EVALUATION OF BIOCHEMICAL AND SEROLOGICAL METHODS TO IDENTIFY AND CLUSTERING YEAST CELLS OF ORAL *Candida* SPECIES BY CHROMagar TEST, SDS-PAGE AND ELISA

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ABSTRACT

The purpose of this work was to evaluate biochemical and serological methods to characterize and identify *Candida* species from the oral cavity. The strains used were five *Candida* species previously identified: *C. albicans*, *C. guilliermondii*, *C. parapsilosis*, *C. krusei*, *C. tropicalis*, and *Kluyveromyces marxianus*, as a negative control. The analyses were conducted through the SDS-PAGE associated with statistical analysis using software, chromogenic medium, and CHROMagar Candida (CA), as a differential medium for the isolation and presumptive identification of clinically important yeasts and an enzyme-linked immunoabsorbent assay (ELISA), using antisera produced against antigens from two *C. albicans strains*. This method enabled the screening of the three *Candida* species: *C. albicans*, *C. tropicalis*, and *C. Krusei*, with 100% of specificity. The ELISA using purified immunoglobulin G showed a high level of cross-reaction against protein extracts of *Candida* species. The SDS-PAGE method allowed the clustering of species-specific isolates using the Simple Matching coefficient, S_{SM} = 1.0. The protein profile analysis by SDS-PAGE increases what is known about the taxonomic relationships among oral yeasts. This methodology showed good reproducibility and allows collection of useful information for numerical analysis on information relevant to clinical application, and epidemiological and systematical studies.

Key words: *Candida* species, protein electrophoresis, numerical analysis, chromogenic medium (CHROMagar Candida), immunoenzimatic assay (ELISA).

RESUMO

Avaliação de métodos bioquímicos e sorológicos na identificação e agrupamento de espécies de *Candida* por CHROMagar Candida, SDS-PAGE e ELISA

Este trabalho teve o propósito de avaliar métodos bioquímicos e sorológicos para serem aplicados na caracterização e identificação de linhagens do gênero *Candida* isoladas da cavidade bucal. As cepas empregadas representam cinco espécies de *Candida* previamente identificadas: *C. albicans*, *C. guilliermondii*, *C. parapsilosis*, *C. krusei* e *C. tropicalis*, utilizando como controle negativo *Kluyveromyces marxianus*. Foram empregadas as técnicas de gel de poliacrilamida (SDS-PAGE) associado à análise estatística em software, CHROMagar Candida (CA), meio cromogênico diferencial descrito para o isolamento e identificação presuntiva de leveduras de importância clínica e um ensaio de imunoabsorção ligado a enzima (ELISA), utilizando antissoro produzido contra extratos protéicos de uma linhagem-

padrão de *Candida* e um isolado de cavidade oral de *C. albicans*. O método mostrou-se adequado para a identificação presuntiva de *C. albicans*, *C. tropicalis* e *C. krusei*, com 100% de sensibilidade e especificidade, com base na coloração e textura das colônias. O método de ELISA utilizando imunoglobulinas G purificadas apresentou alto teor de reação cruzada com as outras espécies de *Candida* estudadas. A análise do perfil protéico por SDS-PAGE permitiu agrupar os isolados da cavidade oral por intermédio do coeficiente "Simple Matching", S_{SM} = 1,0. Os perfis protéicos analisados por SDS-PAGE ampliam os conhecimentos sobre as relações taxonômicas de leveduras isoladas da cavidade oral. Esta metodologia demonstra boa reprodutibilidade e origina informações úteis para aplicação clínica e estudos que envolvem a sistemática e a epidemiologia.

Palavras-chave: espécies de *Candida*, eletroforese de proteínas, análise numérica, meio cromogênico (CHROMagar Candida), ensaio imunoenzimático (ELISA).

INTRODUCTION

Yeast cells, especially *Candida* species, are common in oral cavities and in immunocompromised and immunocompetent individuals (Rinaldi, 1993; Wade, 1997), with a predominance of *C. albicans*. Their isolation from the mouth can be used to investigate reduced salivary flow rate (Parvinen & Larmas, 1981), excessive consumption of fermentable carbohydrates (Samaranayake *et al.*, 1986), dental caries risk, and denture-wearing status (Beighton *et al.*, 1991). Their isolation can also be useful in dealing with infectious diseases, surgeries, antibiotics administration length and medical immunosupression (Odds, 1988; Walsh & Pizzo, 1993) which can trigger the development of candidosis caused by pathogenic *Candida* species. These are therefore, good reasons to evaluate and improve the characterization methods currently used for these microorganisms (Rodrigues, 2000).

Conventional methods for the classification of *Candida* species are based on morphological and physiological features. The most common test used to diagnose *C. albicans* is the analysis of germ tubes, which may be unreliable due to the amount of inoculum and the time required for microscopy examination (Quindós, 1997).

Several alternative methods have been proposed to characterize yeast cells (Kreger-van Rij, 1984; Odds, 1991). Based upon physiology and biochemistry of yeast cells, differential media with chromogenic substrates are used to identify distinct species. The direct identification of yeasts from clinical samples was taken a step further by Odds and Bernaerts, 1994, who reported the use of a new differential and selective medium, CHROMagar Candida (CA), for the isolation and identification of *Candida* species. It has been suggested that contrasting colony color and texture produced by hydrolysis of chromogenic substrates in this medium allows presumptive identification of yeast species isolate (Odds & Bernaerts, 1994; Baumgartner *et al.*, 1996; Pfaller *et al.*, 1996).

Serological reactions can be an important tool in characterizing yeast species (Tsuchiya *et al.*, 1965), and avoiding mistakes that occur in morphological interpretation (Joly, 1954). Ponton & Jones (1993) showed that antigens expressed on the surface of germ tubes could be used in the taxonomy of *Candida* species, even though the serological tests appeared to be less specific.

On the other hand, protein patterns have been proposed by several authors (Shechter, 1973; Vancanneyt *et al.*, 1991; Höfling *et al.*, 1998a; Höfling *et al.*, 1999) as a reliable method for typing strain species. Lee *et al*. (1986) characterized 190 *C. albicans* strains isolated from 142 pacients in the London Hospital based on total protein content and Western blot analysis. This technique used an antiserum produced in rabbbits against a prenssate of *C. albicans* NCTC 3153 serotype A, and was more sensitive than the procedure previously used with these same strains serotyped, morphotyped, and biotyped during an outbreak of systemic *C. albicans* which has been described Burnie *et al.* (1985).

To study the variety of microorganisms in the oral cavities, Maiden & Tanner (1991) used SDS-PAGE and silver staining to identify yeast presence. Another recent report described the characterization of *Candida* species from oral cavities using multilocus enzyme electrophoresis (MLEE) (Rosa *et al.*, 2000). Analysis of the clusters formed based on the protein bands in polyacrylamide gel electrophoresis with sodium dodecyl sulphate (SDS-

PAGE) can be carried out using software which identifies the similarities between species and inter-species (Rosa *et al.*, 2000; Höfling *et al.*, 1998b).

This study reports the evaluation of biochemical and serological methods to identify clustering yeast cells of oral *Candida* species by CHOMagar test, SDS-PAGE, and ELISA using primary antibodies produced in rabbits. These evaluations were accomplished by using as reference patterns *C. albicans*, *C. guilliermondii*, *C. parapsilosis*, *C. krusei*, and *C. tropicalis*.

MATERIAL AND METHODS

Strains

A total of 13 previously identified strains, from the FOP/UNICAMP Fungus Collection Mycoteca recently isolated from human oral cavities of thirteen clinically heal subjects were investigated. Five *Candida* type strains (*C. albicans*, *C. tropicalis*, *C. krusei*, *C. guilliermondii*, and *C. parapsilosis*) from the André Tosello Tropical Foundation for research and technology, Campinas, SP, Brazil, and a negative control strain, *K. marxianus*, from the Yeast Generic Laboratory at ESALQ-USP, Piracicaba, SP, Brazil, were also analyzed. The 19 strains were coded from 1 to 19 (Table 1). All strains were maintained in solid slants of yeast peptone dextrose (YPD) medium (1% w/v yeast extract, 1% w/v peptone, 2% w/v dextrose, 2% w/v agar).

CHROMagar Candida *medium*

The selective CHROMagar *Candida* – CA (Paris, France) was prepared according to manufacturer recommendations (Probac do Brasil). To establish initial chromogenic identification criteria for each species, known reference strains of *Candida* species were plated on CA. All 19 strains were cultured in YPD agar medium for 24 hours at 37ºC, subcultured in the CHROMagar medium for 48 hours at 37ºC. There after, the color and morphology of the colonies were recorded.

TABLE 1
***Candida* species isolated from human oral cavities, *Candida*-type strains and their negative control, with their codes.**

Code	Species name	Source codes
1	*C. albicans*	CBS-562^T
2	*C. albicans*	97-a
3	*C. albicans*	F-72
4	*C. albicans*	E-37
5	*C. albicans*	17-b
6	*C. guilliermondii*	CBS-566^T
7	*C. guilliermondii*	FCF-405
8	*C. guilliermondii*	FCF-152
9	*C. parapsilosis*	CBS-604^T
10	*C. parapsilosis*	21-c
11	*C. parapsilosis*	7-a
12	*C. krusei*	CBS-573^T
13	*C. krusei*	1M-90
14	*C. krusei*	4-c
15	*C. tropicalis*	CBS-94^T
16	*C. tropicalis*	1-b
17	*C. tropicalis*	FCF-430
18	*C. tropicalis*	Ct-4
19	*K. marxianus*	IZ-1339

Statistical evaluation of CA

Colony appearance of CA was analyzed in terms of sensitivity (number of true positives/number of true positives plus the number of false negatives) and specificity (number of true negatives/number of true negatives plus number of false positives).

Protein extraction

All strains were cultured in 250 mL YPD medium for 24 h at 37ºC, in a shaker table under 150 rpm of agitation. The cells were then washed three times with phosphate-buffered saline, PBS (NaCl 8.0 g, KCl 0.2 g, Na_2HPO_4 1.15 g, and KH_2PO_4 0.2 g per liter, pH 7.36), and homogenized with mortar and pestle after the addition of liquid nitrogen. The homogenate was resuspended in 250 mL of PBS and centrifuged at 105,000 x *g* for 60 min at 4ºC to completely remove cell debris (Axelsen, 1973). The supernatants were collected and protein concentration was determined by the Bradford method (Bradford, 1976).

Protein quantification (Bradford´s method adapted by Rodrigues, 2000)

The proteins were diluted 1:100, 2 L of protein plus 198 L of Milli-Q water (Millipore) and microplates of 96 wells were coated with 50 L of the diluted protein extracts from 19 yeast strains and mixed with 200 µL of Bradford reagent (Bio-Rad) per well. After incubation for two minutes at room temperature, the samples in the plates were read with optical densities at 590 nm (OD_{590}) by means of an ELISA reader (Micro plate reader 550, Bio-Rad) and the protein concentration adjusted to 800 µg/mL (Ames, 1974). The proteins were stored at –70ºC for further analysis by SDS-PAGE and ELISA.

SDS-PAGE analysis

The preparation of the proteins for each yeast strain was made according to the Bruneau & Guinet (1989) method. Equal volumes of supernatant and loading buffer (5 mM Tris-HCl, 2.5% 2-β-mercaptoetanol 1.5% SDS, 0.025% bromophenol blue, pH 6.8, were combined and heated for 5 minutes at 95-100ºC, and SDS-PAGE was performed according to Laemmli (1970), using 12% (w/v) polyacrylamide gels. Proteins in the gel were stained with silver nitrate (Vancanneyt, 1991), scanned, and the profiles of each lane were analyzed by densitometry in the Kodak Digital Science Software 1DTM based upon the presence (1) or absence (0) of specific bands. Six standard proteins in the Low Molecular Weight SDS Calibration Kit (MW) with range of 14.4-97 kDa (code 17-0446-01, Amersham-Pharmacia Biotech UK) were used. Similarity dendrograms were built using the unweighted pair-cluster method with arithmetic (UPGMA) with Simple Matching association coefficient, based on band positions computed with the NTSYS software package, version 1.70 (Aplied Biostatistics) for the data (Rohlf, 1992).

Production of antisera

Antigen extraction

Protein extracts from *C. albicans* type-strain (1) and from *C. albicans* isolated from an oral cavity (2), obtained as described above, were used to produce the antisera.

Animals and antisera

Antisera AS_1 and AS_4 were produced in two female New Zealand white rabbits denominated A_1 and A_4, respectively, and immunization was carried out every 14 days by intramuscular injections of 0.5 mg/ml of an oral cavity *C. albicans* strain antigen, emulsified in Freund`s incomplete adjuvant (Sigma), near the lymphonodes region of the hind leg. Two other white female rabbits denominated A_2 and A_3, producing antisera AS_2 and AS_3 respectively, were also immunized with antigen from *C. albicans* (1) type strain, exactly as described by Oliveira (1975). The animals were bled from the marginal ear vein before the first injection to ensure that they were antibody –free. Prior to each immunization, blood samples from the four rabbits were collected; the serum was separated by centrifugation at 4,000 x *g* at 4ºC.

Purification of the rabbit antibodies

Part of the sera (1.5 mL) from the two chosen rabbits with the best titer results were blended in 4 mL of buffer 0.2 M Tris-NaCl (pH 8.2) and the eluate was colleted with buffer 0.2 M Glycine-NaCl (pH 3.0) by a sepharose protein A column (Bio-Rad) (Oi & Herzenberg, 1980) for purification of the immunoglobulin G. Antibody concentration was determined at 280 nm with a proportion of OD/mg IgG = 1.3:1 and the antisera titer was read by ELISA.

Immunoenzymatic assays -ELISA

Polystyrene 96-well microplates were coated with 50 µL of the protein extracts (10 µg/mL) from

19 yeast strains and incubated at 37°C for one hour. After incubation, the plates were blocked by with 200 µL of 1% bovine serum albumin (BSA) in PBS per well for 1 h at 37°C and the plates were washed three times with PBS containing 0.05% Tween 20 and 0.25% gelatine (PBS-T-G). After three additional washes with PBS-T-G, 50 µL samples of 1:200 diluted rabbits sera, produced against the protein extracts of the type-strain (1) and against the isolate from oral cavity (2) of *C. albicans*, were added to each well and incubated for 1 h at 37°C. After being washed as described above, the plates were incubated for 1 h at 37°C with 50 µL of peroxidase conjugated anti-rabbit IgG (Bio-Rad) diluted 1:5.000 in PBS. After incubation, the plates were washed three times with PBS-T-G and 50 µL of substrate solution was added. The substrate solution was prepared immediately before use by dissolving 0.4 mg of o-phenylenediamine (Sigma) per mL in 0.05 M citrate buffer (pH 5.3). The plates were incubated for 20 min in darkness. The reaction was stopped by adding 50 µL of H_2SO_4, and optical densities at 492 nm (OD_{492}) were measured with an ELISA reader (550, Bio Rad). The ELISA procedures were carried out according to established protocols (Crowther, 1995). Reactions were performed using both raw and purified sera in triplicate, and the results are expresssed as mean OD for each determination. Test results were considered positive if the OD exceeded a mean OD + 3 standards (SDs) obtained by the control sera.

RESULTS AND DISCUSSION

CHROMagar test

Strains from *Candida* species were observed after the incubation period in the medium CA and analysis allowed the identification of four groups of microorganisms with more than one species previously identified. Table 2 illustrates the frequency of the strains according to colony morphology and color. The colonies on these media presented a smooth and brilliant present green color (36.84%); colonies with dark blue to blue-gray color smooth brilliant (15.79%); pale pink and purple color, rough opaque with spreading, pale edges (5.26%), and pale pink, purple, and white smooth brilliant colony (42.10%).

The CA demonstrated the presence of samples mistakenly isolated (Tables 2 and 3). The type strain *C. krusei* showed typical morphology, being easy to separate from all other types of yeast colonies and having the following appearance; dry, flat, and rough texture, and spreading colonies with a pale pink color and pale edges. These characteristics were not seen with any other species or isolates, including two isolates, 13 and 14, previously identified as *C. krusei* that showed none of these characteristics except for only the pale pink color. Early recognition of *C. krusei* is very important, because it is intrinsically resistant to fluconazole (Patterson *et al.*, 1996). The CA certainly proved useful in recognizing mistakenly identified or mixed cultures with 100% of specificity and sensibility (Odds & Bernaerts, 1994; Rennie *et al.*, 1998) and may provide additional information in laboratories that do not regularly perform identification beyond the germ tube test.

The CA is able to support the selective growth of yeasts while suppressing the growth of other microorganisms such as bacteria, and at the same time, to maintain viability for further testing. Recognizing mistakenly identified cultures is facilitated because of the clear color discrimination between yeast species. However, further testing is necessary to confirm the identification of other species besides *C. albicans*, *C. tropicalis* and *C. krusei*. These data corroborated those in the literature and showed that this method was highly specific for *C. albicans*, *C. tropicalis*, and *C. krusei* strains (Giusiano & Mangiaterra, 1998; Pfaller *et al.*, 1996; Odds & Bernaerts, 1994).

SDS-PAGE analysis

The protein concentration as determined by the Bradford method in microplate shows that in order to apply them to the SDS-PAGE gels (Table 4), the protein was adjusted to 16 µg/mL to applied in SDS-PAGE. Qualitative interspecific differences among the 19 (nineteen) strains were assessed by the protein profiles and analyzed based on the presence or absence of specific bands.

The application of UPGMA clustering method allowed pre construction of the phenogram with Simple Matching association coefficient (S_{SM}; Sneath & Sokal, 1973; Sokal & Michener, 1958; Boriollo *et al.*, 2000). The dendrogram shown in (Fig. 1) expresses the evaluation of the strains based on SDS-PAGE protein profiles. Three major clusters of similarity were formed, each one showing a Simple Matching coefficient (SM) $S_{SM} = 1.0$ with 100% of similarity. The results expressed by CA and electrophoresis protein profiles associated to a statistical

analysis, showed that at least six of the thirteen cultures isolated previously were mistakenly specified. Only one yeast strain isolated from an oral cavity did not cluster with any reference pattern and has not been identified, thus requiring additional tests not done in this paper.

Reproducibility

The strain profiles of the samples on different gels were reproducible after three repetitions of each electrophoretic running, therefore confirming the viability of the method and its application in clinical and epidemiology studies. Molecular weight marker was applied in all gels providing mean values $S_{SM} = 1.0$.

Immunoenzymatic assays

Test results were considered positive when the OD exceeded a mean OD + 3 standards (SDs) using negative control sera with $OD_{492} = 0.060$; the best dilution to react against all the antigens was 1:200. The sera AS_3 and AS_4 from the 42nd day of immunization from rabbits A_3 and A_4 were chosen for the ELISA method because they had the best antibody titer. The quantitation of the IgG purified by sepharose protein-A in mg of IgG/mL using the equation: 1.3 OD = 1 mg of IgG/mL. Results were 1.48 mg/ml for AS_3, and 1.66 mg/mL for AS_4, at 280 nm.

The indirect ELISA was done using extracellular proteins from different strains to sensitize the microplates. The groups formed by ELISA were the same as those formed by the SDS-PAGE profiles clustered in the dendrogram (Fig. 1). However the high cross reactivity observed among the strains tested against purified polyclonal antibodies by the use of ELISA was not reliable enough for characterizing oral *Candida* species.

The purified immunoglobulin G was tested in a concentration of 10 μg/mL of IgG. Fig. 2 shows the ELISA results expressed in OD_{492} nm using IgG purified by sepharose protein-A produced against the type-strain (1), and an oral cavity strain isolate (2). Four of the five *C. albicans* strains showed high antibody titer. Cluster 1 was formed by four (1, 2, 3, and 4) of the five *C. albicans* strains, two strains from *C. guilliermondii* (7, 8) and *C. tropicalis* (16), expressing high antibody titer and high specificity, reacting with the antisera produced against *C. albicans*. Strain five (5), identified as *C. albicans* and clustered with *C. parapsilosis* (9, 10, and 11) and *C. krusei* (14), formed a second cluster, (Cluster 2) with very low specificity for the produced antisera. *C. tropicalis* (15, 17, and 18) showed high titer, thus forming Cluster 3. Type-strain *C. krusei* (12), *C. guilliermondii* (6) and the *K. marxianus* specie sample (19) did not show any antibody titer.

TABLE 2
Colonies characteristics with CHROMagar test.

Group	Number	Species	Color and morphology (frequency and percentage)
I	1, 2, 3, 4, 7, 8 and 16	*C. albicans*, *C. guilliermondii* *C. tropicalis*	Green, smooth and brilliant (7/19 and 36.84%)
II	15, 17 and 18	*C. tropicalis*	Dark blue to blue-gray, with dark halo in agar, smooth and brilliant (3/19 and 15.79%)
III	5, 6, 13, 14 9, 10, 11 and 19	*C. albicans*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis*, and *K. marxianus*	Pale pink pale pink, purple pale pink pale pink, white pale pink smooth and brilliant (8/19 and 42%)
IV	12	*C. krusei*	Pale pink, purple, rough with spreading, pale edges and opaque (1/19 and 5.26%)

TABLE 3
Sensitivity and specificity for the more frequent species of *Candida.*

Species	Strains	Sensitivity (%)	Specificity (%)
C. albicans	7	100	100
C. tropicalis	3	100	100
C. krusei	1	100	100

TABLE 4
Protein concentration of the yeast strain extracts centrifuged at 105,000 x *g*.

Code	Species name	Source codes	Protein concentration (mg/mL)
1	*C. albicans*	CBS-562	2.20
2	*C. albicans*	97-a	1.97
3	*C. albicans*	F-72	3.32
4	*C. albicans*	E-37	2.14
5	*C. albicans*	17-b	1.73
6	*C. guilliermondii*	CBS-566	2.59
7	*C. guilliermondii*	FCF-405	4.75
8	*C. guilliermondii*	FCF-152	3.56
9	*C. parapsilosis*	CBS-604	3.16
10	*C. parapsilosis*	21-c	1.48
11	*C. parapsilosis*	7-a	2.09
12	*C. krusei*	CBS-573	3.23
13	*C. krusei*	1M-90	4.05
14	*C. krusei*	4-c	0.98
15	*C. tropicalis*	CBS-94	1.74
16	*C. tropicalis*	1-b	0.82
17	*C. tropicalis*	FCF-430	2.21
18	*C. tropicalis*	Ct-4	1.70
19	*K. marxianus*	IZ-1339	5.56

CONCLUSIONS

Although the ELISA is a sensitive and specific method, the use of antibodies produced in rabbits and the immunoglobulin G purified were ineffective in identifying species due to the high level of cross-reaction detected. This is probably due to a limited molecular variability present in the different *Candida* species. The use of monoclonal antibodies would increase the specificity and, thereby, improve differentiation among the species, as showed by Ponton & Jones (1993), where antigens on the surface of germ tubes could be used in the taxonomy of *Candida* species.

The CA test was a fast and efficient first screening method for identifying some clinically important yeast species that occur in the oral cavity. CA test were generally completed in 24 hours, and yield reproducible

results. It was observed four main patterns of reactions in CA test associated to *Candida species*, i. e., green (*C. albicans*), dark blue (*C. tropicalis*), pale pink (*C. krusei* and others *Candida species*). *C. krusei* may be distinguished from other *Candida species* that produce pale pink colonies in CA medium by the rough and spreading morphology of its colonies. The use of CA in clinical dental microbiology would greatly facilitate the study of oral yeast flora, particularly by favouring the discrimination between *C. albicans* and other yeast species, and enabling the presence of mixed yeast populations to be readily recognized and their clinical significance to be assessed.

Based on the data obtained in this study, some previously identified strains may have been mistakenly classified in the wrong species as was strain (5), which in all classified as *C. albicans* but probability is *C. parapsilosis*, and strains (7) and (8), classified as *C. guilliermondii* but which seem to be *C. albicans*. Strain (13), initially classified as *C. krusei*, could not be clustered with any other cluster. Strains could have been incorrectly identified or somehow altered during their culturing, manipulation or storage as has already been mentioned by other authors (Vancanneyt *et al.*, 1991; Vancanneyt *et al*., 1992). These results may be seen in the CA medium, ELISA, and in the SDS-PAGE methods.

The analysis made by SDS-PAGE was the most efficient method for characterizing *Candida* strains from the oral cavity, because these species showed significant differences in protein electrophoretic patterns, and variation by gel showed good reproducibility. The high-resolution electrophoresis combined with proteins in the gel stained with silver nitrate developing nanograms of protein (Vancanneyt *et al.*, 1991), as well as scanned and computer-assisted numerical analysis of the one-dimensional protein electrophoretic patterns proved able to differentiate *Candida* species (Rosa *et al.*, 2000; Höfling *et al.*, 1999), allowing the strains to cluster species-specifically.

To identify yeasts present in the oral cavity or other ecosystems, more than one method should be used since care in the handling of these strains and type-strains is a very important factor in the accuracy of research involving clinical, epidemiological, and taxonomy studies. If the initial screening is performed by CA, specific for three of the main *Candida* species, and this information is associated with SDS-PAGE of cellular proteins, this approach could be used as a convenient and valuable tool for yeast taxonomy. Moreover, this method could be used as a routine procedure for the identification of yeast strains from the oral cavity or other sites since a database of reference proteins has already been constructed.

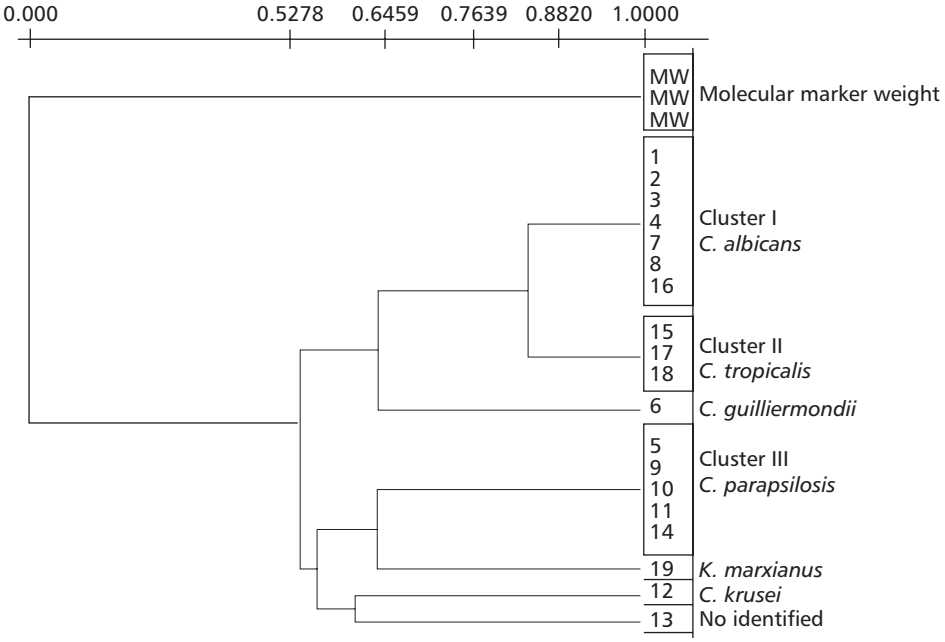

Fig. 1 — Dendrogram of the 19 yeast strains electrophoretic protein profiles obtained by UPGMA using the Simple Matching coefficient (SM).

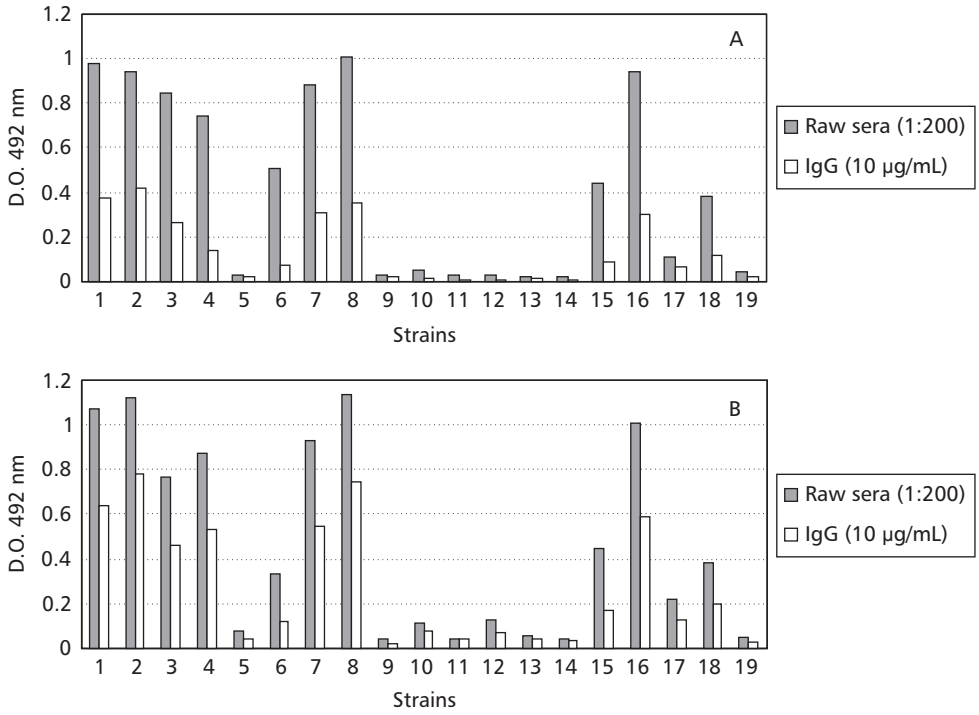

Fig. 2 — ELISA (A and B) – Immunoassay test showing reaction of the antisera against 19 yeast antigens. A. (2) oral cavity purified antisera (IgG/AS_3) and B. (1) type strain purified antisera (IgG/AS_4). Strains used: 1) CBS-562, 2) 97-a, 3) F-72, 4) E-37, 5) 17-b, 6) CBS-566^T, 7) FCF-405, 8) FCF-152, 9) CBS-604^T, 10) 21-c, 11) 7-a, 12) CBS-573^T, 13) 1M-90 14) 4-c, 15) CBS-94, 16) 1-b, 17) FCF-430, 18) Ct-4, and 19) IZ-1339.

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