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Adrian Moody
Myconostica Ltd, South Court, Sharston Road, Sharston, Manchester, M22 4SN, UK
Tel: +44 (0) 161 998 7239 **Fax:** +44 (0) 161 902 2496

Development and Optimisation of a Novel Multiplex Assay for Identifying Candida Species

X.Cui[1], E.Hazlewood[1], D.Ireland[1], A.Moody[1], C.Moore[2], K.Radej[1], D.Tuckwell[1]
[1] Myconostica Ltd., Manchester, M22 4SN, UK [2] The Regional Mycology Laboratory, Wythenshawe Hospital, Manchester M23 9LT, UK

Abstract

Background: Patient mortality from candidaemia can be reduced by early treatment. However, the choice of antifungal therapy is influenced by the species causing the infection, and this can take days to identify. Thus there is a need for a rapid and accurate means of identifying fungal species from patient samples.

Methods: The "MycArray Yeast ID" kit is a DNA hybridisation assay which detects 18 *Candida* and yeast species including; *C. albicans, C. glabrata, C. krusei, C. parapsilosis, C. tropicalis, Histoplasma capsulatum,* and *Cryptococcus neoformans.* Multiple probes are present to cover intra-species genetic variation, and controls, including an internal amplification control, are included. The initial PCR stage can be carried out on DNA extracted from blood cultures or on "tooth-picks" of colonies, and the assay can be completed in under 4 hours.

Results: Assay conditions were established using multiple isolates of each species, giving a system sensitive to 1-2 base differences between probe and target. The array was then further tested and optimised using 20 clinical isolates each of the 5 major *Candida* species: *C. albicans, C. glabrata, C. krusei, C. parapsilosis,* and *C. tropicalis,* using colonies picked from agar plates. Conditions were established which led to the successful identification of approximately 95% of isolates as well as covering known intra-species genetic variation. The sensitivity and specificity of the array was subsequently tested using spiked blood cultures, and the performance was comparable to culture plates, functioning well over a very wide range of cell concentrations.

Conclusion: MycArray Yeast ID is able to identify yeast species with a rapid turnaround. The technique can be routinely applied to clinical samples where yeasts have been seen by Gram stain, to assist in the clinical identification of yeasts and the prescription of appropriate anti-fungal therapy.

Introduction

- Prompt treatment of fungal infections improves patient survival
- Identification of the causative agent of candidaemia has an important effect on the the choice of antifungal
- The MycArray™ Yeast ID kit was developed to rapidly identify and distinguish between Candida species (and other yeasts)
- The kit contains probes for 18 yeasts allowing the simultaneous detection of one or more species from colonies or yeast-positive blood cultures

Methods

Assay Design

- Probe design incorporated sequences in Myconostica's patent portfolio and used a combination of approaches including BLAST searches, multiple alignments and dedicated PERL scripts
- Multiple probes were designed to capture intra-species genetic variation (Table 1)
- The assay includes 3 separate process controls (Table 2)

Assay Optimisation

- Assay conditions were extensively optimised by testing multiple strains of each organisms using colony PCR and DNA extracted from blood culture bottles (Fig.1)
- The assay can be completed in under 4 hrs from PCR to results (Fig.2)

Assay Verification

- Optimised assay conditions were verified using at least 2 strains of each species with colony PCR
- The top 5 candida species have been extensively validated (~20 strains) using colony PCR, spiked blood culture bottles and simulated blood culture positive samples

Length (bases)	20 - 42	
Tm (°C)	58.9 - 60.9	
GC content	33.3 - 65.0 %	
Discrimination	Sensitive to 2 base differences between target and probe	
Number per species	*Candida albicans*	3
	Candida dubliniensis	2
	Candida famata	2
	Candida glabrata	3
	Candida kefyr	2
	Candida krusei	2
	Candida metapsilosis	1
	Candida parapsilosis	1
	Candida parapsilosis group	3
	Candida pelliculosa	2
	Candida rugosa	1
	Candida tropicalis	5
	Candida utilis	2
	Cryptococcus neoformans	2
	Histoplasma capsulatum	1
	Pichia guilliermondii	2
	Rhodotorula mucliaginosa	3
	Saccharomyces cerevisiae	2
	Broad-range fungal probes	3
	Internal Amplification Control	1

Table 1: Properties of probes

Sequence	Function
Three probes for a conserved region within 5.8S	(i) To detect a broad range of fungi, thereby covering rare organisms not represented specifically on the chip. (ii) To act as a control, e.g., absence of a signal could indicate that no PCR product has been added to the array.
Internal Amplification Control	To independently verify that the PCR has functioned properly
Biotin markers (biotin label printed directly onto the chip)	To verify that the final detection stage has worked.

Table 2: Internal process controls

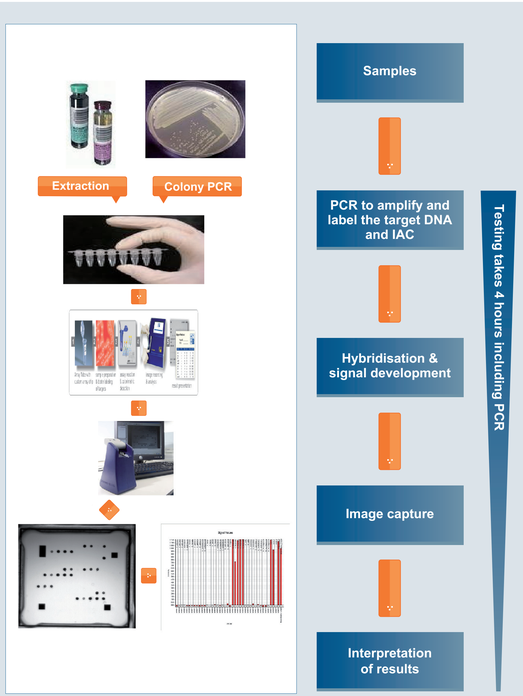

Figure 2: Summary of MycArray™ Yeast ID workflow

Figure 1: Assay optimisation using *C. metapsilosis* 8768 (NCPF) to reduce non-specific signal. The unprocessed image capture by the CCD camera in the Array Tube reader is shown before and after optimisation of hybridisation conditions. Binding to a specific probe is ringed in blue, where as binding to a non-specific probe, which differs by two bases, is ringed in red. Probes are spotted in triplicate. The optimisation of hybridisation conditions leads to removal of the non-specific signal and retention of the specific signal.

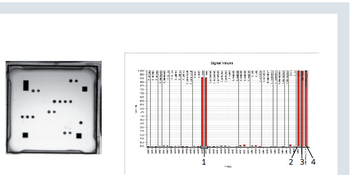

Figure 3: Example data from a colony PCR assay. MycArray™ Yeast ID assay was performed directly on a colony from a *C. krusei* F14237 agar plate. A) CCD camera image. B) Processed graphical representation of the array showing *C. krusei* signal (1); 5.8S control (2); Internal amplification control (3); and biotin control (4)

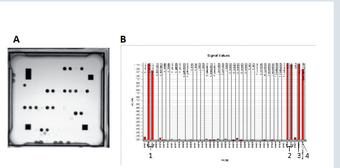

Figure 4: Example data from a yeast-positive blood culture clinical sample. MycArray™ Yeast ID assay was performed on DNA extracted using the MycXtra™ kit. A) CCD camera image. B) Processed graphical representation of the array showing *C. albicans* signal (1); 5.8S controls (2); Internal amplification control (3); and biotin control (4)

Results

- During initial verification the array was 100% specific using colony PCR (Figure 3)
- A discrepancy was seen with F14593 "*C. albicans*" which was identified as *C. glabrata* on the array. Sequence verification confirmed the array result.
- A clinical sample (a yeast-positive blood culture) was tested on the array and found to give a positive signal for *C. albicans* (Figure 4).

Conclusions

- The MycArray Yeast ID has been optimised to speciate 18 clinically relevant yeast species from agar plates and positve blood culture bottles
- The array process can be completed in 4 hrs
- Full validation studies for CE marking are on ongoing