STRAIN COMPARISON AND DIFFERENTIATION USING MULTILOCUS SEQUENCE TYPING (MLST)

When it comes to bacterial identification, the focus is often on identifying unknown bacterial 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 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.

Sequencing of the full 16S rRNA gene (rather than the first 500bp, which is generally used for species identification) provides additional useful data that can highlight differences between bacterial isolates. This can be used to help clarify whether environmental isolates identified as being the same species of bacteria are in fact the same strain, and therefore likely to have arisen from the same source. However, full 16S gene sequencing results are not always conclusive for this purpose. Another approach, which is more definitive, is multilocus sequence typing (MLST).

MLST is a 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.

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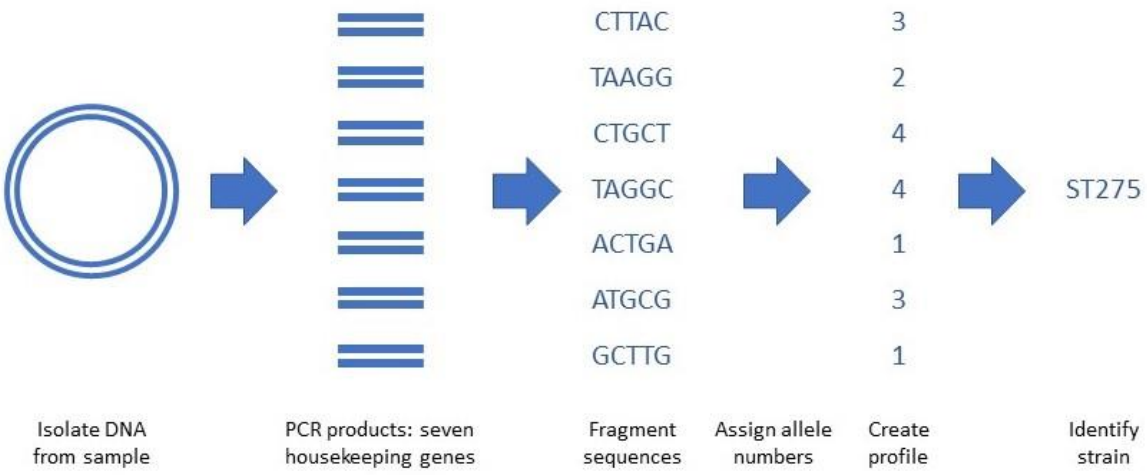
THE MLST PROCESS



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, and it is a useful method for identifying microorganisms to strain level.

The fragments sequenced are approximately 450 – 500 base pairs long, and 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.

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.*