

Candida albicans MORPHOTYPES FROM ORAL CAVITY IN SEVERAL FAMILIES

(*Morfotipos de Candida albicans de la cavidad oral
en varias familias*)

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Key words: *Candida albicans*, *Candida* spp., oral cavity, morphotypes, denture plaque.

SUMMARY

A total of 108 individuals belonging to 22 families consisting of 3 to 8 members each were studied in order to detect *Candida albicans* morphotypes. Yeasts were isolated from the oral cavity of at least one individual per family, with or without teeth.

Following Phongpaichit et al. technique, identical or different morphotypes were detected in 7 families. In 10 families, *C. albicans* was isolated both from the saliva and the removable denture of the same individual. A discontinuous fringe, considered to be a probable virulence factor, was observed in 9 (16,7%) of the *C. albicans* strains, from people having the complete set of teeth, in the saliva of the former and of those using denture. A continuous fringe was observed in 5 (83,3%) of the *C. albicans* strains isolated from a same sites. Morphotyping, a simple and easily executed method which is useful to detect cross-infection, may serve as a "risk indicator" for the occurrence of candidosis.

C. albicans was the prevalent species (86,4%) detected in at least 2 members from 14 families.

INTRODUCTION

The presence of yeast in the human oral cavity usually causes few complications. The infection originated by these microorganisms may be fully asymptomatic, although lesions of the oral mucosa are clearly visible during clinical examination(1). The possible role of yeasts in the development of oral cancer and in infections by microorganisms requires careful attention(2). Patients under treatment with

RESUMEN

Se estudiaron 108 personas provenientes de 22 familias compuestas de 3 a 8 miembros cada una, para la búsqueda de morfotipos de *Candida albicans* de la cavidad bucal con o sin piezas dentarias.

Por la técnica de Phongpaichit et al., morfotipos idénticos o diferentes fueron detectados en 7 grupos familiares. En 10 familias, *C. albicans* fue aislada de la saliva y de prótesis dentarias removibles de un mismo individuo.

La franja discontinua, considerada como probable indicador de virulencia fue detectada en 9 (16,7%) de las cepas de *C. albicans* de personas con dentición completa y de saliva de usuarios y no usuarios de prótesis. La franja continua fue verificada en 5 (83,3%) de las cepas de *C. albicans* aisladas de un mismo sitio. El morfotipo es simple, fácil de ejecutar, puede servir para detectar infección cruzada y como un posible indicador de riesgo de candidosis.

C. albicans fue la especie prevalente (86,4%), detectándose a lo menos en 2 miembros de 14 familias.

cytostatic drugs and radiotherapy are particularly vulnerable to yeast infections(3,4). Other local or systematic predisposing factors such as decreased salivary flow, poor oral hygiene, treatment with corticosteroids, and immunologic deficiency may contribute to the transformation of these microorganisms, usually considered to be commensal, into disease causing agents(5,6).

It has been documented that the frequency of isolation and concentration of yeasts in the mouth increases among

individuals wearing partial or full denture and that this increase is associated with the appearance of erythematous areas in the oral mucosa (7,8). According to Lal *et al.* (9), stomatitis caused by denture, may serve as a model system for the study of infectious processes caused by *Candida albicans* and other *Candida* species in the oral cavity.

Over the last few years several studies have been conducted to characterize yeasts not only in terms of species for taxonomic purposes, but also in terms of pathogenicity and mainly for epidemiological studies. In the case of the phenomenon of morphologic variation of *C. albicans*, the technique called morphotyping is used (11,13). Borronco *et al.* (10) morphotyped *C. albicans* isolated from the oral cavity and noted that different morphotypes were isolated from smokers' denture and without erythematous candidosis, and from smoking and non-smoking denture wearers who presented the disease; a single type was isolated from non-smokers with a healthy oral mucosa wearing no dentures.

Considering that oral infection with yeast may occur by direct or indirect contact between infected and non infected individuals and that dentures may serve as reservoirs of these microorganisms.

In the present study we isolated and identified yeasts from saliva and denture plaque samples obtained from persons belonging to the various family groups to determine the intrafamily similarity of *C. albicans* strains by the morphotyping technique.

MATERIAL AND METHODS

We selected 22 families residing in the town of Ribeirão Preto, S.P., Brazil, of different socioeconomic levels and consisting of at least three members, including the parents, one of whom had to be a wearer of a denture.

Colonies with yeast characteristics isolated in SB20 culture medium modified (14) from saliva and denture plaque samples were grown on agar Sabouraud (Sabouraud Dextrose Agar-Difco) supplemented with 50 mg/L chloramphenicol maintained at room temperature. After development, the yeast colonies were plated on agar Sabouraud for maintenance.

Yeast were identified by classical methods (15) using the following tests: microculture on Cornmeal agar, germ tube in human serum, fermentation/assimilation of different carbohydrates and nitrogen compounds.

Morphotyping was carried out by the technique of Phongpaichit *et al.* (13). *C. albicans* suspensions in sterilized distilled water containing approximately 1.0×10^7 cell/ml., were swabbed on plates containing Malt Extract Agar (Oxoid) and incubated at 30°C for 10 days. The results were read in terms of morphological aspects related to

colony fringe and surface, recorded by means of 4 digit codes according to the method of Hunter *et al.* (12) for later interpretation.

RESULTS

The 22 families selected consisted of 3 to 8 individuals each (mean=3), for a total of 108: 25 of them wearing full dentures and 83 with natural teeth. Thus, the sampling consisted of 133 materials, i.e., 108 saliva samples (83 from dentate individuals, 25 from denture wearers and 25 from the denture plaque).

Yeasts were detected in the oral cavity of at least one individual per family for a total of 61 (56.9%) and the following species were identified: *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. famata*, *C. guilliermondi*, *Candida* spp. and *Trichosporon* spp. (Table 1).

TABLE 1.
Yeasts species isolated from the oral cavity of the various family groups

Species	Families (22)		Individuals (108)	
	Nº	%	Nº	%
<i>Candida albicans</i>	19	86.4	43	39.8
<i>Candida tropicalis</i>	7	31.8	9	8.3
<i>Candida parapsilosis</i>	6	27.3	8	7.4
<i>Candida famata</i>	1	4.5	1	0.9
<i>Candida guilliermondi</i>	1	4.5	1	0.9
<i>Candida</i> spp.	3	13.6	3	2.8
<i>Trichosporon</i> spp.	1	4.5	1	0.9

Twenty of the 25 full denture wearers (80%) carried the yeast, 15 of them presenting it both in saliva and in denture plaque, 3 only in saliva and 2 only in denture plaque. It should be emphasized that 15 of the 20 denture wearers were monocolonized and 5 multicolonized.

Yeasts were isolated from 41 saliva samples from non-denture wearers (49.4%), all of them monocolonized.

C. albicans, the prevalent species, was isolated from 19 families for a total of 54 strains from saliva and/or dentures. Of these 19 families, 14 (73.7%) had at least 2 members colonized by this species. In 10 families (57.9%), *C. albicans* was also isolated from the saliva and denture plaque of the same individual (Table 2).

Typing of the 54 *C. albicans* samples resulted in 20 different morphotypes, the most frequent being number 5330, 5340 and 5240 (Table 3). Table 2, shows that in 7 families (Nº 3, 5, 11, 15, 16, 17, 22), 2 individuals harbored *C. albicans* strains of the same morphotypes. Among the

TABLE 2.
Morphotypes of 54 *Candida albicans* strains isolated from 43 individuals belonging to 19 families

Nº	Family		Individual Nº		Morphotype	
	Nº of individuals				Saliva	Dentures
1	3		1		3240	3240
			3		5340	
2	4		4		5340	
			6		7540	
3	4		9		7320	
			10		5330	
			11		5330	
5	7		21		5240	5240
			22		Nd*	
			24		5330	
			29		7340	
6	7		30		3230	7342
			39		Nd	
			40		5340	
8	5		41		5520	3241
			53		5241	
			54		5541	
10	8		53		5241	5330
11	6		59		3241	
			60		3241	
12	4		63		2240	5330
			64		0000	
15	4		73		5341	5341
			74		5330	
			75		3240	
16	4		77		5340	5340
			78		5330	
			79		5330	
17	7		81		5340	5321
			82		0000	
			83		5340	
			84		5340	
			85		7342	
20	5		96		5222	Nd
			97		7510	
			98		0000	
			100		5240	
22	4		106		5240	
			108		5240	
			14		5240	
4	6		14		5240	
7	5		35		7340	
9	5		46		Nd	0000
13	3		67		Nd	5340
14	5		72		2240	

*Nd: not detected

denture wearers, 4 individuals (Nº 1, 21, 73, 77) carried strains of the same type both in saliva and on dentures (Table 2).

Colonies with discontinuous fringes were observed in

9 strains (16.7%) and colonies with continuous fringes were observed in 45 (83.3%). Of the 9 strains with discontinuous fringes, 2/15 (13.3%) were from denture plaque, 4/14 (28.6%) from the saliva of denture wearers and 3/25 (12.0%) from the saliva of non-denture wearers (Table 3).

TABLE 3.
Morphotypes of 54 *C. albicans* strains, isolated from dentures, saliva of denture wearers and from the saliva of no denture wearers

Morphotype (20)	Denture wearers (20)		No-denture	Total
	Denture (15)	Saliva (14)	Saliva (25)	
0000	2	0	3	5
2240	0	1	1	2
3230	0	1	0	1
3240	1	1	1	3
3241	1	1	1	3
5222	0	1	0	1
5240	1	2	3	6
5241	0	1	0	1
5321	1	0	0	0
5330	3	3	3	9
5340	2	2	5	9
5341	2	1	0	3
5520	0	0	1	1
5541	0	0	1	1
7320	0	0	1	1
7340	0	0	2	2
7342	1	0	1	2
7510	0	0	1	1
7540	0	0	1	1
7641	1	0	0	1

DISCUSSION

Yeasts, and *C. albicans* in particular, are integrating microorganisms of the normal microbiota of the oral cavity, which is altered in patients who carrier dentures, causing the so-called denture stomatitis (DS). In a study of yeast-like fungi among college students from Porto Alegre, Brazil, Santos(16), found yeasts of the genus *Candida* in 40.7% even though they were adults with higher education, dental students with ample information about oral hygiene.

In a study of yeast distribution and concentration among 50 patients with DS, Davenport(17) noted that 49 (98%) saliva samples were positive compared to 30 (60%) from subjects without the disease. *C. albicans* was isolated from 70% of the patients with stomatitis and from 20% of the controls, and other species were detected in 14% and 16% of patients with and without DS, respectively. Narhi et al.(18), reported that high yeast counts in saliva were associated with lesions of the oral mucosa but not with individual complaints of buccal symptoms. They observed that 33% of dentate individuals, 25% of partial denture

carriers and 20% of full denture carriers were also free of this microorganism.

Our results about the incidence of yeasts in the oral cavity of adults (56.9%) agree with data reported by Davenport(17), who isolated these microorganisms from 60% of subjects with no stomatitis. It is more common to find yeasts among denture wearers (80.0%) than in individuals with natural teeth (49.4%) agrees with data reported by Parvinen(7) in Finland.

The prevalent species, *C. albicans*, was isolated from 39.8% of the individuals analyzed in the present study, a lower frequency than that reported by Davenport(17) in this study of saliva from individuals with stomatitis (70%). However, this frequency was higher than that reported by this author for individuals without the disease and also higher than that reported by Arendorf & Walker(19), who isolated this species from 29.6% of the saliva samples studied.

The literature contains many descriptions of techniques for the differentiation of *C. albicans* strains, such as serotyping, resistogram, susceptibility to killer toxins, enzyme production and different techniques at the molecular level (20,21,22,23).

In the present screening of *C. albicans* strains isolated from the members of a family group by the morphotyping technique, we noted that 7 of the 14 families (50%) with at least two individuals colonized by this species, presented individuals with the same morphotype in the oral cavity, and in 3 family groups the morphotypes of the strains of certain individuals were characterized by the fact that they only differed in one digit (Table 2). This fact, by itself, is not indicative of intrafamily transmission, however, the occurrence of these pairings in a restricted cluster such as the family, allows us to infer that some *C. albicans* strains may have been transmitted in an intrafamily manner. The observation that in 4 (36.4%) of the 10 families in which *C. albicans* was isolated from the saliva and/or from the denture of the same individual, the morphotype was identical in the two samples (Table 2), reflects the possibility that the microorganisms were from a single source, the denture, which is considered to be a yeast reservoir in the oral cavity. The detection of different morphotypes in strains isolated from the saliva and the dentures of the same individuals was possibly related to the fact that different sources existed in the oral cavity, such as lesions of the oral mucosa, or that the same individual possibly carried more than one morphotype in the same site.

Hunter et al.(12), using morphotyping for differentiation of *C. albicans* strains, observed a marked association between the presence of discontinuous fringes characterized by the 1st. digit (Nº 1, 2 and 3) of the typing system in colonies of these species isolated from samples obtained from patients who suffered a fatal infection. According to

these investigators, the discontinuous fringe may serve as an indicator of *C. albicans* virulence.

Our data demonstrate that most of the strains (45; 83.3%) had continuous fringes (Table 3), which is not surprising if we consider that all strains were from the oral cavity of apparently healthy individuals. As to the 9 (16.7%) strains with discontinuous fringes, those considered virulent (3; 12.0%) were from the saliva of 25 individuals without dentures, while the other 6 strains were isolated from 4 (20%) of the 20 denture carriers, 2 of them from the dentures and 4 from saliva. These data indicate that 12% of the

individuals with normal teeth and 20% of the denture carriers are susceptible to the disease (candidosis) if no preventive measures are provided for.

Morphotyping, a simple standard method that permits the individualization of isolated strains, can be applied to complement other phenotypic biotyping techniques such as those based on fungal molecular biology.

Thus, morphotyping may serve as a "risk indicator" for the acquisition of candidosis, especially among full denture carriers.

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