High resolution melting analysis for rapid identification of twenty-five *Candida* species from culture

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Introduction

Although *Candida albicans* remains the most common fungal isolate from blood, many studies have described an increasing trend in non-*albicans* infections (Krcmery and Barnes 2002, Leroy *et al.* 2009). Correct identification of *Candida* species is important for targeted antifungal therapy and for epidemiological purposes. The aim of this study was to identify 25 *Candida* sp. from culture using high resolution melting analysis (HRMA).

Materials and Methods

We have developed real-time PCR followed by HRMA for differentiation of 25 *Candida* species. The assay was performed with the reference collections strains confirmed by ITS2 sequencing (**Table 1**).

Results

Real-time PCR with HRMA was performed with *Candida* reference strains in duplicates. Species could be identified by comparing the shapes of the melting curves and values of melting temperatures (Tm) with reference list (Figure 1, Table 1). Mean Tm ± 3SD values together with the shape of the melting curves were conclusive in 16 *Candida* species, while normalized temperature - shifted difference plot against *C. krusei* had to be used to distinguish other 5 species. Only Tm values of two pairs of *Candida* sp.: *C. fabianii*/*C. guilliermondii* and *C. orthopsilosis*/*C. metapsilosis* occurred repeatedly in the narrow ranges, therefore these species within 2 groups could not be reliably distinguished.

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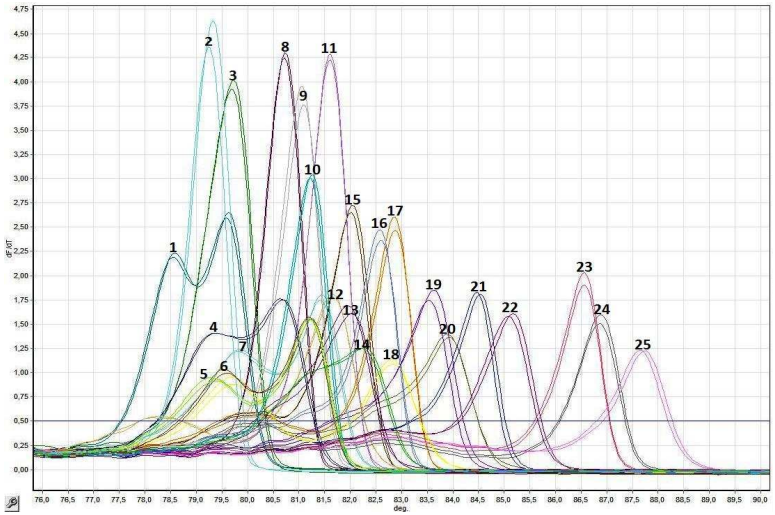

Figure 1: Melting curves of reference *Candida* species:
1) *C. pelliculosa*, 2) *C. zeylanoides*, 3) *C. saitoana*, 4) *C. tropicalis*, 5) *C. metapsilosis*, 6) *C. orthopsilosis*, 7) *C. parapsilosis*, 8) *C. utilis*, 9) *C. fabianii*, 10) *C. guilliermondii*, 11) *C. glabrata*, 12) *C. lipolytica*, 13) *C. intermedia*, 14) *C. pulcherrima*, 15) *C. dubliniensis*, 16) *C. kefyr*, 17) *C. albicans*, 18) *C. catenulata*, 19) *C. lusitaniae*, 20) *C. rugosa*, 21) *C. inconspicua*, 22) *C. californica*, 23) *C. krusei*, 24) *C. norvegensis*, 25) *C. lambica*

Conclusions

Real-time PCR followed by HRMA is a simple, rapid and inexpensive tool to identify *Candida* species. It seems to be a suitable complement to current clinical diagnostic approach based on usage of commercially available biochemical kits. This method should be further validated using clinical isolates.

species	reference strain No.	mean Tm (SD) °C	
		peak 1	peak 2
C. albicans	CCM 8320	80,098 (0,048)	82,938 (0,085)
C. glabrata	CCM 8270	81,705 (0,070)	
C. parapsilosis	CCM 8260	79,863 (0,071)	81,508 (0,061)
C. tropicalis	CCM 8264	79,433 (0,061)	80,738 (0,096)
*C. krusei**	CCY 29-9-17	86,605 (0,043)	
C. guilliermondii	CCY 39-23-6	81,255 (0,039)	
C. fabianii	CCY 38-20-1	81,083 (0,051)	
*C. utilis**	CCY 29-38-74	80,720 (0,073)	
*C. inconspicua**	CCY 26-26-11	84,603 (0,112)	
C. dubliniensis	CCY 29-177-1	80,200 (0,064)	82,090 (0,089)
C. pelliculosa	CCY 29-6-7	78,625 (0,060)	79,683 (0,065)
C. saitoana	CCY 26-9-9	79,695 (0,052)	
C. lusitaniae	CCY 29-59-1	83,663 (0,079)	
C. lipolytica	CCY 29-26-42	78,325 (0,139)	81,710 (0,052)
C. rugosa	CCY 29-15-1	83,980 (0,079)	
*C. norvegensis**	CCY 29-47-2	86,930 (0,087)	
C. catenulata	CCY 29-17-3	79,730 (0,067)	82,850 (0,028)
C. intermedia	CCY 29-12-10	81,968 (0,032)	
C. lambica	CCY 29-97-12	87,678 (0,039)	
C. pulcherrima	CCY 29-2-128	82,178 (0,062)	
C. orthopsilosis	MUCL 49939	79,563 (0,037)	81,208 (0,034)
C. metapsilosis	MUCL 46179	79,333 (0,093)	81,213 (0,083)
C. zeylanoides	MUCL 27735	79,328 (0,063)	
C. kefyr	MUCL 29857	82,658 (0,084)	
*C. californica**	CCY 29-93-4	85,170 (0,083)	

Table 1: Melting temperatures (Tm) of reference *Candida* species
***** normalized temperature - shifted difference plot against *C. krusei* had to be used to distinguish them

References: • Krcmery V., Barnes A.J. Non-albicans *Candida* spp. causing fungaemia: pathogenicity and antifungal resistance. J Hosp Infect. 2002; 50(4):243-60.
• Leroy O., Gangneux J.P., Montravers P., *et al.* Epidemiology, management, and risk factors for death of invasive *Candida* infections in critical care: A multicentre, prospective, observational study in France (2005-2006). Crit Care Med. 2009; 37(5):1612-8.