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Candida/*Candida* biofilms. First description of dual-species *Candida albicans*/*C. rugosa* biofilm

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ABSTRACT

Denture liners have physical properties that favour plaque accumulation and colonization by *Candida* species, irritating oral tissues and causing denture stomatitis. To isolate and determine the incidence of oral *Candida* species in dental prostheses, oral swabs were collected from the dental prostheses of 66 patients. All the strains were screened for their ability to form biofilms; both monospecies and dual-species combinations were tested. *Candida albicans* (63 %) was the most frequently isolated microorganism; *Candida tropicalis* (14 %), *Candida glabrata* (13 %), *Candida rugosa* (5 %), *Candida parapsilosis* (3 %), and *Candida krusei* (2 %) were also detected. The XTT assay showed that *C. albicans* SC5314 possessed a biofilm-forming ability significantly higher ($p < 0.001$) than non-*albicans Candida* strains, after 6 h 37 °C. The total *C. albicans* CFU from a dual-species biofilm was less than the total CFU of a monospecies *C. albicans* biofilm. In contrast to the profuse hyphae verified in monospecies *C. albicans* biofilms, micrographies showed that the *C. albicans*/non-*albicans Candida* biofilms consisted of sparse yeast forms and profuse budding yeast cells that generated a network. These results suggested that *C. albicans* and the tested *Candida* species could co-exist in biofilms displaying apparent antagonism. The study provide the first description of *C. albicans*/*C. rugosa* mixed biofilm.

Introduction

Candida species inhabit the human oral cavity. *Candida albicans* is the species that is predominantly associated with mucosal and systemic fungal infections elicited by yeasts (Miceli *et al.* 2011). Nevertheless non-*albicans Candida* species have arisen a major opportunistic pathogens. *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida krusei* have been frequently isolated from the oral cavity and are often related to biofilm formation on the surface of medical devices and

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tissues (Pires *et al.* 2002; Pires-Gonçalves *et al.* 2007; Zahir & Himratul-Aznita 2013).

Biofilms constitute a structured community of microbial cells enveloped in a self-produced polymeric matrix consisting of extracellular DNA, carbohydrates, proteins, hexosamine, phosphorus, and uronic acid adhered to an inert or living surface (Flemming & Wingender 2010). Biofilm cells inherently have limited exposure to immunological defense and antimicrobial drugs, which could account for the emergence of resistant microorganisms (Lewis 2012). Slow growth, altered cell metabolism regulation due to nutrient limitation and stress conditions, and cell density are other suggested mechanisms of biofilm resistance (Seneviratne *et al.* 2010). In addition, biofilms represent a significant predictor of mortality (Tumbarello *et al.* 2007, 2012; Silva *et al.* 2011) and species that produce biofilms have been correlated with poor outcome of infected patients (Tumbarello *et al.* 2012). For example, *Candida* cells surviving on dentures after chemical or mechanical treatment constitute a highly tolerant cell subpopulation (persistent) that restores the biofilm in the presence of nutrients and causes a relapse of chronic infection (Lewis 2012).

Furthermore, the oral cavity harbours a vast range of microbial species. The residents themselves might interact extensively while forming the biofilm structures, to carry out physiological functions and induce microbial pathogenesis (Kuramitsu *et al.* 2007; Thein *et al.* 2009; Kolenbrander *et al.* 2010). The residents may (i) compete for nutrients; (ii) interact synergistically to stimulate the growth or survival of one or more residents; (iii) produce antagonist compounds that inhibit the growth of another resident; (iv) neutralize a virulence factor produced by another resident; and (v) interfere in the growth-dependent signaling mechanisms of another resident (Kuramitsu *et al.* 2007). It is likely that resident microorganisms interact closely in this scenario, allowing a mixed-species biofilm to grow.

Most studies on polymicrobial biofilms have focused on the relationship between bacteria and *Candida* spp. However, *Candida*–*Candida* co-existence within biofilms has been poorly demonstrated (Kirkpatrick *et al.* 2000; Coco *et al.* 2008; Cuellar-Cruz *et al.* 2012). Thus, this study aimed to examine the frequency of *Candida* spp. on prostheses from denture wearers and to assess the formation of both monospecies and dual-species biofilms consisting of *C. albicans* and *C.* non-*albicans* strains. To our knowledge, this is the first report of mixed biofilm formation by *C. albicans* and *Candida rugosa*.

Materials and methods

Sample

Dental prostheses from 66 patients (33 males and 33 females, aged from 29 to 92 y) attending the outpatient clinics of the Dentistry School of the University of Franca, State of São Paulo, Brazil, were used in this study. All the subjects provided informed consent according to the protocols approved by the Human Subjects Committee of the

Table 1 – Distribution of patients studied.

Strains	Patients (number)																							
	Age range 20–49 y								Age range 50–69 y								Age range 70–99 y							
	Female				Male				Female				Male				Female				Male			
	Tp		Pp		Tp		Pp		Tp		Pp		Tp		Pp		Tp		Pp		Tp		Pp	
	Col	NC	Col	NC	Col	NC	Col	NC	Col	NC	Col	NC	Col	NC	Col	NC	Col	NC	Col	NC	Col	NC	Col	NC
C. albicans	P3			P56	P5			P60	P2 P4 P6*			P47 P48	P13 P21 P22		P9*	P49 P55	P11 P17* P36		P43	P52 P58	P10 P12 P16*			P53 P59
									P14* P15 P18*		P14*		P24 P25*			P61 P64	P39** P40				P23* P26* P27			P65
									P28 P37 P44				P34 P35				P41* P42				P32 P33			
C. glabrata									P18*				P8 P25*				P17* P39** P41*				P19			
C. krusei													P20**											
C. parapsilosis																					P16* P23*			
C. rugosa									P6*		P14*		P20**											
C. tropicalis	P29							P31	P30 P38				P20**		P9*		P39**				P26*			
Absent															P46 P51	P50 P63								
															P54 P01									

Tp: Total prosthesis; Pp: Partial prosthesis; Col: Colonized patient; NC: Non-colonized patient; P: patient; *: patient colonized with two *Candida* species; **: patient colonized with three *Candida* species.