Cell surface hydrophobicity: a predictor of biofilm production in *Candida* isolates?

Biofilm-forming ability undoubtedly represents one of the major medical concerns in clinical practice, being a relevant pitfall for an efficient therapeutic intervention (Donlan & Costerton, 2002). In this scenario, invasive fungal infections, especially those involving *Candida* species, become an important problem in terms of early diagnosis and medical treatment. A large proportion of *Candida* infections are associated with biofilm formation, mainly on the surface of implanted medical devices (Douglas, 2003). Rapid methods for biofilm-forming ability evaluation in clinical isolates could thus improve the clinical outcome of the fungal infection. Biofilm formation is a step-by-step and finely regulated process in which the adhesion phase is a crucial event (Chandra *et al.*, 2001). Hydrophobic interactions play a role in the adherence of micro-organisms to a wide variety of surfaces.

We collected 142 isolates of *Candida albicans*, 138 of *Candida parapsilosis*, 39 of *Candida glabrata* and 37 of *Candida tropicalis*; all strains were from sterile sites (292 from blood, 63 from central venous catheters and 1 from pleural liquid). All isolates were banked for an Italian surveillance study on antifungal susceptibility of yeast isolates from invasive fungal infection in critically ill patients (Borghi *et al.*, 2010).

Protocols for inoculum preparation and biofilm growth followed those previously described (Pierce *et al.*, 2008), using a 96-well polystyrene plate. Biofilm-forming ability was quantified at 48 h by the XTT-reduction assay for counting metabolically active cells, and crystal violet staining for total biomass measurement (Peeters *et al.*, 2008). The experiments were performed with six replicates for each strain and the arithmetic mean of the absorbance values was used (Melo *et al.*, 2010). Microscopic examination of the biofilms was also used to check the efficiency of the two methods used to quantify biofilms.

The ability of yeast cells to adhere to hydrocarbon (octane; Sigma) was used as measure of their cell surface hydrophobicity (CSH) as described by Rosenberg *et al.* (1980). Briefly, the isolates were grown in YPD broth and washed with sterile saline solution as for biofilm assessment. The suspension was then adjusted to approximately 10^8 cells ml^{-1} (OD₆₀₀; A). The optical density of the aqueous phase (B), resulting from mixing the yeast suspension with octane, was determined spectrophotometrically at 600 nm. The CSH index was calculated as: $\text{CSH} = [(A - B)/A] \times 100$.

A regression model was used to evaluate the joint effect of *Candida* species and biofilm production (both as dummy coded variables) on CSH. Because of the heteroscedasticity, the analysis was also performed on ranks to confirm statistical significance. A different behaviour of biofilm production was shown in *Candida* species (interaction term was statistically significant).

No difference in biofilm production was ascribable to the isolation site. CSH mean values among the different species tested are reported in Table 1. Regarding *C. albicans*, about 43.7 % of all isolates showed a good biofilm-forming ability, although no significant differences were observed in cell wall hydrophobicity between biofilm producers and non-producer strains. The relatively low

hydrophobicity of *C. albicans* could be explained by the armamentarium of adhesins that can make up for it, promoting the adhesion process and biofilm formation in such extremely evolved species. In contrast, among the 37.7 % of biofilm producers in the *C. parapsilosis* group, we found a significantly higher percentage of hydrophobicity than in biofilm non-producers. The data for *C. tropicalis* showed a similar trend, with higher CSH values for biofilm-forming isolates and a lower hydrophobicity for non-producers. However, these latter represented only a small proportion of *C. tropicalis* isolates (21.6 %), confirming the high biofilm-forming ability of this species. We obtained a similar result for *C. glabrata*, although for this species the number of biofilm producers (15.4 %) was so scanty as to reduce the data reliability.

The positive correlation observed between biofilm formation and CSH suggested that hydrophobicity plays a major role in biofilm formation in *C. parapsilosis*, is likely to have a role in biofilm formation in *C. tropicalis* and has a lesser role in *C. glabrata* biofilm formation. In these latter, an extensive study on a large number of isolates is mandatory to confirm the data. CSH measurement seems to represent a simple and reliable method to predict biofilm-forming ability, at least in *C. parapsilosis* and *C. tropicalis* isolates from invasive infections. This knowledge could

Table 1. Cell surface hydrophobicity values in biofilm producers (P) and non-producers (NP) for *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *C. glabrata* invasive isolates: regression model results

Species	CSH value (%)		P vs NP Mean difference	t-test	P-value
	NP (mean)	P (mean)			
<i>C. albicans</i>	17.662	19.871	2.208	0.701	0.484
<i>C. parapsilosis</i>	40.267	75.865	35.598	10.884	<0.001
<i>C. tropicalis</i>	44.5	74.759	30.259	4.070	<0.001
<i>C. glabrata</i>	36.212	58.167	21.955	2.657	0.008

help clinicians in the drug choice, due to the well-documented azole resistance of fungal biofilm (Kuhn *et al.*, 2002). Moreover, guidelines for *Candida* bloodstream infection management still recommend medical device removal and replacement, although this procedure is not risk-free (Brass & Edwards, 2010). In this context, especially in patients needing long-term intravenous catheterization, a surrogate marker of biofilm-forming ability could positively influence the decision to remove or replace the device.

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