Collected insights

News, views and tips from NCIMB

What's this concoction? The promise and power of metagenomics.

Dr Daniel Swan, NGS Services Manager, NCIMB

At NCIMB, we routinely identify bacteria that have been found in pharmaceutical, food and other industrial production environments. Accurate identification may help the companies involved trace the source of microbial contamination, and confirm whether repeat incidences arise from the same issue. Often these jobs arrive as pure cultures on plates, and we can pick off colonies to isolate the single species present.

However, sometimes we're faced with a more challenging question. For example, we were recently asked to identify the components of a slime from inside a bioreactor. We may also be asked to analyse microbial populations from settings within the natural environment such as subsea structures.

Within bioreactors, unwanted slimes are generally the result of microbial contamination. There could be one contaminant, or a complex mixed community which has formed a biofilm – a three-dimensional conglomeration that includes not only a mixed community of microbial cells, but also polysaccharides, proteins and lipids. Plating out this kind of slime to isolate individual species for identification can be a very time consuming and ultimately frustrating process. There is no guarantee that all the species present in mixed environmental samples will grow under laboratory conditions.

It's for projects like this that we turn to metagenomics. This approach is also known by other names - microbial community analysis or microbiome profiling are just two. This use of different terminology can be confusing, however, whichever term is used, the principles and the advantages of this method remain the same.

For the more straightforward identification of pure bacterial cultures we use Sanger sequencing for identification. This process involves sequencing a section of one gene,

known as the 16S gene (or D2 LSU for fungal identification). The section is normally the first 500bp, but we can also sequence the whole gene, if required for better taxonomic resolution. But that does require us to have a colony or pure culture to work from in the first place, and as already described, when it comes to biofilms, obtaining those colonies can be challenging.

Metagenomics does away with this intermediate culturing stage, and moves straight from sample to DNA extraction. The quantities of DNA extracted are often very small, especially from environmental samples in cleanroom environments, or from seawater for instance. However, these small quantities of DNA are amplified using a process called polymerase chain reaction (PCR) to create sufficient amounts of the sequences of interest. Much like Sanger sequencing, for metagenomics analysis, we target the 16S gene. Most of the differences between the 16S genes of different bacterial species are concentrated in what are referred to as the variable regions, of which there are nine. As most metagenomic sequencing is performed on Illumina sequencers, which have a maximum read length of 300bp, a variable region, or combination of adjacent variable regions, is chosen for PCR amplification.

The first round of PCR amplifies the majority of 16S sequences present in the starting material to produce a mixed PCR product that represents the mix of bacterial species in your sample. Each fragment of DNA is subsequently sequenced and analysed independently, allowing us to look at each sequence generated and attach a label to it. This is a taxonomic label – in other words a species identifier. This labelling process allows the number of times the sequence occurs to be assessed, and in this way we can understand not just what species are present, but also the abundance of each.

These two measures open the door to other analyses that can help us to gain a much deeper understanding of the situation being studied. The richness of the diversity of species can be calculated. We can look at sampled sites over time, or over treatments, and determine which species are changing between sample groups – effectively looking for differential abundance. We can group samples by their statistical properties – allowing us to classify samples against known types in order to assess where they might have come from.

This method can be applied to many different scenarios - from a contaminated bioreactor or wastewater monitoring, to monitoring of oilfield systems, and analysing the bacteria in your gut. It can be used to analyse the mycobiome (all the fungi present), for invasive species testing (via the CO1 gene instead of the 16S gene), for understanding food spoilage, and even for testing for adulterated meat in the supply chain.

Metagenomics really is a game-changing technology and whether 16S, or other marker genes are used, it can deliver an unparalleled insight into a system under study. However, the process needs to be carefully controlled due to the confounding factors that can enter the experimental system. Running negative controls and replication is advised, and even the selection of the variable region can have unexpected impacts on your downstream data. NCIMB can help you navigate these challenges with our decades of microbiology experience.

If this sounds like something you'd like to explore with us, please contact Dr Daniel Swan (d.swan@ncimb.com) for more information.

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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.

New reference strains available from NCIMB: *gut bacteria and fermented foods*

Researchers from the Host-Microbiota Interactions Laboratory at the Wellcome Sanger Institute recently added 20 strains to NCIMB's reference collection, including a number of novel species and genera.

This research group studies the intestinal microbiota, and the strains they have deposited in the collection are all strict anaerobes that have been isolated from human faecal samples. The strains are from a number of different families and genera including Bacteroides, Enterococcus, Escherichia, Propionibacterium, Sarcina, Bifidobacterium, Gordonibacter, Alistipes, Pediococcus, Lactobacillus and Clostridiaceae.

These strains are an exciting addition to the NCIMB collection. The work that this research group are doing in culturing and isolating gut bacteria is making a significant contribution to the understanding of gut microbiota and its compositional diversity, so we are delighted to be able to make the strains they have deposited available to the wider research community.

We have also recently added 47 strains of lactic acid bacteria to our reference culture collection. Forty-five of these strains were isolated from naturally fermented Chinese pickle, and two were isolated from naturally fermented yoghurt. The strains were all deposited by scientists from the College of Life Sciences, North East Agricultural University, Harbin, China. The new additions include 41 strains of Lactobacilli, five Enterococci and one Weissella species – 46 of them are novel species.

The range of lactic acid bacteria in NCIMB's open collection includes strains isolated from a diverse range of sources in addition to pickles and yoghurt, such as silage, marinated fish, brewery yeast, fermented cassava and lettuce leaves.

We also offer a comprehensive range of services to assess the suitability of strains for use as feed additives or probiotics, including screening for antibiotic resistance and virulence factors.

To find out more about our full range of products services visit www.ncimb.com or contact enquiries@ncimb.com.

Resuscitation of freeze-dried cultures

Most of NCIMB's reference strains are supplied freeze-dried in sealed glass ampoules. This format has a number of advantages – it has a long shelf life and requires no refrigeration during transit. This means most of our strains are available for immediate dispatch and our shipping costs are low.

However, opening ampoules can seem a little daunting if it is not something you do regularly, so we have produced a new step by step photo guide to opening ampoules and resuscitating cultures. You can download the guide from our website or email enquiries@nicmb.com to request a copy.

Aflatoxins in silage

The presence of aflatoxins in silage can present a serious health risk for both animals and humans. Scientists from the University of Torino, and the Institute of Sciences of Food production in Italy, used NCIMB 40788 *Lactobacillus buchneri* in a study that aimed to evaluate the origin of aflatoxins in corn silage. The fresh herbage was subjected to different lactic acid bacteria treatments prior to being ensiled for 250 days. The pre-ensiled material, the silages at silo opening, and samples of the silages after seven and 14 days of exposure to air, were then analysed.

The authors found that the inoculation with lactic acid bacteria increased the aerobic stability of the silages and delayed the onset of aerobic microbial degradation, which in turn indirectly reduced the risk of *Aspergillus flavus* outgrowth and afalotoxin B1 production after silage opening.

The paper has been published in the Journal of Dairy Science: 102, pp 1176 – 1193, Ferrero *et al* (2019) Increase in aflatoxins due to *Aspergillus* section *Flavi* multiplication during the aerobic deterioration of corn silage treated with different bacteria inocula.

Probiotics and microbial additive testing

NCIMB has launched a new package of services to support manufacturers of probiotic products and feed additives in assessing the suitability of candidate strains and complying with European Food Safety Authority (EFSA) FEEDAP guidelines. FEEDAP is the Panel on Additives and Products or Substances used in Animal Feed.

Our package includes a range of tests and analyses that provide valuable information about the functional characteristics of strains. Individual packages can be tailored to specific requirements. For example, strains used in probiotic products must survive the acidic gastric environment if they are to reach the small intestine and colonize the host, and our package of tests includes low pH survivability assays in simulated gastric fluid.

We also undertake mucin adhesion assessment, providing further information about the capability of strains to colonise the intestinal epithelium. The auto-aggregation ability of bacteria is key to maintaining the bacterial population within the gut and so we also offer aggregation testing within our probiotic package.

A high-quality genome sequence offers the ultimate characterisation and understanding of strains and is a key consideration in the responsible use of microbial additives with respect to limiting the spread of antimicrobial resistance in accordance with EFSA FEEDAP guidelines. We undertake genome sequencing, assembly and annotation, including prediction of antimicrobial resistance genes and virulence factors. Combining predictions from whole-genome sequencing with our lab-based MIC tests helps you understand the potential of intrinsic and acquired antimicrobial resistance.

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