



IN THIS ISSUE:

AUTUMN 2018

Pages 1 & 2

Next generation sequencing

Page 3

Depositing seeds for patent applications

Page 3

New reference strains available from NCIMB

Page 4

NCIMB strains in the press

Page 4

ISO 9001 2015



Next generation sequencing: revolutionising microbiology

Dr Daniel Swan, Bioinformatics Delivery Manager, NCIMB

NCIMB has launched a new Next Generation Sequencing (NGS) service with bioinformatics analysis. The development of NGS has revolutionised genomic research, but what exactly are the benefits of NGS, and what value can it offer our customers? NCIMB's Bioinformatics Delivery Manager, Dr Daniel Swan, takes a brief look back at the development of sequencing technologies and highlights some of the applications.

Microbiologists have always exploited new technologies in order to understand the impact and processes of bacterial systems. In 1977, Carl Woese revolutionised microbial taxonomy, using rRNA genes as a marker to classify archaea as a separate domain from bacteria. The same year marked the development of DNA sequencing by Fred Sanger. By the early 1990s

“Sanger sequencing” had led to the study of microbial communities through sequencing of the 16S ribosomal DNA gene, and this was followed by the first bacterial genome sequence (*Haemophilus influenzae*) in 1995.

The impact of Sanger sequencing on modern biology has been huge. Its automation enabled production scale sequencing, and a major landmark was reached in 2000 when, after 13 years and an investment of \$2.7bn, the human genome was completed.

As the technology matured and costs reduced, Sanger sequencing technology started to become more widely used for routine analysis, and 16S sequencing became accepted as the “gold standard” for bacterial identification. This is still a core NCIMB service for our pharma customers who require identification of unknown isolates to Good

Manufacturing Practice (GMP) standards.

The next generation

NGS platforms were launched commercially in 2005, and this development opened the floodgates to new applications, an increased understanding of microbial biodiversity, and an industry brimming with genome data.

The term “next-generation sequencing” refers to a number of different technologies, that all have one thing in common – they offer a step change in the scale of data production from a single machine. A state of the art, automated Sanger sequencing platform, running at full capacity can generate 1,536 DNA sequences a day – a total of 1,536,000 bases (1.5Mb). The first NGS platform gave a single lab the ability to analyse 20,000,000 bases (20Mb) in 5.5 hours, using a machine with a much

smaller footprint. The development in throughput has continued at a rapid pace and at NCIMB we can produce 14Gb of data in a single run, a huge advance again from early NGS machines.

In addition, the old adage of 'Better, faster, cheaper – pick two' was a poor predictor for sequencing as NGS has delivered all three - better accuracy, higher throughput and lower costs. The lower cost is a combination of both the underlying technologies used (clonal amplification of DNA strands on immobilised surfaces; super-sensitive methods of detecting the bases added during sequencing) and also the massive uptake of the new platforms as science became hungry for sequence data.

An enabling technology

The rapid throughput of NGS allows genomic diversity to be looked at across thousands of individuals, and this has a myriad of applications. Within the microbiology community, it has enabled researchers to understand the minimum genome required for bacterial life. With respect to clinical applications, NGS has enabled rapid responses to outbreaks of pathogens, and much more finely grained taxonomic classification of serovars.

The link between the human microbiome and human health has been in the news in recent months, with improved understanding set to revolutionise the treatment of conditions like recurrent *Clostridium difficile* infection. These leaps in understanding of the microbial ecology of the human gut have only really been possible as a result of NGS.

NGS has also revolutionised environmental microbiology, providing new insights into the diversity of extreme marine environments and soil microbe communities.

NGS services at NCIMB

Much of our NGS work focuses on understanding the makeup of bacterial whole genomes. Whole-genome sequencing is the ultimate in organism characterisation. It allows us to take a deep dive into a strain, and one application we are very interested in, is screening strains for the production of industrially or clinically important secondary metabolites. We offer this as a service to customers, and also work in collaboration with other researchers to explore the potential of our own culture collection.

Another critical application of whole-genome sequencing is in characterising probiotic products for human or animal use. Screening genomes for the presence of anti-microbial resistance genes and virulence factors is essential, as it is crucial that strains used do not facilitate the spread of resistance or pose other health risks.

We also use our experience in microbial ecology to sequence and understand the makeup and effects of microbial communities in environments, with 16S sequencing at NGS scale. This has applications in environmental monitoring programmes and we use it to analyse samples from niches as diverse as oil and gas reservoirs and production facilities, soil, and the human gut. In the case of the oil and gas industry, community analysis can help operating companies assess the likelihood of reservoir souring and corrosion, and take preventative steps to safeguard the environment and potentially avoid much greater costs.

Importantly, at NCIMB, we have the bioinformatics capability and expertise to get the most from your NGS data, no matter what your research or commercial aim.



16S community analysis can help operating companies assess the likelihood of reservoir souring and corrosion, and take preventative steps to safeguard the environment and potentially avoid much greater costs.



ABOUT THE AUTHOR

Dr Daniel Swan joined NCIMB in 2017. Daniel is an experienced bioinformatician who has been generating and analysing DNA sequence data since 1995. He is responsible for developing and maintaining the analysis infrastructure that supports NCIMB's NGS platforms as well as providing NGS and bioinformatics consultation for R&D partners. He is passionate about sequencing all of the reference strains in the NCIMB collection.

Depositing seeds for patent applications – how many is enough?



Patents offer assignees exclusive rights to their inventions in exchange for detailed disclosure. A detailed description with drawings meets this requirement for many inventions, but in the case of inventions that are, or require the use of biological material, a detailed disclosure involves the deposit of this material in a recognised institution, from where samples can be requested. NCIMB is such a recognised institution and holds the status of International Depository Authority (IDA) under the Budapest Treaty. We store patent deposits

of bacteria, fungi, bacteriophages, plasmids, plant cell tissue culture and plant seeds.

So how many seeds should be deposited when a patent application is made? It is important that enough seeds are made available to furnish any requests that may be made, taking into account the likelihood of a reduction in the germination rate of stored seeds over time.

A minimum of 250 seeds is required for an initial deposit, and while the US Patent and Trademark Office requires a minimum of 2500 seeds to be deposited prior to the issue of a patent, the International Board of Genetic Resources recommends a minimum of 4000 seeds for long term storage.

A germination rate of at least 85% is normally required, but deposits may be accepted in certain circumstances where this is impossible to achieve.

For more information about patent deposits contact enquiries@ncimb.com or visit our website www.ncimb.com.

New reference strains available from NCIMB

Three new strains are now available from the National Collection of Industrial, Food and Marine Bacteria – one hydrocarbon degrading *Halomonas* species and two strains of *Salinisphaera*.

The *Halomonas* species, NCIMB 15113, was deposited by researchers from the Department of Microbiology and Plant Biology at the University of Oklahoma. It was isolated from a produced water storage tank in Barnett Shale, North Texas USA, and has the ability to degrade a variety of straight-chain alkanes.

The two *Salinisphaera* strains, NCIMB 15097 *Salinisphaera ulaidhensis* and NCIMB 15098 *Salinisphaera belfastensis*, were both isolated from brine taken from the Kilroot salt mine in Northern Ireland. The Kilroot mine produces hundreds of thousands of tonnes of rock salt, which is used to de-ice roads throughout Ireland and the UK, and both of these strains are capable of growth at high salt concentrations.

NCIMB Ltd manages the National Collection of Industrial, Food and Marine Bacteria: the UK's biggest repository for reference strains of environmental and industrially useful bacteria, plasmids and bacteriophages. The collection is continuously expanding as a result of new accessions from the international research community.

To purchase strains or for information on how to deposit strains with NCIMB, contact enquiries@ncimb.com or visit our website www.ncimb.com.

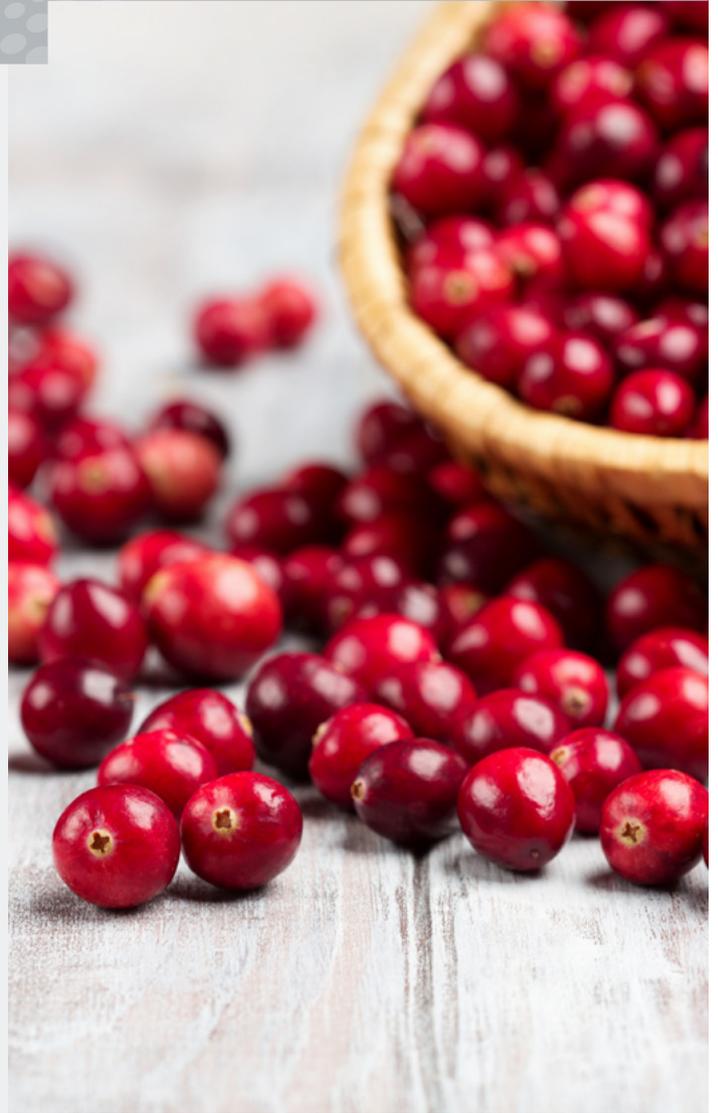


NCIMB strains in the press

A team of researchers at the University of Reading have used NCIMB 8826 *Lactobacillus plantarum* in a study of cell survival in low pH fruit juices during refrigerated storage. *Lactobacillus* species are commonly used in probiotic foods, which are often dairy products. However, many people do not consume dairy products and there has been a growing interest in the use of fruit juices as a means of delivering probiotic microorganisms.

The study, which has been published in Food Research International, found that pre-treatment of stationary phase cells of *Lactobacillus plantarum* with citric acid significantly improved subsequent cell survival in several highly acidic fruit juices including cranberry, pomegranate and lemon and lime. Cranberry juice, which had the lowest pH of the juices used in the study, was found to be the most toxic towards *Lactobacillus plantarum*.

The authors state that, although the mechanism for this adaptation is still unclear, a significant increase of a cellular cyclopropane fatty acid, and a significant upregulation of cyclopropane synthase were observed during acid adaptation. They say it is likely that these changes led to a decrease in membrane fluidity and to lower membrane permeability.



ISO 9001 2015

We are pleased to announce that NCIMB has successfully achieved the transition from ISO 9001: 2008 to the newly revised ISO 9001:2015 standard. ISO 9001:2015 sets out the criteria for a quality management system and is based on a number of quality management principles including a strong customer focus, and continual improvement.

Our commitment to high quality standards is central to our operation and is an essential requirement for many of our customers. We are therefore delighted to be able to report that the transition to the revised standard went without a hitch!



NCIMB Limited
Ferguson Building
Craibstone Estate
Bucksburn
Aberdeen AB21 9YA

t. +44 (0) 1224 711100
e. enquiries@ncimb.com
w. www.ncimb.com

 [@ncimb](https://twitter.com/ncimb)

Autumn 2018