Differences in adhesion and biofilm formation among *Candida albicans* and *Candida dubliniensis*

M. Henriques¹, B. Fontinha¹, J. Azeredo¹ and R. Oliveira¹

¹ Centre of Biological Engineering, University of Minho, Campus Gualtar, 4710-057 Braga, Portugal
(E-mail: mcrh@deb.uminho.pt; belindafontinha@tugmail.com; jazeredo@deb.uminho.pt; roliveira@deb.uminho.pt)

Abstract

*Candida dubliniensis* is an opportunistic yeast that can cause serious systemic diseases. This recently found yeast has been mismatched with *Candida albicans* once it presented similar genotypic and phenotypic characteristics. In the normal body flora, these yeasts exist predominantly in the form of biofilm. The main goal of this work was the evaluation of the ability for initial adhesion and biofilm formation by both *Candida dubliniensis* (two strains) and *Candida albicans* (one strain). To mimic the oral conditions the biofilms were formed on acrylic coupons in artificial saliva and using Sabouraud Dextrose Broth (SDB) as control medium. The biofilms were formed during 72 hours and compared in terms of total number of cells (using crystal violet) and activity (measured with XTT). In terms of initial adhesion, the three strains showed no statistical differences. However, the biofilm formed by *Candida dubliniensis* 7988 behaved similarly to the biofilm of *Candida albicans*, while the biofilm formed by *Candida dubliniensis* 7987 had a distinct evolution, either in SDB or saliva. The results show that *Candida dubliniensis* biofilm formation differs among strains and is not directly correlated with initial adhesion.

Keywords

Biofilm; *Candida albicans; Candida dubliniensis*; biomass (CV); activity (XTT)

INTRODUCTION

Biofilms, structured microbial communities that are attached to a surface (Douglas, 2003), are the prevalent formation of microorganisms in nature. They are responsible for the development of clinical infections (Kuhn et al., 2002). In 1995, Sullivan et al., (Sullivan et al., 1995) described a new *Candida* species, *Candida dubliniensis*, responsible for oral diseases in HIV-infected individuals. Although *Candida albicans* is largely studied as adhesion and biofilm formation are concerned, considering *Candida dubliniensis* only few studies have been reported (Kirkpatrick et al., 2000; Ramage et al., 2001).

MATERIALS AND METHODS

The strains used were *Candida albicans* 12A, a clinical isolate kindly provided by the Department of Biology of the University of Minho and *Candida dubliniensis* 7987 and *Candida dubliniensis* 7988, obtained from Centraalbureau voor Schimmelcultures (CBS).

Initial adhesion

Yeast cells adhesion was performed according to Henriques et. al. (Henriques et al., 2004). The number of adhered cells to acrylic was automatically enumerated under epifluorescence microscopy.
Biofilm formation
Cells were sub cultured in Sabouraud dextrose agar for 24h at 37°C and then were grown in Sabouraud dextrose broth (SDB) at 37°C and 150 rpm for 18h. Cells were harvested by centrifugation (5000 rpm, 10 min) and resuspended in SDB to $5 \times 10^7$ cells/ml. The biofilm was grown in acrylic coupons (8×8 mm$^2$) in 24 well plates with 1 ml of cells suspension in each well. The medium (SDB) was changed each 12h and the biofilm analysed after 7, 14, 24, 48 and 72h of formation.

Quantification of biofilm mass and activity
Crystal violet (Stepanovic et al., 2000) was used to quantify the total cell number and XTT (Kuhn et al., 2002) (with 50 μg/μl of XTT and 5 μg/μl of PMS, for 3h) was used to quantify cellular activity.

RESULTS AND DISCUSSION
To mimic the oral conditions the assays were performed using a medium of artificial saliva, with SDB medium as a control, once it is the most appropriate medium for Candida species.
Comparing both media, the three strains studied presented different behaviours of biofilm mass accumulation (Figure 1).

![Graphs showing biofilm mass accumulation](image)

**Figure 1** – Values of absorbance of crystal violet obtained for biofilms formed in SDB (a) or Saliva (b) after 7, 14, 24, 48 and 72h for Candida albicans 12 A (alb12), Candida dubliniensis 7987 (dub 87) and Candida dubliniensis 7988 (dub 88).

It is interesting to notice that the two strains of Candida dubliniensis presented different behaviours in both media. In SDB medium the biofilm mass of Candida albicans and Candida dubliniensis 7988 increased until 72h and Candida dubliniensis 7987 reached a plateau after 48 h (Figure 1a), while in artificial saliva this behaviour was the opposite. In fact, Candida albicans and Candida dubliniensis 7988 presented a plateau after 48h, but for Candida dubliniensis 7987 the biofilm mass increased until 72h.

The biofilms were also quantified in terms of cell activity, either in SDB medium (Figure 2a) or artificial saliva (Figure 2b).
Figure 2 – Values of absorbance of XTT obtained for biofilms formed in SDB (a) or artificial saliva (b) after 7, 14, 24, 48 and 72h for Candida albicans 12 A ( alb 12), Candida dubliniensis 7987 (dub 87) and Candida dubliniensis 7988 (dub 88).

As in the case of biofilm mass accumulation, the strain Candida dubliniensis 7987 had a different behaviour in terms of cellular activity. Considering SDB, that strain presented a plateau after 48h, but the activity of Candida albicans 12A and Candida dubliniensis 7988 decrease after that time. While the cellular activity of Candida dubliniensis 7987 increased until 72h, the other two strains presented a plateau after 48h, in the case of artificial saliva.

Comparing the results obtained in terms of biofilm mass and biofilm activity it can be concluded that cell activity is not dependent on cell number. In the case of a mature biofilm, the number of total cells might be high but their activity can be low, for instances the cells in the deeper layer can be less active, due to diffusional limitations.

One of the main goals of this work was the comparison of biofilm formation with adhesion ability for the same strain and among strains. The assays of initial adhesion (1h) were performed in artificial saliva and in water as a control (Figure 3).

Figure 3 – Number of adhered cells of Candida albicans 12 A ( alb 12), Candida dubliniensis 7987 (dub 87) and Candida dubliniensis 7988 (dub 88) to acrylic in the presence of water and artificial saliva.

The number of initially adhered cells of Candida albicans and both Candida dubliniensis
to acrylic presented no statistical differences, either in water and artificial saliva. However, the mass accumulation during biofilm formation was significantly different for *Candida dubliniensis* 7987. The number of initially adhered cells increased in the presence of artificial saliva, while in the case of biofilm there were no significant differences concerning biomass accumulation.

**CONCLUSIONS**
Considering the number of initially adhered cells to acrylic, the strains studied presented no statistical differences compared to each other, either in water or saliva. However, in the case of biofilm formation *Candida dubliniensis* 7987 presented a different behaviour from *Candida albicans* and *Candida dubliniensis* 7988, both behaving similarly. This difference was obtained both in terms of biomass accumulation and cellular activity and for both media (SBD and Saliva). As a general conclusion, the number of initially adhered cells had no direct correlation with biofilm evolution.

**REFERENCES**


