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Need to know if isolates of the same species are from the same source? MLST can be a valuable tool.

When it comes to bacterial identification, the focus is often on identifying unknown isolates to the species level. However, strain comparison and differentiation can be valuable in a range of circumstances. NCIMB's Identification Services Manager, VIKKI MITCHELL, takes a look at one of the methods available for this – MLST.

When it comes to investigating process contaminants, comparing them to previous isolates and tracing their source, strain comparison and differentiation can provide valuable information over and above species level identification. It can also be a very important process with respect to the industrial use of bacteria. For example,

companies may specify the presence of a particular strain of bacteria in their probiotic foods or use a specific strain within a patented process. In these circumstances, testing may be required to ensure that there is no strain drift and that the correct strain is being used or is present in the product. In the case of patented processes that involve bacteria, it is also vital that the strain can be accurately identified if the patent is contested or infringement is suspected.

For species level identification, there are a number of approaches: phenotypic identification, based on metabolic differences, proteotypic, which uses a peptide mass fingerprint, and genotypic, which uses DNA sequencing to

identify microorganisms. At NCIMB we favour the genotypic approach, as we believe it gives the most accurate and reliable results. New additions to our reference collection undergo 16S rRNA sequencing, and it is the approach we use for our identification service.

For strain to strain comparison, a genetic approach is really the only one to consider. Multilocus sequence typing (MLST) is a sequence-based strain typing method that was first proposed 20 years ago. Much initial interest in the method was focused on its value as a means of pathogen outbreak tracking. However, it is a useful technique for any application where strain information is needed, and at NCIMB we regularly use it when strain comparison is required by our

customers. For example, it is especially valuable for very common environmental isolates – if several different strains of common environmental isolates are present in a manufacturing facility, investigation results may be misleading or inconclusive if strain typing has not been undertaken.

MLST uses the sequences of internal fragments of (typically) seven essential, single-copy, housekeeping genes i.e. genes required for processes that are essential for cell operation, to characterise isolates. Different strains of the same bacterial species show enough variation within each of these housekeeping genes i.e. at each of the seven loci, to create a vast number of distinct allelic profiles. In an MLST scheme, each possible sequence observed at each of the loci is assigned a unique allele number. The allele numbers at each of the seven loci together create the allelic profile that can be used to unambiguously

characterise strains. Similarly to 16S rDNA sequencing, the development of this technique has been underpinned by online data sharing. Central databases have been established to which users can submit strain information and new allele sequences. Strains are identified by comparing the sequence profiles obtained with previously published data. The amount of sequence data is increasing all the time, and the technique has become a very valuable tool - not only for research and pathogen outbreak tracking, but also for use in environmental monitoring programmes, and when unambiguous confirmation of strains used in manufacturing processes is required.

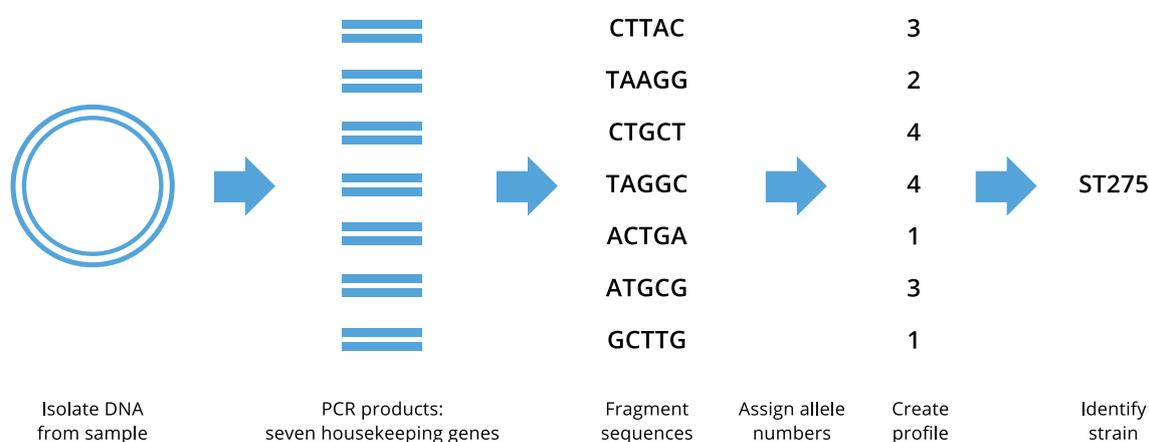
A good example of this technique working well is when we were recently asked to compare several different isolates of *Staphylococcus epidermis* from a manufacturing facility. Following MLST analysis, it was clear which of the isolates were the same strain, and

this information could be used in the investigation to identify the source. MLST results are usually available within a week to ten working days of receiving samples.

Contact enquiries@ncimb.com for more information.



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The MLST process



ABOUT THE AUTHOR

Vikki Mitchell joined NCIMB in 2005. She leads a team of scientists responsible for delivering NCIMB's identification services and sequencing new deposits to the UK's National Collection of Industrial Food and Marine Bacteria.

Vikki holds a BSc (Hons) degree in Applied Biosciences and Management, and an MSc in Instrumental Analytical Techniques; DNA Analysis, Proteomics and Metabolomics from the Robert Gordon University in Aberdeen.

New additions to the National Collection of Industrial, Food and Marine Bacteria (NCIMB)



The School of Natural and Environmental Sciences at Newcastle University have added 15 new strains from interesting and extreme environments to the NCIMB open collection. Microbes that thrive in extreme environments can be a source of industrially useful enzymes. An example of this is *Thermus aquaticus*, a microbe isolated from a hot spring in Yellowstone National Park, which is the source of the heat-resistant enzyme Taq DNA polymerase. As this is used in the polymerase chain reaction (PCR) DNA amplification technique, it has become one of the most important enzymes in molecular biology. These new strains are therefore an exciting addition to the collection.

The new accessions, which were all isolated in Indonesia, include:

- Three *Streptomyces* species (NCIMB 15210; NCIMB 15211; NCIMB 15212); and one *Arthrobacter* sp. (NCIMB 15209) isolated from the Parangkusumo sand dunes;
- Five species isolated from hot spring sediment (*Micrococcus* sp. NCIMB 15213; *Rhodococcus* sp. NCIMB 15214; *Micromonospora* sp. NCIMB 15215; *Pseudonocardia* sp. NCIMB 15216; *Actinospica* sp. NCIMB 15218);
- Two *Dermacoccus* sp. (NCIMB 15219; NCIMB 15220), and *Verrucosispora* sp. NCIMB 15223, isolated from arid sand dunes;
- *Kytococcus* sp. NCIMB 15217 and *Amycolatopsis* sp. NCIMB 15222 isolated from a saline mud volcano;
- *Amycolatopsis* sp. NCIMB 15221 isolated from volcanic sand dunes.

If you would like to find out more about these strains contact enquiries@ncimb.com or visit our website www.ncimb.com.

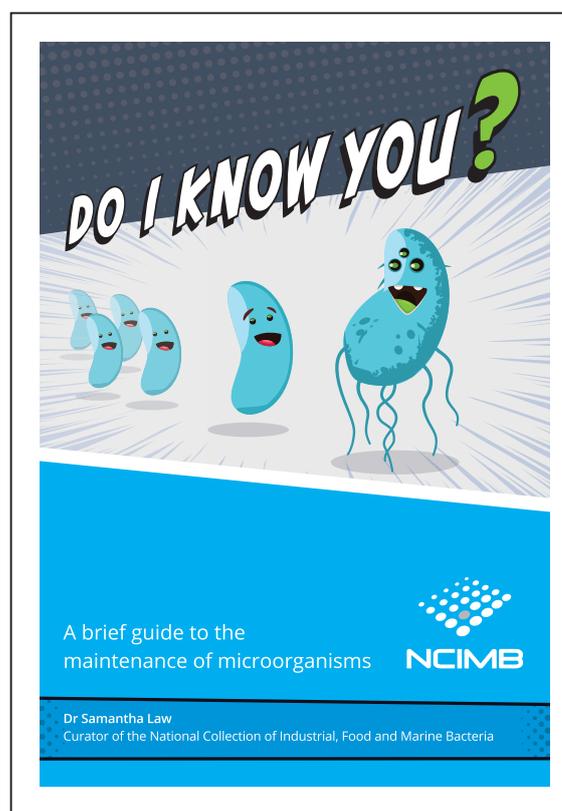
Do I know you?

When you start a new job, or employ a new member of staff, checks are carried out. Employers usually ask for references, and evidence of qualifications. New recruits may even be asked to show their passport of driving license.

All these checks are carried out to ensure that new recruits are who they say they are and that they are qualified to do the job that they have been appointed to. So why should you be any less careful about the strains that you work with in the laboratory? After all, discovering, that your cultures have become contaminated or strain drift has occurred can ruin years of hard work.

Our culture collection curator, Dr Samantha Law, has written a brief and lighthearted guide to the maintenance of microorganisms. The booklet, titled "Do I know you?" highlights the pitfalls of repeated subculturing, and outlines the basic steps you can take to avoid strain drift, and make sure that you know the strains that you are working with in the laboratory.

Please contact enquiries@ncimb.com if you would like us to send you a copy of the guide.



NCIMB strains in the press

The complete genome sequence of NCIMB 700965 *Lactobacillus plantarum* has been deposited in DDB/ENA/GenBank under GenBank accession numbers CP023490 (chromosome) and CP023491 to CP023495 (plasmids).

Strains of the species *Lactobacillus plantarum* are reported to be used in the production of many fermented foods such as Kimchi and have been investigated for their probiotic properties. They also synthesize a variety of bactericidal peptides (bacteriocins) called plantaricins that cause cell death in closely related bacteria. Plantaricins have recently been investigated in order to understand more about their potential as a food preservatives and antimicrobial candidates for combating bacterial infections in humans.

Strain NCIMB 700965 was originally isolated from cheddar cheese in 1939 and, because of its sensitivity to plantaricins, has been used as an indicator for plantaricin biosynthesis by other *L. plantarum* strains.



NCIMB 700965 was originally isolated from cheddar cheese

This work was undertaken by Dustin Heeney and Maria Marco from the Department of Food Science and Technology at the University of California. Reference: Complete Genome Sequence of the Plantaricin-Sensitive Strain *Lactobacillus plantarum* NCIMB 700965 Dustin D. Heeney, Maria L. Marco Microbiology Resource Announcements May 2019, 8 (21) e01724-18; DOI: 10.1128/MRA.01724-18

NCIMB hosts Institute of Corrosion visit

We were delighted to host a visit from the Institute of Corrosion Aberdeen branch at the start of September. Microbiologically influenced corrosion (MIC) is a complex and difficult to predict process that impacts the oil and gas industry. Effective management to prevent the costly and damaging failures that are associated with MIC therefore relies on regular monitoring of the microbial populations present, and in recent years new tools have become available for this task. Techniques available for monitoring oilfield microbial populations include the most probable number (mpn) technique, quantitative polymerase chain reaction (qPCR) and next generation sequencing. The different techniques all give different kinds information about the microbial population so which is the best approach?



The visit included a presentation from our next generation sequencing services manager Dr Daniel Swan who outlined the individual techniques available before introducing an innovative approach that combines different analytical methods, and can bring an unparalleled insight to the management and mitigation of microbially influenced corrosion. Delegates then enjoyed a tour of the NCIMB facilities and a brief introduction to the history and importance of our culture collection.

Earlier in the summer we also welcomed Qa3's on-site chemistry team for a day of oilfield microbiology training. Delegates were given an introduction to the topic of oilfield microbiology as well as some hands-on training in the laboratory.

For more information about the services we offer to the oil and gas sector contact enquiries@ncimb.com

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Autumn 2019