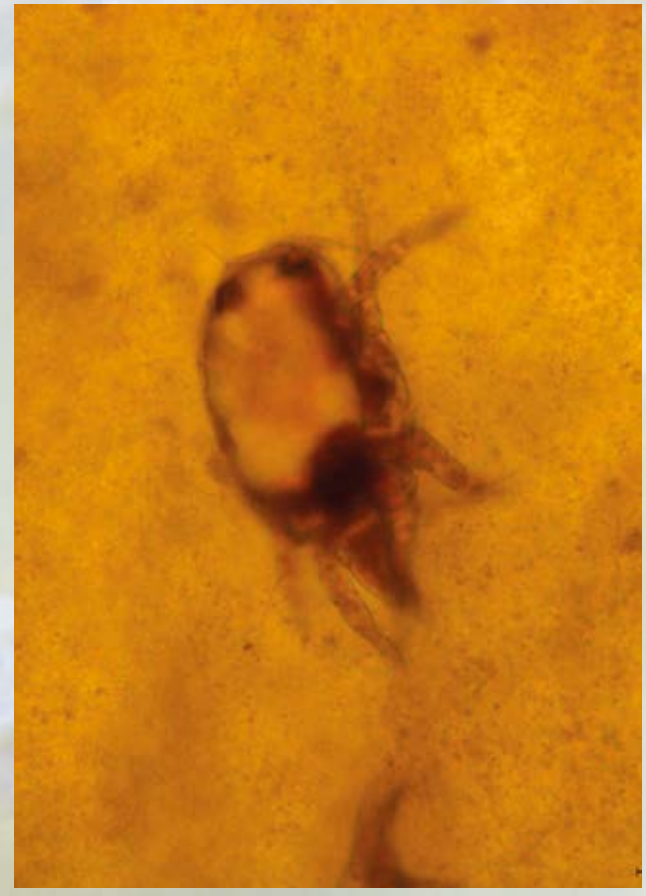
At the end of six weeks the vinegar is ready for use. The liquid should be placed in a pan and brought to the boil, then simmered for 10 minutes before consumption. It produces a lovely sweet-tasting vinegar.

All this got me thinking, not just about what exactly this plant is biologically, but also about the history and importance of vinegar production – I had to find out more about the vinegar plant.

A Google search led me to a paper entitled 'Notes on the vinegar plant' by Spencer Thomson MD, published in the Association Medical Journal in 1853. In this paper the author describes the vinegar plant as a '*peculiar fungoid development*', which '*when well-developed resembles nothing so much as a thick unbrowned pancake*'. On initial observation, that seems to be a fairly accurate description of the microbial artefact now in NCIMB's care. The author goes on to say that the plants are '*now extensively used in many parts of the country for the domestic manufacture of vinegar*'.

Perhaps it is not surprising that there was a craze for home vinegar production in the 19th century – it's much more than a condiment. In recent years there has been a resurgence of interest in vinegar as a natural cleaning product, but in the 19th century there may have been little alternative to the use of vinegar for a variety of domestic applications.

Some of the uses of vinegar at the time are illustrated by a book, which was also authored by Dr Thomson, and published around the same time. His '*Dictionary of Domestic Medicine and Household Surgery*' makes several references to the use of vinegar for the treatment of ailments, including corns and sore throats, and as a solvent for medicinal substances. Much earlier use of vinegar as a disinfectant has also been described. In 1665, bubonic plague arrived in the Derbyshire village of Eyam. To minimize cross-infection, food and



A mite found in the vinegar plant

Background: growth of mixed yeast colonies on yeast mould agar

other supplies were left at the boundary of the quarantined village, and coins for payment are said to have been left there, in vinegar, to disinfect them.

Back in 1853, Dr Thomson studied the vinegar plant under a microscope. He found it to '*consist of spores and minute cells, in various stages of development, lying among interlacing and branched filaments*'. He described them as being '*precisely similar to those seen in the vegetative and mycelium stages of a common mucor, or of the Penicilium glaucum, only they are more minute*', and proposed that '*the vinegar plant, therefore, is simply an expanded thallus formed by an aggregation of spores and cells entangled in the filaments developed by the budding forth of the latter; the whole apparently being connected by mucilaginous matter*'.

He also investigated how the domestic manufacture of vinegar production was affected by the age of the plant, whether or not it was affected by the addition of yeast and the use of various sugar solutions. He concludes by saying that '*the observations in the foregoing paper I offer rather as suggestive than otherwise. The subject is one which does not appear to have undergone any special investigation, and my chief objective is to direct to it the attention of those who have more leisure time than myself to devote to such enquiries*'.

One hundred and fifty three years later I decided that I should pick up this investigation where Dr Thomson left off. However, the industrial production of vinegar for human consumption generally relies on the use of acetic acid bacteria. The National Collection of Industrial Food and Marine Bacteria includes a varied collection of acetic acid producing bacteria dating back to the 1940s, including strains isolated from East African vinegar brew, ditch water in The Netherlands and soil below a bamboo grove of a burial mound in Japan. I am much less familiar with the concept of production of vinegar by fungi, and so I decided to get in touch with Dr Andy Taylor from the James Hutton Institute. He is a specialist in environmental fungi and agreed to undertake further investigation of the plant.

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On examining the vinegar plant, using **differential interference contrast microscopy**, some filamentous material could be seen, covered in a mix of cells

On examining the vinegar plant, using differential interference contrast microscopy, some filamentous material could be seen, covered in a mix of cells. However, no filamentous fungi were found to be present.

I also plated out samples from the plant on both yeast mould agar and nutrient agar. Growth on the nutrient agar showed a mixture of colonies – both yeast and bacteria, while growth on the yeast mould agar showed two distinct yeast colony types. Gram staining revealed that the bacterial colonies included a mix of Gram-positive and Gram-negative rod-shaped cells.

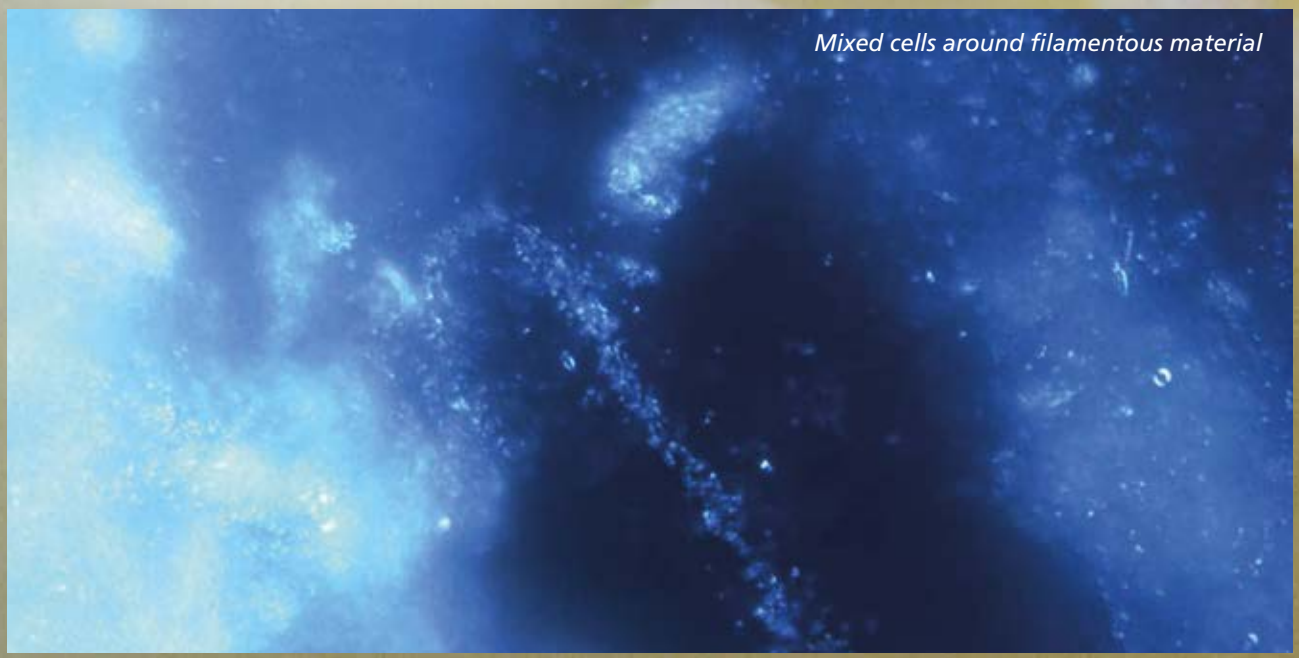
Our vinegar "plant", therefore, appears to be a diverse mixed population of yeasts and bacteria with no evidence of the filamentous fungi described by Dr Thomson. It seems likely that in our plant the yeasts are responsible for converting the sugar solution to alcohol, which is then oxidized by the action of the bacteria to form vinegar. However, further investigation would be required to confirm that.

Although we did not observe any filamentous fungi, Dr Thomson did also describe a mixture of cells. He could have been dealing with a completely different form of vinegar plant, or it could be that vinegar production in the plant he described was due to the action of yeasts and bacteria, with the filamentous element being an extraneous contaminant. Since the plant would not have been kept in controlled conditions, it could have easily been supporting the growth of unrelated organisms. We were surprised to find a noteworthy number of mites in our vinegar plant – none of them living, so perhaps mite extermination is another useful domestic application of vinegar.

In the words of Dr Thomson, '*the observations in the foregoing paper I offer rather as suggestive than otherwise*'. The subject is one which does not appear to have undergone any special investigation, and my chief objective is to direct to it the attention of those who have more leisure time than myself to devote to such enquiries.

Thanks to Dr Andy Taylor and Dr Astrid Taylor from the James Hutton Institute, and Mr Welbourne, for their contributions to this article.

Samantha Law
NCIMB



Mixed cells around filamentous material

Spreading the word on the **benefits of bioscience**

Post-election we will be continuing to raise the profile of biology in all its forms and promote to all the UK governing legislatures the ways biosciences contribute to everyday life, society and the economy. Our Westminster Parliamentary Links Day (23 June) will be an initial focal point for direct parliamentary engagement, convening representatives from scientific organizations and those who form science policy.

In March, at our Voice of the Future event, the Science and Technology Committee explained to young researchers that they often keep their parliamentary colleagues informed about scientific issues relevant to public policy. It is also the responsibility of the science community to make their voices heard, not only in policy discussions, but also in the media and public spheres, in order to ensure the public recognize the wider value of biology and in turn can influence the political agenda.

In June we will be at the Cheltenham Science Festival with newly developed activities on GM and stem cells. We will also be asking '***Can eating insects save the world?***' at the Glasgow Science Festival and meeting hundreds of science curious families at the Big Bang Fairs in Leicester and the South East.

It is the responsibility of the science community to make their voices heard, not only in policy discussions, but also in the media and public spheres

BIOFocus

Public engagement and science communication is particularly important around animal research. At the time of writing we are running a student essay competition looking for members to write about animal research to raise awareness and understanding with the added incentive of winning training to support their future career.

It's great when engagement with science starts at school. Currently our Curriculum Committee are working towards the development of an 'ideal' biology curriculum which spans primary and secondary education. The Committee is made up of representatives from higher education across the biosciences, secondary and primary schools, research, curriculum design and development and industry, as well as representatives on maths, physics, chemistry and biomedical sciences.

At the moment the Committee is looking at how they can engage with a wide range of stakeholders about what should be included for the study of biology at the 16–19 age range. We have put together a primary working group and are looking for undergraduate and A level/highers level representatives to become part of a Student Committee. We hope that we will be able to create a continuous progressive curriculum.

The biggest week in the biology calendar, in terms of public engagement, has to be Biology Week – which we will be organizing to celebrate the life sciences for the fourth year running in October 2015. Last year the Biology Week banner flew over more than 100 events and activities all over the UK and beyond. Everyone from children to research scientists got involved in debates, bug hunts, competitions, dinosaur digs and Big Biology Days, sharing a passion for biology. We were thrilled so many members of our MOs got involved in some great events and activities, and we are keen to involve even more of you this Biology Week: Saturday 10 – Sunday 18 October. Ways to get involved include: organizing your own event, running a hands-on activity at a Big Biology Day, creating an online educational resource, doing a pub quiz, or anything else you can think of!

Please contact **natashalittle@societyofbiology.org** if you would like to discuss being a part of Biology Week 2015.

Mark Downs FSB
Chief Executive, Society of Biology

Journal**WATCH**

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Journal of Applied Microbiology

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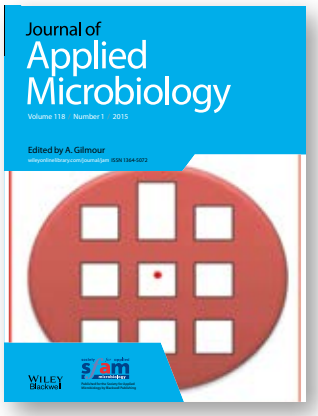
Antimicrobial activity of hop extracts against foodborne pathogens for meat applications

B. Kramer *et al*.



The objective of this study was the fundamental investigation of the antimicrobial efficiency of various hop extracts against selected foodborne pathogens *in vitro*, as well as their activity against *Listeria monocytogenes* in a model meat marinade and on marinated pork tenderloins. We found that ß-acid containing hop extracts have proven to possess a high antimicrobial activity against Gram-positive bacteria *in vitro* and in a practice-related application for food preservation. This suggests antimicrobial hop extracts could be used as natural preservatives in food applications to extend the shelf life and to increase the safety of fresh products.

http://onlinelibrary.wiley.com/doi/10.1111/jam.12717/abstract

A *Streptococcus uberis* transposon mutant screen reveals a negative role for LiaR homologue in biofilm formation

T. Salomäki *et al*.

The environmental pathogen *Streptococcus uberis* causes intramammary infections in dairy cows. Because biofilm growth might contribute to *Strep. uberis* mastitis, we conducted a biological screen to identify genes potentially involved in the regulation of biofilm growth. The research concluded the DNA-binding protein LiaR is a potential regulator of biofilm formation by *Strep. uberis*. In significance, several molecular primary and downstream targets involved in biofilm formation by *Strep. uberis* were identified. This provides a solid foundation for further studies on the regulation of biofilm formation in this important pathogen.

http://onlinelibrary.wiley.com/doi/10.1111/jam.12664/abstract

Letters in Applied Microbiology

www.lettersappliedmicro.com

Preservative activity of lavender hydrosols in moisturizing body gels

A. Kunicka-Styczyńska *et al*.

The presented research proved the antimicrobial activity of hydrosols obtained from fresh or dried *Lavandula angustifolia* herbs or flowers in moisturizing body gel. The study shows the usefulness of lavender hydrosols as a natural, ecologically friendly component of cosmetics with potential preservative activity in formulations. Hydrosols are commonly regarded as waste in the production of essential oils. The use of lavender hydrosols in the cosmetic industry as a replacement for the water phase in cosmetics may not only result in expenses reduction for chemical stabilizers and preservatives but also in a substantial decrease in sewage disposal.

http://onlinelibrary.wiley.com/doi/10.1111/lam.12346/abstract

Method for monitoring deletions in the aflatoxin biosynthesis gene cluster of *Aspergillus flavus* with multiplex PCR

K. A. Callicott and P. J. Cotty

Aflatoxins, potent naturally occurring carcinogens, cause significant agricultural problems. The most effective method for preventing contamination of crops with aflatoxins is through use of atoxigenic strains of *Aspergillus flavus* to alter the population structure of this species and reduce incidences of aflatoxin producers. Cluster amplification pattern (CAP) is a rapid multiplex PCR method for identifying and monitoring indels associated with atoxigenicity in *A. flavus*. Compared to previous techniques, the method reported allows for increased resolution, reduced cost and greater speed in monitoring the stability of atoxigenic strains, incidences of indel mediated atoxigenicity and the structure of *A. flavus* populations.

http://onlinelibrary.wiley.com/doi/10.1111/lam.12337/abstract

Microbial Biotechnology

www.microbialbiotech.com

Evaluation of industrial *Saccharomyces cerevisiae* strains as the chassis cell for second-generation bioethanol production

H. Li *et al*.

To develop a suitable *Saccharomyces cerevisiae* industrial strain as a chassis cell for ethanol production using lignocellulosic materials, 32 wild-type strains were evaluated for their glucose-fermenting ability, their tolerance to the stresses they might encounter in lignocellulosic hydrolysate fermentation and their genetic background for pentose metabolism. The strain BSIF, isolated from tropical fruit in Thailand, was selected out of the distinctly different strains studied for its promising characteristics. Compared with other strains, this strain exhibited superior tolerance to high temperature, hyperosmotic stress and oxidative stress, better growth performance in lignocellulosic hydrolysate, and better xylose utilization capacity when an initial xylose metabolic pathway was introduced, and in combination with additional results, indicate that this strain is an excellent chassis strain for lignocellulosic ethanol production.

http://onlinelibrary.wiley.com/doi/10.1111/1751-7915.12245/abstract

Synthetic biology approaches to improve biocatalyst identification in metagenomic library screening

M-E. Guazzaroni, R. Silva-Rocha and R. J. Ward

There is a growing demand for enzymes with improved catalytic performance or tolerance to process-specific parameters, and biotechnology plays a crucial role in the development of biocatalysts for use in industry, agriculture, medicine and energy generation. Metagenomics takes advantage of the wealth of genetic and biochemical diversity present in the genomes of microorganisms found in environmental samples, and provides a set of new technologies directed towards screening for new catalytic activities from environmental samples with potential biotechnology applications. However, biased and low level expression of heterologous proteins in *E. coli* together with the use of non-optimal cloning vectors for the construction of metagenomic libraries generally results in an extremely low success rate for enzyme identification. The bottleneck arising from inefficient screening of enzymatic activities has been addressed from several perspectives; however, the limitations related to biased expression in heterologous hosts cannot be overcome by using a single approach, but rather requires the synergetic implementation of multiple methodologies. Here, we review some of the principal constraints regarding the discovery of new enzymes in metagenomic libraries and discuss how these might be resolved by using synthetic biology methods.

http://onlinelibrary.wiley.com/doi/10.1111/1751-7915.12146/abstract

Environmental Microbiology

www.env-micro.com

The evolving dynamics of the microbial community in the cystic fibrosis lung

L. McGuigan and M. Callaghan

The cystic fibrosis (CF) lung is a niche colonized by a diverse group of organisms, with a more limited number of species including *Pseudomonas aeruginosa* dominating in adult patients. Whether all members of this microbial community play a direct or indirect role in pulmonary decline has yet to be fully elucidated, but investigations of their

interactions with both co-colonizing species and with host cells are beginning to shed light on their virulence potential. This review highlights the recent developments in CF microbiology focusing on the cooperative, competitive and adaptive interactions of established and emerging pathogens in the lung microbiome.

http://onlinelibrary.wiley.com/doi/10.1111/1462-2920.12504/abstract

Bacterial networks and co-occurrence relationships in the lettuce root microbiota

M. Cardinale *et al*.

Lettuce is one of the most common raw foods worldwide, but is occasionally also involved in pathogen outbreaks. This research looks to understand the correlative structure of the bacterial community as a network, by studying the root microbiota of eight ancient and modern *Lactuca sativa* cultivars and the wild ancestor *Lactuca serriola* by pyrosequencing of 16S rRNA gene amplicon libraries. The lettuce microbiota was dominated by Proteobacteria and Bacteriodetes, as well as abundant Chloroflexi and Actinobacteria. Spearman correlations between operational taxonomic units (OTUs) showed that co-occurrence prevailed over co-exclusion, and complementary fluorescence *in situ* hybridization-confocal laser scanning microscopy (FISH-CLSM) analyses revealed that this pattern results from both potential interactions and habitat sharing. The approach to combine co-occurrence analysis and FISH-CLSM allows reliably reconstructing and interpreting microbial interaction networks.

http://onlinelibrary.wiley.com/doi/10.1111/1462-2920.12686/abstract

Environmental Microbiology Reports

www.env-micro-reports.com

Different bulk and active bacterial communities in cryoconite from the margin and interior of the Greenland ice sheet

M. Stibal *et al*.

Biological processes in the supraglacial ecosystem, including cryoconite, contribute to nutrient cycling within the cryosphere and may affect surface melting, yet little is known of the diversity of the active microbes in these environments. We examined the bacterial abundance and community composition of cryoconite over a melt season at two contrasting sites at the margin and in the interior of the Greenland ice sheet, using sequence analysis and quantitative PCR of coextracted 16S rDNA and rRNA. Significant differences were found between bulk (rDNA) and potentially active (rRNA) communities, and between communities sampled from the two sites. The bulk alpha diversity was higher in the margin site community, suggesting that local sources may be contributing towards the gene pool in addition to long-distance transport.

http://onlinelibrary.wiley.com/doi/10.1111/1758-2229.12246/abstract

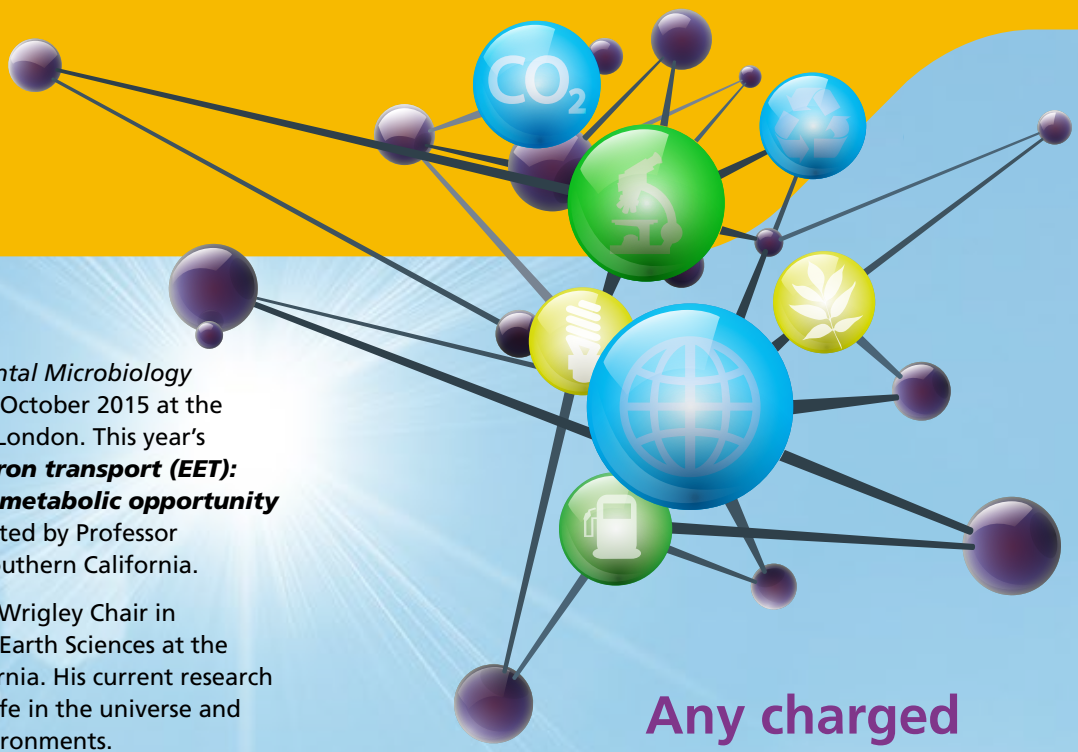
Evaluation of revised PCR primers for more inclusive quantification of ammonia-oxidizing archaea and bacteria

K. A. Meinhardt *et al*.

Ammonia-oxidizing archaea (AOA) and bacteria (AOB) fill key roles in the nitrogen cycle. Thus, well-vetted methods for characterizing their distribution are essential for framing studies of their significance in natural and managed systems. Quantification of the gene coding for one subunit of the ammonia monooxygenase (*amoA*) by PCR is frequently employed to enumerate the two groups. However, variable amplification of sequence variants comprising this conserved genetic marker for ammonia oxidizers potentially compromises within- and between-system comparisons. We compared the performance of newly designed non-degenerate quantitative PCR primer sets to existing primer sets commonly used to quantify the *amoA* of AOA and AOB using a collection of plasmids and soil DNA samples. The new AOA primer set provided improved quantification of model mixtures of different *amoA* sequence variants and an increased detection of *amoA* in DNA recovered from soils. Although both primer sets for the AOB provided similar results for many comparisons, the new primers demonstrated increased detection in environmental application. Thus, the new primer sets should provide a useful complement to primers now commonly used to characterize the environmental distribution of AOA and AOB.

http://onlinelibrary.wiley.com/doi/10.1111/1758-2229.12259/abstract

Melissa McCulloch
Wiley-Blackwell



Environmental Microbiology LECTURE 2015

The annual S*f*AM *Environmental Microbiology Lecture* will take place on 13 October 2015 at the Royal Society of Medicine in London. This year's lecture, '***Extracellular electron transport (EET): opening new windows of metabolic opportunity for microbes***' will be presented by Professor Ken Nealson, University of Southern California.

Ken Nealson is holder of the Wrigley Chair in Geobiology and Professor of Earth Sciences at the University of Southern California. His current research focuses on the evolution of life in the universe and microbial life in extreme environments.

He gained recognition in the 1970s with his ground-breaking PhD by discovering the mechanism by which bacteria communicate with each other. But, Nealson's collective body of research has become even more acclaimed since the discovery of quorum sensing. He has revealed new organisms and even spent time advising NASA on how to detect life on other planets.

You can find dozens of brilliant papers that bear his name including many on the marine bacteria *Shewanella* he discovered in Lake Oneida, New York, in 1988.

Nealson's findings since *Shewanella* have defied many long-held assumptions on bacterial metabolism including showing how bacteria can modify insoluble chemicals to produce electricity. His current research on *Shewanella* focuses on a metabolic process called extracellular electron transport, also known as EET.


Professor Ken Nealson

In the S*f*AM *Environmental Microbiology Lecture* Professor Nealson will raise the captivating possibility that in some of these organisms, EET involves specialized proteins that allow the flow of electrons from the inner membrane, across the periplasm and the outer membrane to (or from) insoluble electron acceptors or donors, previously thought to be inaccessible to microbes.

Any charged surface may be regarded as a potential microbial "nutrient"

He will describe how EET has thus opened up new "*windows of metabolic opportunity*" and that any charged surface may be regarded as a potential microbial "nutrient". His research places him firmly in the forefront of bioelectricity generation and/or bioremediation, using bioelectrochemical systems such as microbial fuel cells.

Although he has received many awards, the Waksman "Outstanding Educator Award" from the Society for Industrial Microbiology and the D. C. White "Research and Mentoring" award from the American Society for Microbiology stand out for Nealson.

He sees his greatest accomplishment as the training of some outstanding young people in science who continue to change the intellectual landscape in microbiology.

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

Winter Meeting REPORT 2015

Morning session

The Winter Meeting was opened with 'Professor James Oliver presenting the Denver Russell Memorial Lecture. His lecture on the ecology and pathogenesis of *Vibrio. vulnificus* enlightened the audience on this deadly pathogen. *V. vulnificus* was introduced as an organism that can cause the most fatal foodborne disease from contaminated seafood such as oysters, often proving life threatening in individuals with underlying liver disease. Virulence factors such as the ability to scavenge serum iron and the presence of a capsule were discussed in terms of their role in pathogenesis. James went on to explain why most cases are found in men and that this gender difference regarding lethality is seen in both humans and rats, with oestrogen most likely helping to protect females. The genomics were explored by showing major differences of the sequence within the virulence region of the gene between clinical and environmental samples following their exposure to seawater and human serum. We were then told of the role of salinity and temperature in the occurrence of *V. vulnificus*, where salinity is critical to bacterial survival. So few cases are seen in the Mediterranean Sea for this reason as the high salinity of water inhibits survival despite *V. vulnificus* being an obligate halophile. We were left with some food for thought, when James explained that global warming is influencing the occurrence of *V. vulnificus* worldwide as elevated temperatures causes resuscitation of the pathogen allowing it to thrive.

Lorna Fewtrell then delivered a talk on the microbiology of urban UK floodwater. She explained that there is very little data on the microbiology of urban floodwater. Crude sewage and foul water from the River Ouse was found to contain *Cryptosoridium*, *Salmonella* and *Campylobacter*. Opportunistic sampling of summer floodwater in Gloucestershire revealed similar organism profiles to those of raw sewage. Lorna described how people react during a flood with most people staying until their area or home is flooded and very few people actually leaving before the flood. This behaviour leads to more people being exposed to

the higher bacterial levels found in sediment compared with that in water. Rotavirus is often the most likely pathogen to cause illness and was the reason for illness in 2.5% of the population during the Carlisle floods in 2005. An eye-opening view of flood issues was closed by reporting that psychological stress is often overlooked, and is a much more common health issue in adults affected by flooding compared with any other health impact.

The morning session was brought to a close by Frances Lucy on potential waterborne pathogen risks in recreational water. Driven by housing, agriculture and recreational activities she explained why we need to biomonitor recreational waters. An eight-year longitudinal study showed an increase in outbreaks of *Cryptosporidium*, *Giardia* and pathogenic microsporidia, with the source often being environmental, coming from sewage and agriculture. She went on to discuss how we can take advantage of organisms at the bottom of waterbeds to assess water quality. Zebra mussels and hog louse were appreciated for their ability to act as a filter and as a tool for assessing pollution in recreational waters, with hog louse especially renowned for eating faecal matter. An enlightening talk was concluded with the reiteration that climate change is a major issue for waterborne pathogen risks.

Sabrina Roberts

Afternoon session

In a break from tradition, there was a single afternoon session this year, chaired by a Member of the Meetings Subcommittee, Mike Dempsey. Four talks continued the theme, exploring differing environments associated with water.

David Lees, Centre for Environment, Fisheries and Aquaculture Science (CEFAS), started the session with **Contamination of bivalve shellfish with enteric viruses**. After acknowledging that hepatitis A is a significant problem, David's talk concentrated on norovirus which accounts for 55% of viral gastroenteritis associated with bivalve shellfish, especially oysters.

Earlier risk assessments which relied on *E. coli* as a faecal indicator have now been replaced by PCR methods for detecting viruses in the shellfish. Standardization of methods at EU level has been implemented, but further work is needed to address viability and determine safe levels.

Outbreaks continue to occur and a contributory factor to shellfish contamination is the proximity of harvesting sites to areas of human pollution resulting from sewage spills.

In the second talk, **Microbial risks associated with spring waters and private supplies**, Paul Hunter, University of East Anglia, sought to dispel the romantic notion of spring water being pure. He described the categorization of private supplies based on water volume or number of users and mentioned some risk factors such as poor maintenance and run-off involving sheep faeces.

Contamination of public water supplies is predominantly associated with *Cryptosporidium* spp. but private water contamination is associated with a diverse range of agents including *Campylobacter* spp. Motivation to improve the safety of private supplies is lacking since those regularly consuming the water have become immunized by prolonged low-level exposure to pathogens. However, children and visitors will lack this immunity and are likely to succumb to gastrointestinal infections. Elderly people who retire to rural areas from urban areas with public water supplies are also vulnerable.

Biofilm problems in dental unit water systems and their practical control was the title of the third talk by David Coleman, University of Dublin. The microbiological issues relating to these complex pieces of equipment can be divided into two groups: those involving water supply and those involving suction to remove water and debris. The former have largely been resolved but the latter are not yet fully recognized.

Common environmental isolates from dental units include strongly catalase-positive *Sphingomonas* spp. Biofilm formation is enhanced by the multi-component fittings which feature many blind spots. Bacterial growth is also enhanced by the heat generated by the units.

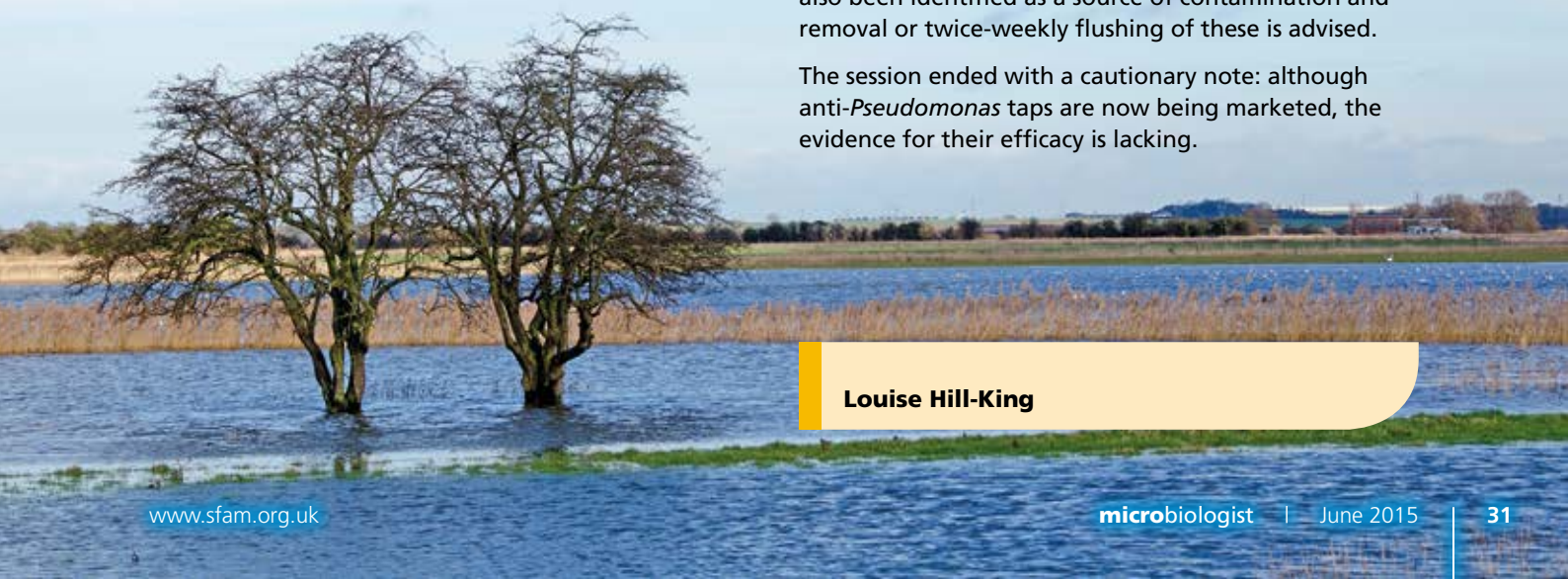
Automation is an important consideration as dental workers lack the time for cumbersome cleaning procedures. Automation also addresses the pitfall of stagnation which occurs with manual methods when units are out of action during weekends.

David described the system adopted in his setting which utilizes electrolysed water. He has been monitoring 400 such units 4 times a week and found them to be effective and cost-saving.

The final speaker of the day, Jimmy Walker, PHE, spoke about ***Pseudomonas aeruginosa* in hospital waters**. Much of the talk concerned the lessons learned from a well-publicized outbreak in Belfast in which four neonates died.

Investigation of components such as solenoids, flow straighteners and sensors has led to improved tap design and work is ongoing at Porton Down using a test rig with 27 tap outlets. Under-used water outlets have also been identified as a source of contamination and removal or twice-weekly flushing of these is advised.

The session ended with a cautionary note: although anti-*Pseudomonas* taps are now being marketed, the evidence for their efficacy is lacking.



Louise Hill-King

*Sf*AM Summer Conference 2015

29 June to 2 July 2015, Intercontinental Dublin

Monday 29 June 2015

11:00 – 17:00	**Workshop: Opportunities for impact**
18:00 – 19:00	***The growth and applications of functional metagenomics*** Don Cowan, *University of Pretoria, South Africa*
19:00 – 20:00	**Drinks reception and buffet**
20:30 onwards	**Quiz night**

Tuesday 30 June 2015

SESSION 1 **DEVELOPING A NEW UNDERSTANDING OF FERMENTATIONS**

Chair: Tim Aldsworth

09:00 – 09:35	**Use of metagenomics to study microbial fermentations** Danilo Ercolini, *University of Naples, Italy*
09:35 – 10:10	**Functionality of multi-level diversity in complex fermentation starters** Eddy Smid, *Wageningen University, The Netherlands*
10:10 – 11:05	**Tea, coffee and trade exhibition**
11:05 – 11:40	**Food poisoning and fermented foods** Kathie Grant, *Public Health England, UK*
11:40 – 12:15	**Systems engineering of cheese consortia** Alan Ward, *Chung Ang University, Korea*
12:15 – 13:15	**Lunch, trade exhibition and posters**

SESSION 2 **IMPROVING FERMENTATIONS**

Chair: Linda Thomas

13:15 – 13:50	**Generating superior yeasts for the fermentation industry – from beer, wine and chocolate to biofuels** Kevin Verstrepen, *University of Leuven/VIB, Belgium*
13:50 – 14:25	**Development of phage-insensitive cultures for the fermentation industry** Paul Ross, *TEAGASC, Ireland*
14:25 – 14:45	**Tea, coffee and trade exhibition**
14:45 – 15:20	**Bacteriocins: providing innate immunity for food systems** Colin Hill, *University College Cork, Ireland*
15:20 – 15:55	**Gene-trait matching: predicting functional properties of microbial strains** Sacha van Hijum, *NIZO food research, The Netherlands*
16:00 – 17:00	**Attended poster session A**
17:00 – 18:00	**Early career scientist session**
17:00 – 19:30	**Trade exhibition with wine, buffet and a competition**

Wednesday 1 July 2015

SESSION 3 **THE ROLE OF MICROFLORA**

Chair: Damilo Ercolini

09:00 – 09:35	**The role of non-starter organisms in cheese ripening** Paul McSweeney, *University College Cork, Ireland*
09:35 – 10:10	**Commercial- scale cucumber fermentation without sodium chloride** Ilenys Perez-Diaz, *USDA-ARS Food Science Research Unit, USA*

FERMENTED FOODS and BEVERAGES

Thursday 2 July 2015

10:10 – 10:45	**Cocoa bean fermentation** Luc de Vuyst, *Brussels University, Belgium*
10:45 – 11:05	**Tea, coffee and posters**
11.05 – 11.40	**Sourdough fermentation** Marco Gobbetti, *University of Bari, Italy*
11:40 – 12:15	**Adaptation of the starter culture *Staphylococcus xylosus* to meat products** Régine Talon, *INRA, France*
12.15 – 13.15	**Lunch and networking**
13.15 – 13.50	**Lactic acid bacteria – friend or foe in wet cassava starch** Andrew Graffham, *NRI, University of Greenwich, UK*
13:50 – 14:25	**Fermented soya bean products** Rob Nout, *Wageningen University, The Netherlands*
14:30 – 15:30	**Student Member oral presentations**
15:30 – 16:30	**Attended poster session B**

*Sf*AM AWARD LECTURES

Chair:	Christine Dodd, *SfAM President*
16:30 – 16:35	**Introduction to the New Lecturer Research Grant** Christine Dodd
16:35 – 17.10	***Sf*AM New Lecturer Research Grant Lecture** Robert Fagan, *University of Sheffield, UK*
17:10 – 17:15	**Introduction to the W H Pierce Prize** Christine Dodd
17:15 – 17:45	**W H Pierce Prize Lecture** *TBC*
17:45 – 18:15	**Annual General Meeting**
19:00 onwards	**Drinks reception and conference dinner**

SESSION 4 TRADITIONAL FERMENTATIONS

Chair:	Christine Dodd, *University of Nottingham, UK*
09:00 – 09:35	**Bacterial diversity and functions during production of traditional fermented West African cereal foods** Folarin Oguntoyinbo *Univeristy of Lagos, Nigeria*
09:35 – 10:10	**Fermentation of cassava** Linda Nicolaides, *NRI, University of Greenwich, UK*
10:10 – 10:45	**Tea and coffee**
10:45 – 11:20	**The microbiology of Bandji, traditional palm wine of the palm tree *Borassus akeassii*** Irene Ouoba
11:20 – 11:55	**Craft brewing – an industry sector in fermentation** Jerry Avis, *University of Nottingham, UK*
12.00 – 13.00	**Lunch and depart**

Closing date for registration:
Friday 12 June 2015
Book now at:
www.sfam.org.uk/summer

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*Sf*AM **AGM Agenda**

84th Annual General Meeting of the Society for Applied Microbiology
1 July 2015, 5.45 pm Intercontinental Dublin.

1. Apologies for absence.

2. Approval of minutes published September 2014 *Microbiologist* of the 83rd Annual Meeting held in Brighton, 2014.

3. Matters arising from the previous minutes.

4. Report of the Trustees of the Society 2014:

(i) Report of the Honorary President.
(ii) Report of the Honorary General Secretary.
(iii) Report of the Honorary Meetings Secretary.
(iv) Report of the Honorary Treasurer.

5. Adoption of the 2014 Annual Report.

6. Election of new Members (including Honorary Members), deaths and resignations.

7. Nomination and election of Trustees:

(i) Honorary General Secretary.
(ii) Honorary Meetings Secretary.
(iii) Honorary Treasurer.

8. Nomination and election of new Committee Members.

9. Any other business*.

****To ensure the meeting keeps to time items of any other business must be raised with the Honorary General Secretary at least 24 hours before the start of the meeting.***

GRANTS SPOTLIGHT: SCIENTIFIC MEETING ATTENDANCE GRANT OR PRESIDENT'S FUND?

DEADLINES IN 2015
PRESIDENT'S FUND: 19 June, 25 September, 31 December
SCIENTIFIC MEETING ATTENDANCE GRANT: 4 weeks prior to event

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

The **Scientific Meeting Attendance Grant** will fund your travel, accommodation and registration fees at any relevant scientific meeting, including *Sf*AM meetings, up to £300. If caring responsibilities would prevent you from attending, we will increase the upper limit to £600 and include the cost of alternative arrangements for care of your dependent(s).

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

For more information about all our grants and awards, please visit **www.sfam.org.uk/grants**.

PECS events at the Summer Conference 2015

Where: **Dublin, Ireland**
When: **29 June to 2 July**

Summary of events

Monday 29 June:	Icebreaker event (after the *JAM* Lecture)
Tuesday 30 June:	17:00 – 18:00 Early Career Scientist Workshop – Creating your personal brand
Wednesday 1 July:	Open PECS Committee Meeting

As the summer approaches you may have started to think about and plan for the conferences you will be attending. We hope that many of you reading this will have the opportunity to attend the S*f*AM Summer Conference which this year will be held in Dublin. As part of the conference the PECS Committee intend to provide a full and varied programme of activities for Student and Early Career Scientist Members of the Society.

Our programme of events kicks off on the evening of Monday 29 June during the drinks reception after the *Journal of Applied Microbiology* Lecture. We will be holding an icebreaker session which will take place in a location adjacent to the main reception and is an excellent opportunity to meet some of the other Early Career Members of the Society who will be attending the conference. It is a great way to meet people and a fun start to the week.

Our main event for the conference will be the Early Career Scientist session which takes place on the afternoon of Tuesday 30 June from 5pm – 6pm. This year's session will focus on networking and ways to improve your employability.

In the current economic climate it is important for all of us to ensure that when we apply for jobs we give ourselves the best chance possible. Following on from last year's extremely popular CV clinic, we plan to expand to discuss the importance of 'Creating your own self brand'. One area which is becoming more important in recent years is how you present yourself on social media platforms such as Twitter, LinkedIn and Facebook. Use of social media can be a double-edged sword, carefully handled they can be an excellent way of promoting yourself to potential employers; however, photos of drunken nights out can be off-putting. The session will be a workshop that will cover several different ways of creating your self brand and making a good impression at an interview and through networking.

You will learn how to apply marketing principles to develop your personal brand and create a virtual platform. Understand and highlight your core and transferable skills to utilize as part of the STAR interview approach. Recognize the alternative ways to find relevant job opportunities and how to use social media effectively. Identify the benefits of digital networking strategies to enhance your job search with LinkedIn 101. Finally, apply what you've learnt, join in and network at the 30 second pitchathon – how many connections will you make? We hope you find this workshop useful!

On Wednesday we will be holding our annual open PECS Committee Meeting that all students and Early Career delegates are encouraged to attend. You will have the chance to meet us all in person as well as find out how you can get involved with PECS and share your ideas and feedback.

Finally, if you want to ask us any questions about the events for Early Career Scientists at the Summer Conference or anything else to do with PECS please email us at **pecs@sfam.org.uk**. We hope to see as many of you as possible in Dublin in June.

Ali Ryan
PECS Publications Officer

Fermented food and drink: an overview

If you were to ask someone to list their top 10 favourite foods and drinks, I do not doubt that fermented ones would figure highly in that list. The range of fermented products is extensive and, to a non-microbiologist, in some cases quite surprising. Even as a recently graduated microbiologist, some years ago, it came as a surprise to me to learn that some of my favourite foods were actually the result of a fermentation process! Fermented foods and drinks include as diverse a range of products as chocolate and kimchi, in addition to the usual suspects of bread, beer, cheese, wine and yoghurt, not to mention all of the traditional fermentations such as manioc. Arguably, fermented foods and drinks could be described as the first biotechnology, with evidence suggesting a fermented drink being made as far back as 6,000–7,000 BC in China (McGovern *et al*., 2004). It has been estimated that fermented foods and drinks form up to 30% of daily food intake globally (Campbell-Platt, 1994).

Microorganisms make use of fermentative pathways as a means to regenerate intracellular $NAD(P)^+$ supplies, without using oxygen as a terminal electron acceptor, since without a ready supply of this molecule metabolism would cease (Johnson & Steele, 2001). The range of metabolites produced as a result of the regeneration of $NAD(P)^+$ through fermentation is very broad. Generally in food and drink fermentations, we make use of organisms that excrete lactic acid, acetic acid, ethanol or CO_2 as their major metabolic products. The benefits of these fermentations in food production are numerous. Acidification, as well as adding a tangy flavour to a food can also help to suppress the growth of spoilage organisms and pathogens. Production of ethanol also aids in suppressing spoilers and pathogens, and would have helped to provide a safe source of potable liquid, quite apart from its mood-altering effects. Moreover, some of the organisms involved in food fermentations are known to produce inhibitory compounds, such as bacteriocins, that can help to

Arguably, fermented foods and drinks could be described as the first biotechnology

suppress spoilers and pathogens. Production of CO_2 in bread helps to make a lighter and more digestible product than a plain flour and water dough, as anyone who has attempted to make, bake and eat dough as a child can attest! The fermentation of lactose in milk by lactic acid bacteria (LAB) can also help to make fermented milk products more digestible, especially for the lactose intolerant, by reducing the load of this sugar in the product.

A very diverse range of microorganisms are involved in the different types of food fermentations. In some fermentations a simple microflora is used, for example, *Saccharomyces cerevisiae* – baker's yeast – is used to make bread. In other fermentations, though, the microflora can be very complex, for example, in chocolate many species are involved and species succession occurs through three broad phases before the final product is achieved. In the initial phase of cocoa fermentation, yeasts ferment the polysaccharide pulp around the beans, yielding ethanol and breaking down the pulp. Succession of yeasts by LAB brings about the second phase of the fermentation, during which citrate is metabolized and a slight rise in pH occurs. This rise in pH, and inclusion of oxygen by physical turning of the bean mass, allows the third phase of the fermentation to develop. In the third phase of fermentation, acetic acid bacteria (AAB) predominate and convert the ethanol to acetic acid. The combination of elevated temperature as a result of AAB activity, and diffusion of ethanol and acetic acid into the beans, kills the embryos and starts to impart the cocoa flavour through the breakdown of cell walls and release of endogenous enzymes (Thompson *et al*., 2001; Crafack *et al*., 2014). This process is complex and all phases are required to yield a good cocoa flavour. However, the majority of species involved in the fermentation do not seem to come from the plant material itself, but are inoculated from fomites during harvesting and fermentation.

In commercial fermentations, especially, such as dairy, beer, and to some extent wine, a starter culture is added to the system so as to achieve a more homogeneous and reproducible product. Even when a starter culture is used, the native microflora of the raw material can still have a significant influence on the organoleptic properties of the finished product. Pre-production processing can help to ameliorate the influence of the native flora; for example, pasteurization will reduce the numbers of native milk microbes in dairy fermentations and hop boiling will reduce the numbers of bacteria and wild yeasts during the production of beer. This pre-processing can help the manufacturer to make a product with more reproducible characteristics and also improve safety by reducing the numbers of potential pathogens in the raw material. However, pre-processing will also reduce species diversity and so may reduce the range of complex secondary, flavour-enhancing, fermentations that can occur.

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Food fermentations contribute a significant sum to the global economy

Recently, in Western economies a movement has developed towards the production of "artisanal" products, such as breads, beers and cheeses, which make much more use of the native microflora. On the other hand, manufacturers of traditional fermented foods, such as chocolate and kimchi, have been seeking to isolate starter organisms that could help to yield a more reproducible product. To this end, modern molecular techniques are helping to yield new insights into the developing microflora of these products over and above what is possible with traditional isolation and identification techniques.

Fermentation of both foods and drinks can run into a variety of problems. Bacteriophage can kill important species and lead to a failure of the process, for example, in dairy fermentations. Alternatively, "wild" strains of the production organism may contaminate the process and outcompete the intended strain leading to unpredictable composition and flavour profiles, such as, in beer and wine fermentations. Moreover, some strains of *Saccharomyces*, known as "killer yeasts" are able to produce lethal factors which will kill receptive strains of *Saccharomyces* and potentially lead to undesirable flavours or poor alcohol yields. Consequently, impeccable hygiene must be maintained at all times during production to prevent these problems from arising. In addition, biotechnologists are seeking new strains of production organisms that are resistant to these problems whilst still offering desirable flavour and safety profiles.

In conclusion, food fermentations contribute a significant sum to the global economy in addition to yielding products with enhanced flavour, texture, shelf life and safety. The range of foods which are produced using fermentation is extensive, and both the microflora, and the metabolites, involved are diverse. Starter cultures are used to initiate some fermentations and to yield reproducible products, but are not available for all fermentations. Consequently, both traditional and molecular techniques are being employed to better understand the microflora of complex fermentations and thus hopefully allow more accurate control of them for commercial processing.

Tim Aldsworth
Coventry University

In chocolate many species are involved and species succession occurs through three broad phases before the final product is achieved

The place of fermented foods in the food supply of developing countries

Figure 1

In developing countries the preparation of fermented foods is a widespread tradition, a sustainable post-harvest value addition and a component of food security. The simple microbial and enzymatic processes responsible for the fermentation processes are widely used for the production of foods and flavourings as well as improved preservation, palatability, digestibility, detoxification and nutritional intake. As a relatively efficient, low-cost process that increases shelf life and decreases the need for refrigeration it is a highly appropriate technique for use in developing countries where sophisticated equipment is limited.

Countries in these regions have many years' experience producing different traditional fermented foods either naturally or by adding starter cultures that contain various microorganisms. Low-tech mechanical devices are still predominantly used for production and include grating, pressing and drying, such as the small-scale techniques for cassava fermentation (Figures 1 and 2).

If the endeavour to supply sufficient food to a growing human population is to have a less deleterious effect on the agricultural landscape then a proper understanding of under-utilized crops and new methodologies of production is certainly required. With the world human population drastically increasing, the corresponding reduction in available agricultural space has far-reaching consequences on food supply. The processing of a large diverse agricultural harvest is vitally important to support food availability. This becomes even more apparent in the developing world region where drought-resistant crops such as cassava (*Manihot esculenta*) and sorghum (*Sorghum bicolor*) are grown to provide a basic diet for over half a billion people. Ironically, it appears there is limited industrial large-scale processing for most of the agro products leading to large economic losses of up to 30–50%. This, as a consequence, leads to poverty and hunger.

Traditional food fermentation methods, and the value addition to harvested agricultural produce, has a long history throughout the developing world and fermented foods are produced by many household or small-scale cottage-type businesses as an important part of the diet. Not only do they make a major contribution to the protein requirements of the population but are also an important source of fibre, carbohydrate, fatty acids, minerals, vitamins and antioxidants.

Although vitamin fortification introduction in most industrialized countries has ensured specific vitamin-

deficient diseases in the general population are rare, in the developing world these continue to be major health problems. Many of these diseases are caused by a lack of B vitamins which are not synthesized in the body and must be obtained by nutrition, in fact biotin deficiency is almost never seen outside the developing world. However, the microbial fermentation of vegetable proteins leads to a significant increase in this class of vitamins, including an estimated 14-fold increase in biotin levels (Etkin, 2008). Methionine- and lysine-producing lactobacilli strains have also been isolated and recommended for use in the production of traditional fermented cereals consumed as infant complementary food (Odunfa *et al*., 2001). By way of supplying these, and other vitamins, antioxidants and essential amino acids, fermented foods play an important role in the prevention and development of chronic diseases such as cancer, cardiovascular disease (hypertension) and the pathogenesis of HIV (Willcox *et al*., 2004; Najgebauer-Lejko *et al*., 2014).

Consumption of contaminated foods contributes to a vicious cycle of disease and malnutrition particulary affecting poorer regions. Phylogenetic diversity studies on the microbiota responsible for the biochemical changes during the fermentation of these foods, reveal a predominance of members of Firmicutes especially the

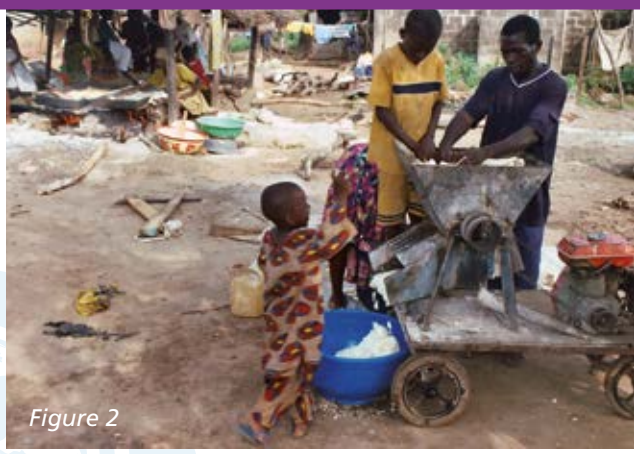

Figure 2

diverse strains of Lactobacillales and Bacillales. Whilst the Western world can afford to can and freeze much of its food, developing economies are becoming increasingly more reliant on the fermentation process to meet their nutritional and food safety needs. Lactic acid bacteria (LAB) is a common method of preserving many foods in Africa due to the high prevelance of bacteriocinogenic strains formed in the fermentation process.

During food processing, the production of microbiologically safe and stable foods is a daunting challenge to traditional food fermentation in the developing world as a result of poor sanitation, regulation and standardization. Apart from the regulation of processes using good manufacturing practices (GMP), the use of starter cultures in complement with back-slopping (inserting a small fraction of a previous fermentation into the start of a new fermentation) to replace uncontrolled natural fermentation has been suggested. This will make fermentation predictable, encourage well-defined microbial strains to dominate, improve consistency, safety and nutritional qualities. Overall, this can increase the attractiveness of traditional fermented foods and guarantee livelihood.

The place fermented foods has in the developing world is vital, as a profitable part of the economy and in terms of food quality, preservation, nutrition and flavour. The technology employed still needs to be further developed to enhance processing speeds and minimize waste in a rural poor-resource setting. The evidence relating to health benefits of the microbial fermentation of foods must be correctly relayed to communities, and investigations into appropriate starter cultures and back-slopping methods will ensure safe practice.

What is certain is that many people from developing countries will be continuing to enjoy the benefits of consuming fermented foods and drinks for a very long time.

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A renaissance in fermented milk: **KEFIR makes a comeback**

It's easy to grow at home and its advocates say it has amazing health benefits. High in probiotics, people drink it to control digestive complaints. What is this creamy, cottage cheese-like substance and why is it taking up residence in so many fridges?

Kefir is a fermented drink made by adding kefir grains to milk. Research has suggested that kefir grains are formed by the attachment of bacteria and yeast, which come together under biofilms in a matrix of proteins, sugars and lipids. Added to fresh milk, these grains ferment the milk, creating a slightly alcoholic, acidic and fizzy yoghurt-like drink. Following fermentation, little lactose remains in the milk, so most lactose-intolerant people are able to drink it.

Kefir has been popular in Russia for hundreds of years, with its origins in the Caucasus Mountains. It first garnered widespread acclaim in the early 20th century – the first kefir drink was bottled and sold in Moscow in 1908. Kefir is now enjoying a second wave of popularity; a simple search returns almost 8 million web pages, most of which sing the praises of this microbial product. And with 100,000 videos on YouTube to help you get started making kefir, it's definitely trending.

Although it's easy to grow, kefir can be difficult to study because the bacteria and yeast form complex symbiotic relationships. Despite this, researchers were already investigating kefir for its effect on the microflora before the term probiotics was coined. In 1984, an early study in *Journal of Applied Bacteriology* examined the make-up of the kefir grains. It showed that the yeasts and lactobacilli contained in kefir grains are held together in a matrix that is predominantly composed of carbohydrate produced by the bacteria. These carbohydrates separate out the yeast and bacteria in the grains, resulting in sheet-like structures, with yeast on one side and bacteria on the other.

Knowing the structure and function of kefir grains could open the door to useful applications. In 2007, research published in *Journal of Applied Microbiology* suggested that kefir could be used to "extend the shelf life of dairy products with reduced or no salt content."

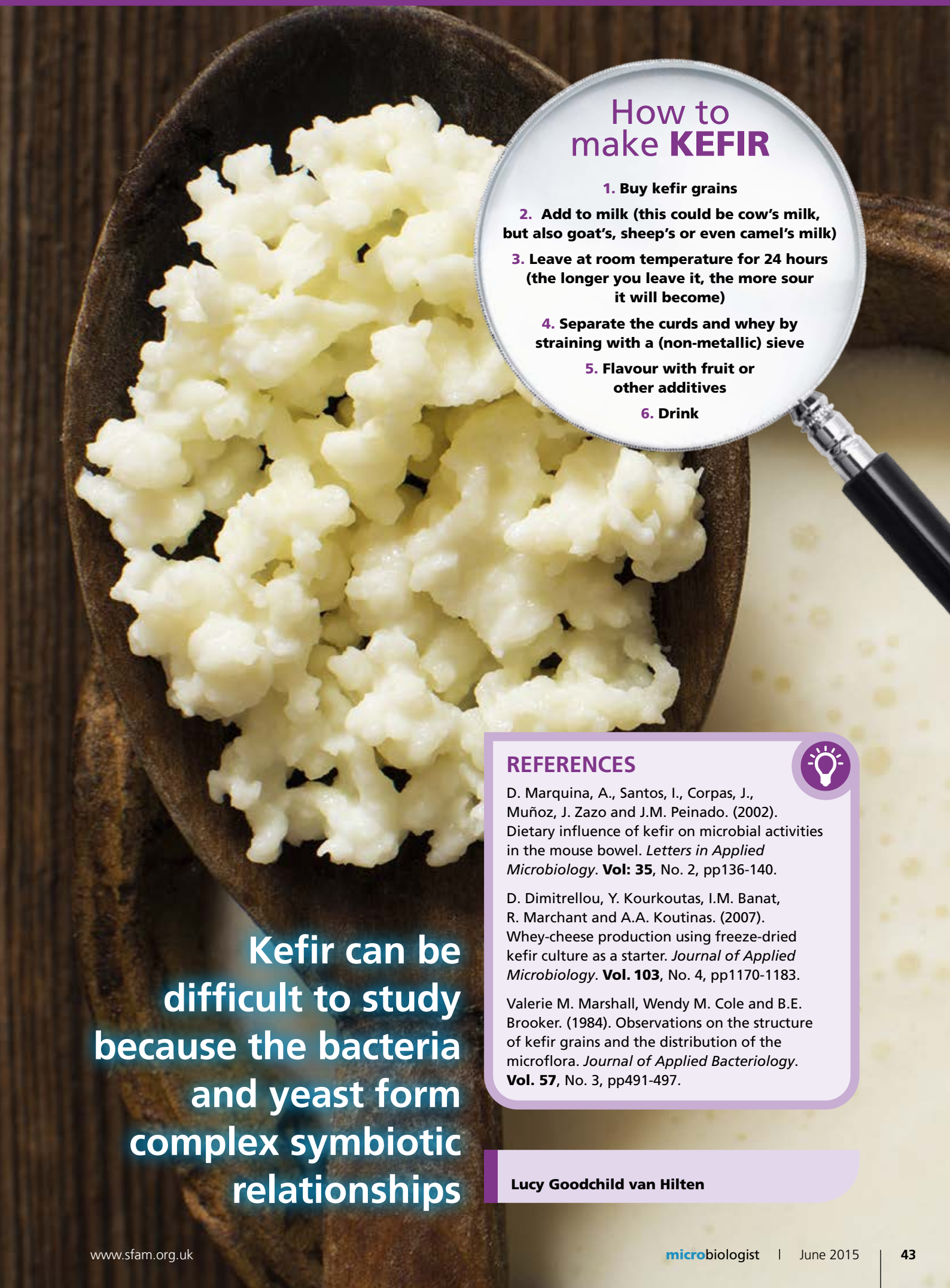
Rather than being focused on the composition of the grains, most attention has been on the health benefits of kefir. The first long-term study of the effects of kefir on mouse gut microflora was published in *Letters in Applied Microbiology* in 2002. The study revealed that mice that consumed kefir had a significantly increased number of lactic acid bacteria in the gut. Kefir also decreased the number of sulfite-reducing *Clostridia* by 100-fold.

The research so far supports some of the health benefit claims, which are in line with the reported benefits of probiotics. Reported benefits include boosting the immune system, killing *Candida*, improving lactose digestion and combating allergies. The calcium it contains may help strengthen bones, the tryptophan could help you relax and the vitamins may have various health benefits.

While this is tempting to test out at home, the research on the health benefits of kefir is still limited, and many of the claims could also apply to milk or yoghurt. Yet sales of kefir have spiked: Lifeway, the company that supplies most of the USA's kefir stock, reported sales of $130 million in 2014, up from $58 million in 2009. So what makes kefir so special? Perhaps the answer is in its alternative production.

'Home-grown' is enjoying a renaissance – more and more people are taking to their allotments, gardens and balconies to cultivate their own food, maintaining control over what they eat and supporting the growing field of agroecology. This approach to food extends to other areas too – urban beekeeping has seen a significant increase in recent years.

Kefir can be grown easily at home. Buying kefir grains online is straightforward, and the only ingredients you need in addition to the grains are milk and additives to enhance taste of the drink. Kefir seems simple, cheap, healthy and appealing. Further research is needed to uncover the secrets of this drink, but for now millions of people are happy to test it out at home.



How to make **KEFIR**

1. **Buy kefir grains**
2. **Add to milk (this could be cow's milk, but also goat's, sheep's or even camel's milk)**
3. **Leave at room temperature for 24 hours (the longer you leave it, the more sour it will become)**
4. **Separate the curds and whey by straining with a (non-metallic) sieve**
5. **Flavour with fruit or other additives**
6. **Drink**

Kefir can be difficult to study because the bacteria and yeast form complex symbiotic relationships

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Lucy Goodchild van Hilten

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Full details of all the Society's grants and awards, together with application forms, can be found on the website at **www.sfam.org.uk/grants**.

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

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CAREERS

From rolling hills to lion mountains

Microbiology perhaps wasn't the most common profession for someone growing up in the Cotswold town of Wotton-Under-Edge, although the town does have a link with one of the microbiological pioneers – Edward Jenner. Jenner was educated in Wotton and spent much of his life in the nearby town of Berkeley so little surprise that his work and the history of vaccination had an ever-present influence on my own education.

The UK Foot and Mouth Disease outbreak of 2001 also piqued my interest in microbiology as, coming from fairly rural surroundings, it was fascinating to watch and try to understand the control measures being put into place in my immediate environment. I have vivid memories of dipping my feet in foul-smelling disinfectant when going into school and other public buildings, as well as watching tens of pyres smoulder through the night from the vantage of Wotton Hill. Even though the amount of microbiology taught in the curriculum at the time was fairly low, I was still fortunate to be able to focus my self-directed learning to microorganisms and disease – no matter how tenuous the link! These relatively brief experiences fuelled my decision to study microbiology more in depth when choosing what to study at university. The course that I chose focused on the more exotic pathogens and neglected tropical diseases, and it was during this time that I was first introduced to the biology and pathogenesis of some of the organisms that would later form my niche.

Looking for a job in such a specialist area as a graduate seemed a daunting task, especially considering the negative environment brought about by the economic recession at the time. For months I was unable to get acknowledged by employers let alone secure interviews. I did, however, get my chance in December of that year with an interview for what was then the Health Protection Agency at Porton Down near Salisbury. I was familiar with the work of the Special Pathogens Reference Unit from my studies and from people coming to speak at the university about their work with exotic pathogens.

Port Loko team leaders wearing the full PPE used by the healthcare workers working in the Ebola wards: Daniel Carter on the left and Bruno Pichon on the right.

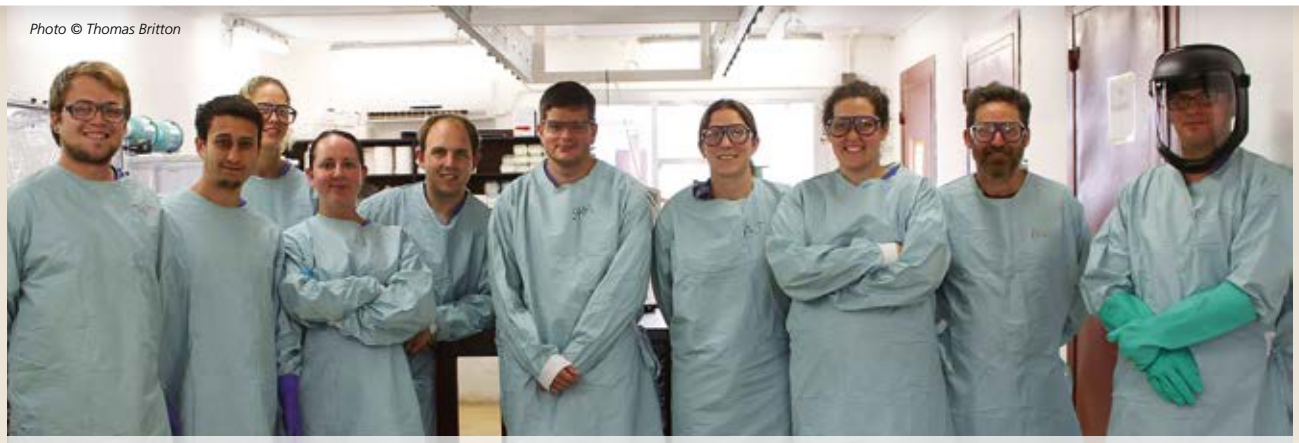

Photo © Thomas Britton

Port Loko Lab team in the lab just before it went live: (left to right) Thomas Britton, Marlon Martinez, Kimberley Steeds, Lisa McLean, Daniel Carter, Samuel Collins, Katy-Ann Thompson, Lauren Cowley, Bruno Pichon and Richard Loy.

I started the morning of the interview feeling positive about the day ahead – until I stepped outside. There had been heavy snowfall overnight and I spent two hours shovelling my way off the driveway and 50m further down to the main road. Needless to say, my positive mood melted away with the snow as all my preparation work had been forgotten by the time that I got to the interview. Despite this, I must have said something right because I received an offer of employment through the post on Christmas Eve – not so many "Bah! Humbug!" comments from me that year!

After another frantic episode of trying to relocate to Salisbury straight after the Christmas period, I finally started working for what is now Public Health England's Rare and Imported Pathogens Lab (RIPL) as a Biomedical Scientist in mid-January 2010. I wasn't sure what to expect as I had only ever had an hour-long visit to a diagnostic lab before and I knew that the assays and techniques used here were somewhat different to the norm. For example, the laboratory had a substantial repertoire of in-house PCR and immunofluorescence assays – processes that I had only encountered in theory. I joined the department at the height of the 2009 anthrax outbreak in injecting drug users in the UK and this was my first exposure to diagnostic microbiology. With the high number and variety of samples all having to be processed using Class III microbiological safety cabinets my learning curve was steep to say the least. It was fascinating to have such a personal insight and impact on the evolving situation in Scotland and then as cases began to appear in England. Working intensively in this environment and with such experienced colleagues has given me a firm foothold and has proven useful so many times throughout my relatively short career.

Using these skills I've been fortunate enough to be involved with some of the more novel causes of infection in returning travellers that RIPL specializes in. For example, I have worked on a wide range of cases such as imported cases of Congo-Crimean Haemorrhagic Fever virus (Afghanistan, 2012 and Bulgaria, 2014), several Dengue virus outbreaks (India/Pakistan and Madeira, 2012), Zika virus (South Pacific, 2014) and a reappearance of anthrax in intravenous drug users in 2012. I have also been involved in the 2014 Ebola outbreak in West Africa through the testing of samples from infected healthcare workers (August and December 2014).

I was privileged enough to be seconded to Sierra Leone as part of PHE's involvement with the UK Ebola response. I was deployed to the district of Port Loko during November and December 2014 to assist with the establishment of an Ebola diagnostic laboratory in a new Ebola treatment centre. When our team arrived at the centre all we found was a construction site, but within two weeks we had set up a UK-standard diagnostic laboratory. The quick turnaround was outstanding. Not only were we able to provide a service to the local treatment centre but we also had the capability to test hundreds of samples from the community. The success that we were able to achieve is testament to the efforts of all involved and especially the dedication and hard work of my team mates. I've always been sceptical when speaking to others who have done humanitarian work about the impact on their own lives. However, I honestly have to put this down as the most insightful and humbling experience of my life so far and I would definitely recommend such work to others when it becomes available.

Reflecting on my career, especially when I was asked to write this piece, I always focus on the fact that I've only been working for five years and that I have little to say. However, having to write to a word limit quite poignantly reminds me of what I have done, what I have achieved and how fortunate I have been to have had these experiences. I was always concerned that specializing too far would limit my opportunities and make me less versatile; on the contrary, I've quickly found that if you're engaged and passionate about your niche there are a plethora of avenues to explore.

Dan Carter
Public Health England

STUDENTS into WORK GRANT REPORT

Linezolid resistance in staphylococci

Following completion of the third year of my Microbiology degree at Glasgow Caledonian University, I was generously awarded funding from SfAM in order to gain experience as part of a research team working on a six-week project within the School of Health and Life Sciences. I am interested in pursuing a career in microbial research and this project gave me insight into working with both pathogens and antimicrobials in a research setting.

Linezolid, the first antibiotic of the synthetic oxazolidinone family, is an antimicrobial agent effective against a number of multi-drug resistant Gram-positive organisms including *Staph. aureus* strains which are no longer fully susceptible to more widely used antimicrobials such as meticillin and vancomycin (Diekema & Jones, 2001). Linezolid functions by inhibiting bacterial protein synthesis through the inhibition of ribosomal initiation complexes during translation, in the case of *Staph. aureus*, complexes involving 70S ribosomes are inhibited (Clemett & Markham, 2000). Against most susceptible organisms linezolid has a bacteriostatic effect (Clemett & Markham, 2000). Given that linezolid is used when resistance to other antimicrobials renders their use ineffective, the possible occurrence of resistance to linezolid is troubling, though resistance levels remain low at 1.2% for coagulase-negative staphylococci and *Staph. aureus* (Flamm *et al*., 2011a; 2011b).

The work carried out during this project aimed to investigate the occurrence of linezolid resistance among clinical staphylococcal isolates from around Scotland.

Forty two clinical staphylococcal strains, comprising 11 *Staph. epidermidis* and 31 *Staph. aureus* (of which 21 had been submitted to the Scottish Meticillin Resistant *Staphylococcus aureus* Reference Laboratory (SMRSARL) in Glasgow for further investigation due to atypical linezolid susceptibility profiles) were tested for linezolid susceptibility using several methods which included MIC, MBC, time-kill assays, antibiotic-mediated cell death in biofilms and exposure assays to evaluate MIC-creep.

The MIC of the strains were determined both by the microbroth dilution method, using 96-well microtitre plates, and through the use of linezolid E-test strips. The MBC were determined by sub-culturing from the MIC plates onto fresh antibiotic-free agar. From these results it was apparent that no resistance was present among any of the strains, therefore the reduced susceptibility profiles originally detected in those strains submitted to SMRSARL were likely to be the result of exposure to linezolid during treatment before subsequent loss after sub-culturing in the absence of linezolid.

Six *Staph. aureus* strains were tested using a serial-passage technique that was continued for 12 days to determine whether linezolid resistance was inducible. Aliquots of $1x10^5$ cells/ml of each of these strains was inoculated into 0.5 ml of broth supplemented with 0.5 mg/l linezolid, equivalent to 0.5xMIC of each isolate. Each day, the previous culture was used to inoculate a fresh broth containing 0.5 mg/l linezolid until day 8 when the MIC, after re-testing by E-test, had risen from 1 mg/l to 2 mg/l. At this point the cultures were inoculated into broth containing 1 mg/l linezolid. By day 12, the MIC for each strain remained at 2 mg/l, but this was still an increase from the starting MIC of 1 mg/l. As demonstrated in this experiment, it is likely that exposure to linezolid during treatment prompted temporary inducible resistance in those isolates which initially presented atypical susceptibility profiles.

Three of the isolates were subjected to linezolid time-kill assays: i) culture turbidity measured in parallel with viable-cell counts over 7 hours using a single isolate exposed to 1xMIC alongside an untreated

control culture, ii) culture turbidity of a control strain and two test strains exposed to a range of linezolid concentrations, including untreated controls, in replicate for 15 hours, were measured using an automated plate-reader. The assay allowed the study of multiple linezolid concentrations above and below the MIC of each isolate, allowing observation of a slowing of growth at concentrations below the MIC and minimal killing effect at concentrations greater than the MIC. The results indicated that linezolid had a bacteriostatic effect in keeping with the literature.

Sixteen *Staph. aureus* and 8 *Staph. epidermidis* isolates were grown as biofilms (12 wells per isolate) in duplicate using 96-well microtitre plates incubated at 37°C for 24 hours. After incubation the biofilms were washed and treated with linezolid at 1x, 4x and 10xMIC in triplicate with a control of untreated biofilms. The plates were again incubated for 24 hours at 37°C before being washed and stained with resazurin; a metabolic dye which is reduced to resorufin, a highly fluorescent compound, following incubation (Mariscal *et al*., 2009). The fluorescence of treated and untreated biofilm-associated cells was compared to give an indication of linezolid-induced cell death. While all of the strains showed increased tolerance to linezolid after biofilm formation, a number of strains demonstrated minimal loss of cell viability following exposure to the antibiotic even at concentrations 10x greater than would normally be required to inhibit planktonic populations.

From the results obtained during this study it can be seen that: i) although only a low number of isolates were tested, no resistance to linezolid was detected, ii) when reduced susceptibility had previously been detected in clinical isolates this was not maintained after removal of the antibiotic, iii) linezolid was bacteriostatic in its activity, even at high concentrations, iv) there was evidence to suggest that prolonged exposure to linezolid would lead to reduced susceptibility and v) biofilm associated cells were tolerant to high linezolid concentrations.

I would like to thank SfAM for awarding me the Students into Work Grant as it has allowed me to gain first-hand experience in a research lab environment as well as giving me an insight into the possibility of continuing on to study at PhD level. I would also like to thank my supervisor Dr Sue Lang for offering me the opportunity to undertake this project and thanks are also due to Andrew Anyakwo, Kirsty Skinner and Victoria Cruikshank for their invaluable help throughout the project. The skills I have learned on this project and the practical experience of skills from my previous studies should prove invaluable to me in the future.

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Stuart Simms
Glasgow Caledonian University

Membership CHANGES

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DEATHS
We were saddened to learn of the deaths of the following Member of the Society:

S. Morgan-Jones

Contributions and feedback

The *Microbiologist* Editorial Group welcomes contributions and feedback from all our Members.

Please contact **paul@sfam.org.uk** with ideas.

Corporate NEWS

The latest news, view and microbiological developments from our Corporate Members

Bacterial Identification and Characterisation

APHA Scientific offers a comprehensive service that provides detailed bacterial identification and characterisation for a variety of applications including the development of vaccines, characterisation of probiotics and intellectual property protection. The service includes:

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Real-time qPCR with BioConnections

BioConnections and Primerdesign, two British companies, are making real-time PCR more accessible for more tests in more laboratories.

The genesig q16 is a 16 well real-time quantitative PCR machine. Although small (it has a 12 cm footprint) and incredibly easy to use this real-time PCR machine matches larger machines in performance.

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To find out more about these new products and how they can help in your laboratory testing please visit the BioConnections website, alternatively contact us by email or by telephone.

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Redipor Prepared Media; Meeting Customer and Regulatory Expectations

Manufactured at Cherwell Laboratories' ISO 9001 registered site in Bicester, Redipor® Prepared Microbiological Media has built a reputation within the pharmaceutical and related industries for high quality products supported by technical expertise and a friendly, customer-focused service. The 2015-16 Redipor price list is now available on request from www.cherwell-labs.co.uk alongside further information on Cherwell's other environmental monitoring, validation and cleanroom bio-decontamination products.

Offering an extensive selection of both industry standard products and those with a unique formulation or presentation, the Redipor range provides a flexible solution for environmental monitoring, sterility testing of products, operator validation and process validation. The range includes petri dishes (55mm, 90mm and 140mm) and contact plates, plus bottled media, broth bags and ampoules, with all products subjected to a full array of QC tests, including comprehensive growth tests. With over 40 years' industry experience, Cherwell can also discuss specific requirements and work with individual customers to ensure their needs are met.

Despite significant growth, fuelled by increasing demand for Redipor prepared media throughout the UK and across Europe, Cherwell has retained flexible production methods and managed stock levels, ensuring they can continue to deliver cost effective, timely solutions for customers of all sizes.

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Laboratory tests on the Whitley Internal HEPA Filtration System prove that it achieves a rapid and substantial reduction in bacterial contamination of the workstation atmosphere when challenged with high concentrations of bacterial cells or spores.

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In our laboratory tests, exhaust filters of a Whitley Workstation were deliberately challenged with high concentrations of aerosolized bacterial cells and their discharge through the exhaust valve filters quantified. The results demonstrated that the system achieved a substantial reduction in bacterial loading of better than 99.999% compared to what would have been released in the absence of filters.

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New appointments extend customer offering at Lab M

21 April 2015; Heywood, UK: Microbiology media supplier, Lab M, has made a significant number of new appointments to enhance its offering of specialist products and services to customers worldwide, reflecting its continued growth as a global supplier of culture media, supplements and microbiology products.

The investment in a number of specialists joining its Research and Development (R&D), Quality Control (QC) will ensure the development of new products as well as the extension of existing ranges. Increasing the sales teams means customers will benefit from the development of a wider range of microbiology products to meet their business needs along with enhanced expertise and customer support excellence offered by Lab M's team.

Melanie Patterson joins the Lab M board as Head of Business Development, bringing a wealth of industry experience and knowledge of product management to develop Lab M's offering to meet customer requirements worldwide and drive growth in a competitive and changing market.

The company has also expanded its export sales team and recruited a new Account Manager covering the northern region of the UK to strengthen its service to customers both at home and overseas.

Further Information

Visit: www.labm.com
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Leatherhead Food Research: Food Safety & Product Integrity

Leatherhead's food safety portfolio provides a comprehensive range of products and services to help food and drink companies maintain the highest possible standards of safety and stability in their products. We provide a broad array of microbiological food testing, analysis and consultation covering microbiological food safety, training and advice, and bespoke testing, if required.

Our main focus areas include:

- Shelf life and challenge testing (including *Cl. botulinum*)
- Microbial inactivation kinetics (to aid food processing)
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- Enteric virus research and detection
- Antimicrobial screening and alternative natural preservation technologies
- HACCP training and studies including troubleshooting and audits
- Biofilm study and control
- Method development and validation of rapid detection methods
- Validating and advice on cleaning and disinfection procedures

Additionally, our Food Safety & Product Integrity Department is supported by expertise from across Leatherhead. For example, we can check the safety/shelf life of a reformulation, whilst ensuring that it meets the required sensory and nutritional properties. We can also provide regulatory advice so that the product meets the required legislation for the countries in which is it sold.

Further information

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Polywipes™ are the subject of many studies, and have successfully recovered pathogens including *Salmonella*, *Listeria*, *MRSA*, *Acinetobacter* and *Clostridium difficile* for both culture and molecular studies. They are convenient to use, and to store, and the bright blue colour ensures they cannot be easily lost in critical areas. The construction of the sponge materials prevents shedding of fragments due to abrasion.

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100 years of the bacteriophage

This year marks the 100th anniversary of the discovery of bacteriophages by British bacteriologist Frederick W Twort. In 1915 his paper "An investigation on the nature of ultra-microscopic viruses" was published in the Lancet, although it was not until 1917 that Félix d'Hérelle proposed the term bacteriophage. D'Hérelle has been credited with pioneering the development of phage therapy with the use of a phage preparation to treat dysentery.

While much work on phage therapy continued in the former Soviet Union during the 20th century, elsewhere attention turned to the development of antibiotics.

Today, however, with repeated warnings about growing microbial resistance to antibiotics, we could be seeing a resurgence of interest in the clinical use of bacteriophages. For example, the EU funded Phagoburn project was launched in 2013, with the aim of evaluating phage therapy for the treatment of infected burn wounds.

NCIMB's open collection includes a number of bacteriophages in addition to phage hosts, and we also accept bacteriophages as patent deposits under the Budapest treaty, or for confidential and secure offsite storage. For more information about depositing bacteriophages, or to find out about those that we already hold in our collection contact Dr Sam Law. **S.Law@ncimb.com**.

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Neogen introduces NeoFilm® for rapid microbial testing

AYR, Scotland, 10 March, 2015 – Neogen Europe now offers a range of easy-to-use, rapid microbial tests.

Neogen's NeoFilm® microbial tests are simple to prepare and interpret with a broad range of applications. The tests feature a streamlined workflow with easy colony enumeration. NeoFilm tests are available for coliform, *E. coli*/coliform, aerobic counts, plus yeast and mould. Each test has been validated through the AOAC Research Institute.

"NeoFilm has been designed with the user in mind, and offers advantages such as greater visual clarity and easy enumeration," said Neogen Europe's Sales & Marketing Director Dr. Steve Chambers. "Each test film is colour-coded for easy identification and the required incubation time is printed on the test to simplify the whole procedure. The advanced design of NeoFilm simplifies workflow by allowing test films to be stacked as they are inoculated. This frees up valuable bench space and eliminates any wait time between inoculation and incubation. Unlike other tests, NeoFilm doesn't require a 'spreader' and there is no gelling or set-up time, making them much easier to handle. The biggest advantage is the clarity of the results that NeoFilm provides."

NeoFilm tests detect and quantify microbiological organisms in environmental samples, process materials and finished products. The protocol is straightforward with minimal training required for microbiology teams. Diluted samples are inoculated onto thin films, incubated, and then any colonies are counted giving an accurate result with minimal work.

In its recent AOAC validation, the performance of NeoFilm for Yeast and Mould was compared to the Food and Drug Administration's (FDA's) Bacteriological Analytical Manual (BAM) reference culture procedure for its ability to detect yeasts and moulds in breaded chicken nuggets, dry pet food, orange juice concentrate, yogurt and cake mix. Results of the internal and independent laboratory studies demonstrate the NeoFilm method is an effective test for the enumeration of yeasts and moulds in the food matrices tested.

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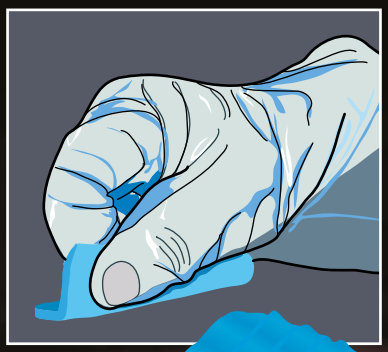

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