Mycobiome sequencing – can we use it for diagnosing fungal infection?

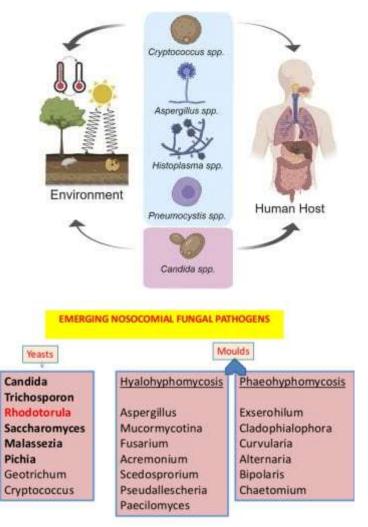
Danielle Weaver Manchester Fungal Infection Group



Diagnosing fungal infections

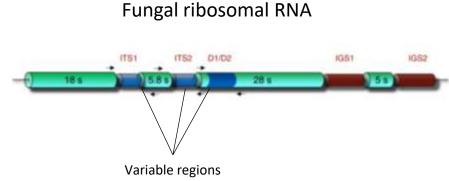
- Direct microscopy and culture methods
 - time consuming
 - require technical expertise
- GM and β-D-Glucan testing
 - fail to detect some fungal pathogens
 - don't identify the organism causing the positive result

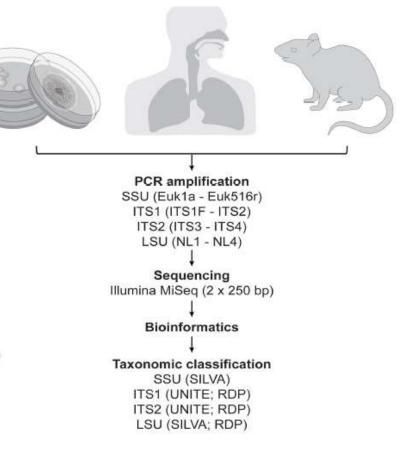
 New diagnostics with broad species coverage & good speciation are warranted



Mycobiome sequencing

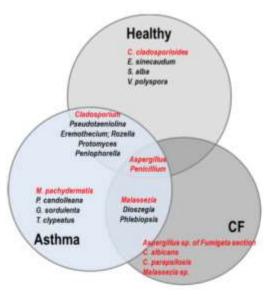
- Mycobiome the fungal community in and on an organism
- A few targets have been reported in the literature – all are ribosomal RNA regions
- The Internal transcribed spacer (ITS) regions have become standard, particularly ITS1

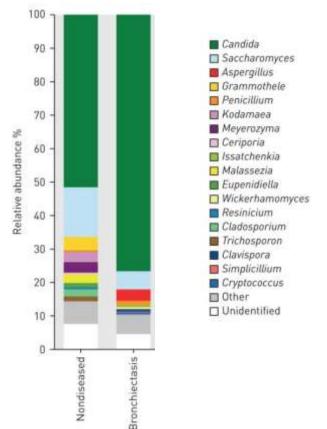




Mycobiome varies in health & disease – Can we harness it for diagnostics?

- High throughput sample processing
- Sensitive = good limit of detection
- Specificity depends on chosen primers
- Potential to provide a complete picture of all species within a sample in a quantitative manner

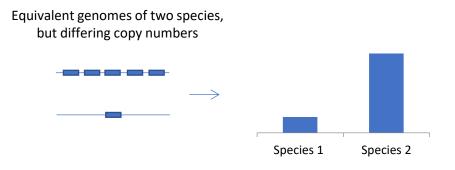




doi: 10.3389/fmicb.2015.00089

Pitfalls of ITS for use in an NGS fungal diagnostic

- Amplicon not consistent in length PCR bias against some species
- Variable copy number in fungal genomes (13-fold between *A. fumigatus* isolates!)

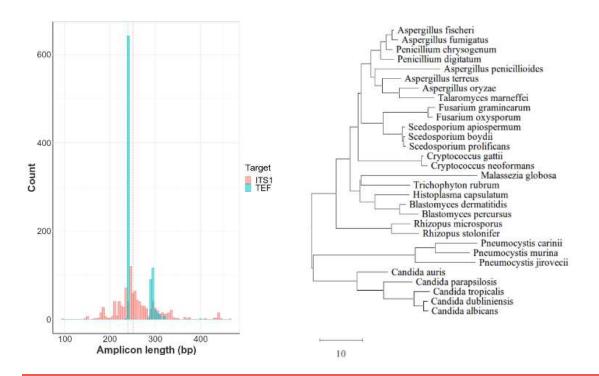


Accurate quantification is key to differentiate between members of mycobiome which may be transient due to inhalation and those which are infecting

A novel amplicon target, TEF

• Single copy gene

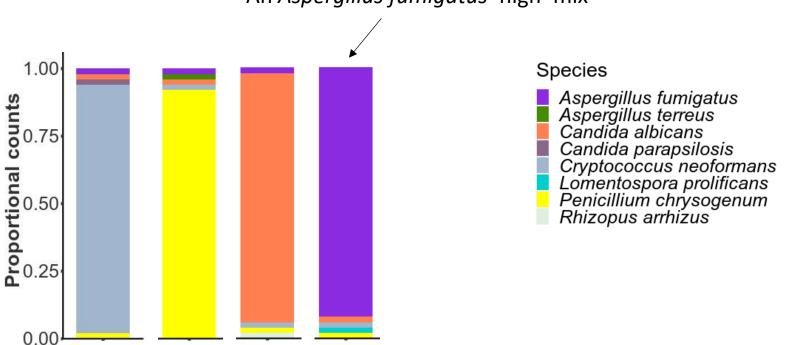
- More consistently sized amplicon
- Can distinguish between closely related fungal pathogens
- Small amplicon size means can use Illumina benchtop sequencer: iSeq100





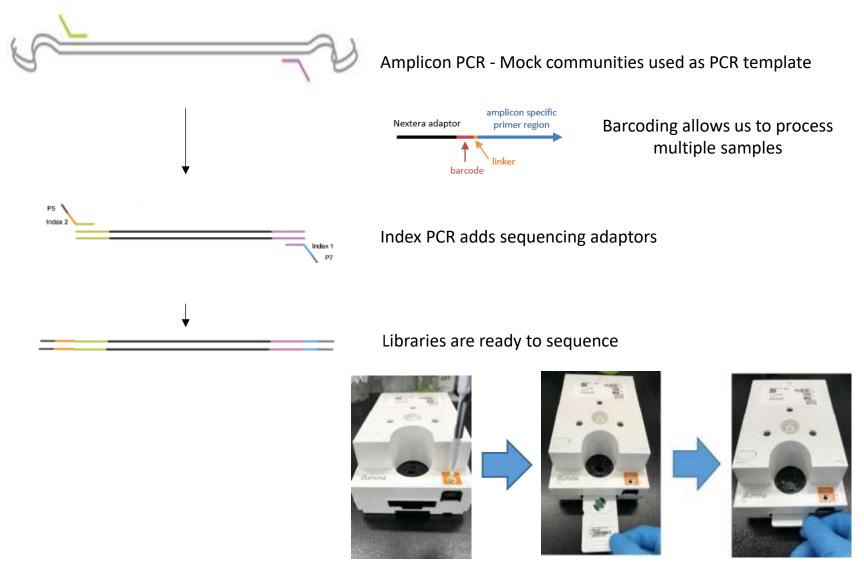
Mock fungal communities for assay validation

- Mock fungal communities mixes of genomic DNA from 5 species
- Dilute genomic DNA to haploid genome equivalents & combine
- Each mock has a single species at high abundance and all remaining species are at low abundance (2%)

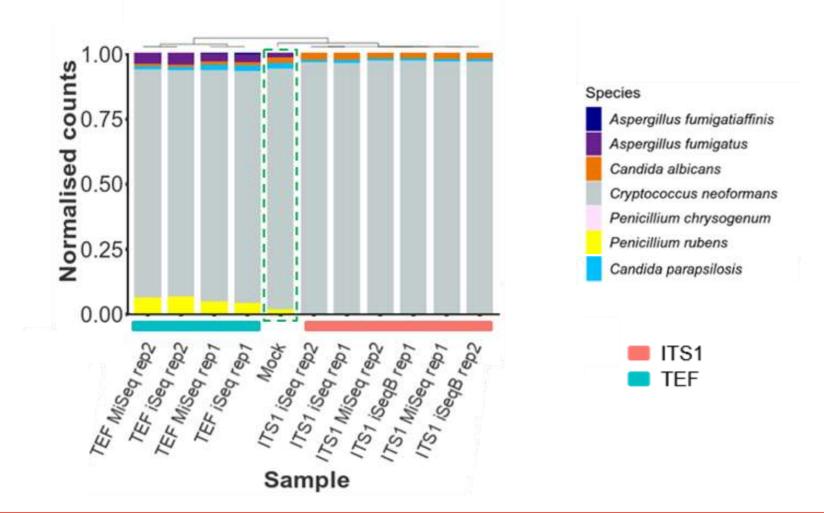


An Aspergillus fumigatus 'high' mix

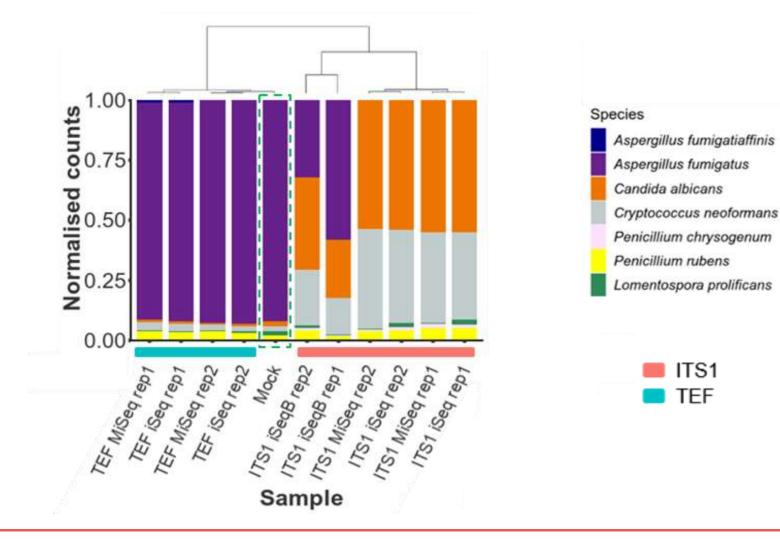
Sequencing library preparation



Mock community analysis identifies ITS1 under-representation of filamentous fungi



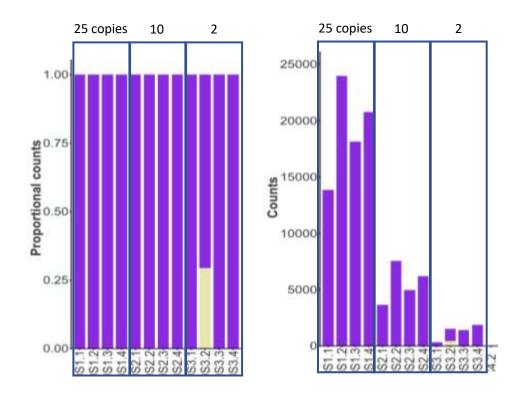
Mock community analysis identifies ITS1 under-representation of filamentous fungi



Issue translates into poor ITS1 species identification rates

				Sequencer	¹⁰⁰ Sequencer			
		- 2			Target	80		iSeq
			100	100	Aspergillus fischeri	c0		MiSeq
00	100	100	50	50	Aspergillus flavus		T	
ã	0	0	100	100	Aspergillus fumigatiaffinis	40	Tar	ITS1
o l	28		100	100	Aspergillus fumigatus	20		TEF
00	100	100	100	100	Aspergillus penicillioides	0		
0	0	0	100	100	Aspergillus terreus			
00	100	100	100	100	Candida albicans			
50	66	66	100	100	Candida auris			
00	100	100	100	100	Candida glabrata			
00	100	100	100	100	Candida parapsilosis			
92	100	100	100	100	Cryptococcus neoformans			
60	66	66	83	100	Fusarium oxysporum			
00	100	75	100	100	Lomentospora prolificans			
33	50	33	100	100	Penicillium rubens			
00	100	100	100	100	Pichia kudriavzevii			
14	25	37	100	100	Rhizopus arrhizus			
00	0	100	100	100	Saccharomyces cerevisiae			
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Determining assay limit of detection



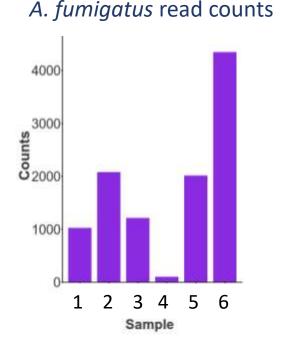
- Cystic fibrosis sputum sample spiked with *A. fumigatus* DNA
- 2, 10 or 25 haploid genome equivalents per PCR reaction

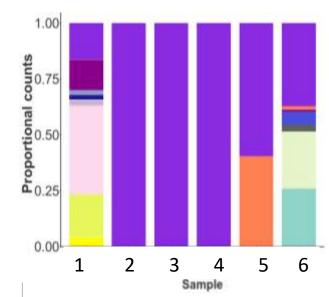
Species

Aspergillus fumigatus Malassezia restricta

Assay validation with clinical samples

Patient samples positive by Aspergillus qPCR





Relative abundance of all Fungi

Species
Aspergillus furnigatus
Candida albicans

- Candida glabrata
- Chaetomium cochliodes
- Lecanicillium dimorphum Malassezia restricta
- Malassezia sympodialis
- Penicillium arizonense-or-jensenii
- Penicillium brevicompactum Penicillium commune-or-palitans
- Penicillium crustosum
- Penicillium expansum
- Penicillium olsonii Penicillium rolfsii-or-janthinellum
- Penicillium rubens
- Penicillium vulpinum
- Saccharomyces cerevisiae
- Scedosporium boydii
- Scedosporium dehoogii

Assay can detect A. fumigatus

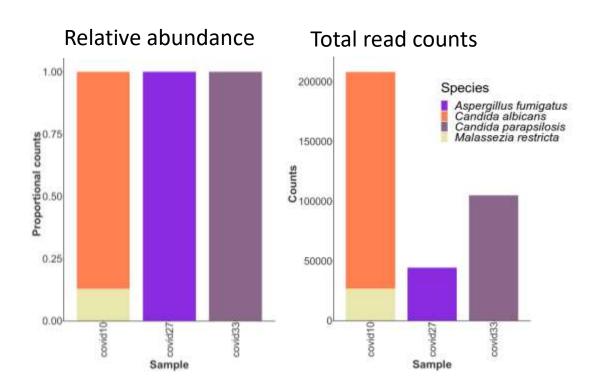
..and also other clinically relevant species

Searching for fungi in covid patient samples

- Covid-associated pulmonary aspergillosis (CAPA) reported in literature varying incidence
- · 32 covid patient samples
- 4 extraction negative controls
- 6 no template controls

Output: 3 samples with significant fungal counts 1) *C. albicans* 2) *A. fumigatus* 3) *C. parapsilosis*

Potential CAPA incidence of ~3%

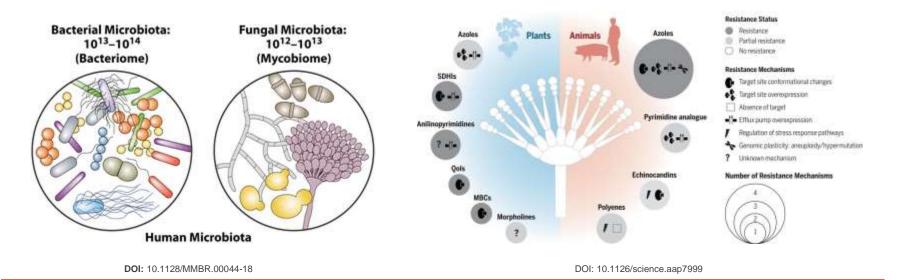


Summary

- Mycobiome sequencing is a promising tool to provide fungal diagnostics with broad range and speciation.
- The established ITS1 target can significantly under-represent filamentous fungi in mixed communities, leading to poor ID rates
- Novel target can identify filamentous fungi, even when present at 2%, and can ID as few as 2 copies of *A. fumigatus* in a sputum sample.
- Assay validation with clinical samples is ongoing preliminary data shows it can identify *A. fumigatus* but also reveal additional fungal pathogens - eg. *Scedosporium* – which may inform drug treatments

Looking to the future

- Combined fungal and bacterial pathogen identification?
- Combined pathogen ID & resistance screening?
- Drug discovery?



Acknowledgements

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Thanks for listening!