Collected insights

News, views and tips from NCIMB

Fungal identification methods explained

Vikki Mitchell, Identification Services Manager, NCIMB

Species level identification of fungi has long been considered challenging and, in contrast to bacteria, fungal isolates obtained from environmental monitoring programmes are often identified to genus rather than species level. More detailed identification of environmental isolates can be desirable - so what are the difficulties when it comes to species level identification of fungi?

Bacteria are commonly identified by sequencing of the 16S ribosomal DNA, and the approaches that have been developed for genotypic identification of yeasts and moulds also involve sequencing sections of ribosomal DNA. Fungal ribosomes have a large and small subunit. The ribosomal RNA (rRNA) operon, that is the DNA that codes for ribosomal RNA, has three rRNA sequences and two internal transcribed spacer regions: ITS1 and ITS2.

Two distinct approaches have emerged for genetic identification of fungi: sequencing of the D2 region of the large subunit ribosomal DNA (D2 LSU) and sequencing of either one or both of the internal transcribed spacer regions (ITS).

D2 LSU sequencing is probably the most widely used approach for fungal identification at present. Similarly to 16s rDNA sequencing for bacteria, data obtained can be analysed using a validated commercial database that has been built using reference strains, as well as publically available but non validated sources such as the EMBL-EBI (European Molecular Biology Laboratory - European Bioinformatics Institute) database. The ITS approach however, generally relies more heavily on the use of data obtained from unvalidated public databases.

While I find it is usually possible to obtain a species level identification of yeasts using D2 LSU sequencing, we sometimes can't get that level of identification for moulds and in these cases, it is often possible to obtain species level identification using ITS sequencing. Generally, there is a higher level of differentiation between ITS sequences. Unlike ribosomal DNA, ITS sequences have no functional role, and consequently have accumulated a greater level of mutation, which aids identification.

A good example of moulds that are difficult to identify to species level using D2 LSU comes from the genus *Penicillium* - a commonly occurring genus amongst environmental isolates.

I have found *Penicillium camemberti*, *Penicillium clavigerum*, *Penicillium commune*, *Penicillium corylophilum* and *Penicillium crustosum* have all matched a single isolate sequence at 100% similarity. However, when ITS sequencing has subsequently been undertaken, a species level result has been achieved – in one specific example the isolate was identified as *Penicillium crustosum* with a 100% match.

The above example is an illustration of where D2 LSU sequencing cannot differentiate between different species of the same genera, but in some cases, D2 LSU sequencing alone cannot distinguish between different, but closely related, genera. For example, I have found several species of *Cladosporium* and *Mycosphaerella* have all matched to the same isolate sequence. *Cladosporium* is a large genus that has been reported to be the most common fungal component isolated from air, and is therefore also quite commonly found in the course of environmental monitoring programs. Again, I have found ITS sequencing to be successful in providing a species level identification - one isolate which matched to *Cladosporium cladosporioides*, *Cladosporium herbarum*, *Cladosporium oxysporum*, *Mycosphaerella aronici* and *Mycosphaerella tassiana*, was identified as *Cladosporium cladosporioides* when ITS was used.

In another example, we found that it was not possible to differentiate between an even larger group of closely related genera. Searching the non-validated EMBL database for D2 LSU sequences failed to distinguish between *Saccothecium sepincola*, *Mycosphaerella sojae*, *Pithomyces chartarum*, *Pleospora gaeumannii*, *Leptosphaerulina saccharicola*, *Heterophoma adonidis*, *Nothophoma quercina* and *Leptosphaerulina australis*. These eight species, from seven different genera, all matched a single D2 LSU isolate sequence at 100% similarity. In this case however, ITS sequencing did not result in a species level match either – the isolate matched to *Leptosphaerulina saccharicola*, *Leptosphaerulina australis*, *Leptosphaerulina chartarum* and *Leptosphaerulina trifolii*. It was, however, successful in narrowing the identification down to *Leptosphaerulina* species rather than several different genera – a substantial improvement on the initial result obtained.

As many fungi do match very well to the validated D2 LSU database, at present, we recommend the use of D2 LSU sequencing in the first instance. If a match is not found using the validated database, we would analyse the results against the non-validated EMBL database, before considering whether to follow up with ITS sequencing. We always refer to any relevant published papers for additional supporting information when using unvalidated databases for identification purposes.

The decision on whether to progress to ITS sequencing if a species or genus level identification cannot be obtained using D2 LSU sequencing is really dependant on the individual circumstances and whether family, genus or species level identification is required. For example, it may be requested when investigating excursions from normal populations or contamination issues. While ITS does not always provide a species level match, the examples above illustrate that it has been successful in doing so with some commonly isolated fungi, and where a species level match has not been found it has given an improved result. Validated databases of ITS sequences are being developed and in future, our experience suggests that this may result in increased popularity of the method.

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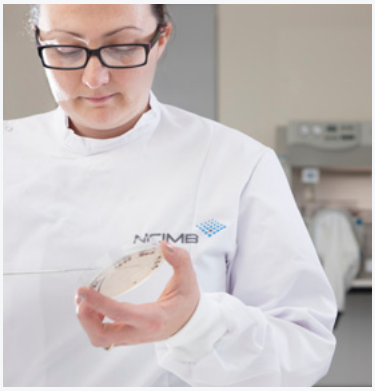

ABOUT THE AUTHOR

Vikki Mitchell joined NCIMB in 2005. She leads a team of scientists responsible 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.

NCIMB appoints bioinformatics expert

NCIMB is delighted to welcome Dr Daniel Swan in a new role as bioinformatics delivery manager.

Daniel is responsible for the delivery and continued development of NCIMB's bioinformatics services and comes directly from running one of the UK's largest genomics facilities. His expertise is the analysis of next-generation sequencing (NGS) data, which is revolutionising our understanding of microbial communities and environmental microbiology.

Commenting on his appointment Daniel said: "NGS has an exciting role to play in realising the full potential of NCIMB's culture collection, offering both industry and academic researchers a deep dive into the biology of the strains through fully sequenced genomes. I will also be developing NCIMB's bacterial and fungal identification services, extending the existing strain identification with high-throughput 'metagenomics' approaches which will provide even greater resolution of microbial communities and their functions in production environments."

For more information about NCIMB's bioinformatics capabilities contact:

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Investigating probiotic gum for healthy gums

NCIMB is collaborating with leading probiotics manufacturer Protexin in a project that aims to identify strains of bacteria that could neutralise the harmful effects of dental biofilms. The project is supported by Innovate UK.

Dental biofilms, in the form of plaque, are commonly associated with tooth decay and gum disease. Regular brushing is recommended to remove plaque and avoid oral disease. However, dental biofilms reform quickly after brushing, and many people do not brush thoroughly or frequently enough to avoid problems.

The mouth is one of the most heavily colonised parts of our bodies in terms of the human microbiome, and the microbes present are important for health. This project is investigating strains held within the NCIMB culture collection for their potential to shift composition of oral biofilms towards a healthier oral flora, and combat dental caries. Strains found to be effective could be delivered in the form of a lozenge or gum.

NCIMB offers microbiology, chemical analysis and biomaterial storage services. We collaborate with companies, universities and research organisations to carry out research and development projects and are particularly interested in investigating novel uses of our strains.

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NCIMB strains in the press

The 'M' in NCIMB stands for marine. A new type strain, NCIMB 15095 or *Colwellia echini* sp. nov., was isolated from a sea urchin intestine, and deposited in the NCIMB reference collection by researchers from the University of Copenhagen. The strain solubilises agar and carrageenan. It is now described with its published draft genome.

Christiansen *et al*., *Colwellia echini* sp. nov., an agar and carrageenan solubilizing bacterium isolated from sea urchin. International Journal of Systematic and Evolutionary Microbiology 68: 687-691

Novel marine methylotrophs added to the National Collection of Industrial, Food and Marine Bacteria

Four novel species of methylotrophs have been added to the National Collection of Industrial, Food and Marine Bacteria. They are NCIMB 15078 *Methyloceanibacter methanicus*; NCIMB 15077 *Methyloceanibacter stevinii*; NCIMB 15076 *Methyloceanibacter superfactus* and NCIMB 15075 *Methyloceanibacter marginalis*.

The four type strains were deposited by Bran Vekeman from the Laboratory of Microbiology, University of Gent. They were isolated from North Sea sediment.

Marine methylotrophs play a key role in the carbon cycle by metabolizing reduced one-carbon compounds that are found in high concentrations in marine environments. More information about the new species can be found in the paper: Vekeman *et al* 2016, New *Methyloceanibacter* diversity from North Sea sediments includes methanotroph containing soley the soluble methane monoozygenase. Environ Microbiol. 2016 Dec;18(12):4523-4536. doi: 10.1111/1462-2920.13485.

The authors state that despite their crucial role in marine carbon cycling, to date only a handful of marine methanol oxidizers and even fewer methane oxidizers, have been isolated and physiologically characterized.

The National Collection of Industrial, Food and Marine Bacteria is 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 and includes many strains that have applications within the pharmaceutical and personal care sectors.

To purchase strains or for information on how to deposit strains with NCIMB contact enquiries@ncimb.com or visit our culture collection pages: www.ncimb.com

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