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ELECTROPHORETIC PROTEIN PATTERNS AND NUMERICAL ANALYSIS OF *Candida albicans* FROM THE ORAL CAVITIES OF HEALTHY CHILDREN

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SUMMARY

The aim of this research was to evaluate the protein polymorphism degree among seventy-five *C. albicans* strains from healthy children oral cavities of five socioeconomic categories from eight schools (private and public) in Piracicaba city, São Paulo State, in order to identify *C. albicans* subspecies and their similarities in infantile population groups and to establish their possible dissemination route. Cell cultures were grown in YEPD medium, collected by centrifugation, and washed with cold saline solution. The whole-cell proteins were extracted by cell disruption, using glass beads and submitted to SDS-PAGE technique. After electrophoresis, the protein bands were stained with Coomassie-blue and analyzed by statistics package NTSYS-pc version 1.70 software. Similarity matrix and dendrogram were generated by using the Dice similarity coefficient and UPGMA algorithm, respectively, which made it possible to evaluate the similarity or intra-specific polymorphism degrees, based on whole-cell protein fingerprinting of *C. albicans* oral isolates. A total of 13 major phenons (clusters) were analyzed, according to their homogeneous (socioeconomic category and/or same school) and heterogeneous (distinct socioeconomic categories and/or schools) characteristics. Regarding to the social epidemiological aspect, the cluster composition showed higher similarities ($0.788 < S_D \leq 1.0$) among *C. albicans* strains isolated from healthy children independent of their socioeconomic bases (high, medium, or low). Isolates of high similarity were not found in oral cavities from healthy children of social stratum A and D, B and D, or C and E. This may be explained by an absence of a dissemination route among these children. Geographically, some healthy children among identical and different schools (private and public) also are carriers of similar strains but such similarity was not found among other isolates from children from certain schools. These data may reflect a restricted dissemination route of these microorganisms in some groups of healthy scholars, which may be dependent of either socioeconomic categories or geographic site of each child. In contrast to the higher similarity, the lower similarity or higher polymorphism degree ($0.499 \leq S_D < 0.788$) of protein profiles was shown in 23 (30.6%) *C. albicans* oral isolates. Considering the social epidemiological aspect, 42.1%, 41.7%, 26.6%, 23.5%, and 16.7% were isolates from children concerning to socioeconomic categories A, D, C, B, and E, respectively, and geographically, 63.6%, 50%, 33.3%, 33.3%, 30%, 25%, and 14.3% were isolates from children from schools LAE (Liceu Colégio Albert Einstein), MA (E.E.P.S.G. "Prof. Elias de Melo Ayres"), CS (E.E.P.G. "Prof. Carlos Sodero"), AV (Alphaville), HF (E.E.P.S.G. "Honorato Faustino), FMC (E.E.P.G. "Prof. Francisco Mariano da Costa"), and MEP (E.E.P.S.G. "Prof. Manasses Ephraim Pereira), respectively. Such results suggest a higher protein polymorphism degree among some strains isolated from healthy children independent of their socioeconomic strata or geographic sites. Complementary studies, involving healthy students and their families, teachers, servants, hygiene and nutritional habits must be done in order to establish the sources of such colonization patterns in population groups of healthy children. The whole-cell protein profile obtained by SDS-PAGE associated with computer-assisted numerical analysis may provide additional criteria for the taxonomic and epidemiological studies of *C. albicans*.

KEYWORDS: Numerical analysis; SDS-PAGE; *C. albicans*; Healthy children; Oral cavity.

INTRODUCTION

Different types of electrophoretic techniques have been used for the characterization or typing of *Candida* species including separation of chromosomes, DNA fragments, isoenzymes, cell-wall glycoproteins and whole-cell proteins[4,7,8,27,28,32,45,49]. Regarding whole-cell proteins, their separation has been employed satisfactorily in the characterization of bacteria and yeasts[12,21,23,56,57,58,59]. The resulting electrophoretic profiles can be plotted into a binary value matrix that, with computer-assisted support, produces comparative results expressed as similarity or cophenetic correlation matrixes and phenograms[24]. Several investigators employed electrophoretic analysis of whole-cell proteins in the fungi

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taxonomy[18,20,48,59]. In addition, variations in this technique made it possible to analyze the intra- and inter-specific variability of *Candida* species. Radiolabeling proteins with [^{35}S] methionine were used satisfactorily in the differential identification of *Candida* species and other yeasts[49]. The polyacrylamide gel electrophoresis (SDS-PAGE) also has been used in the identification of oral yeasts. This technique showed high specificity in addition to the rapid acquisition of significant data for classification[28]. In several cases, unidimensional electropherograms of whole-cell proteins and DNA-DNA hybridization data were equalized as their discriminatory capacities[9,22,23,24,35]. Bacterial strains with 90-100% of similarity in DNA sequences generally have identical protein patterns, while those with at least 70% of similarity in DNA sequences have similar protein profiles. Such observations showed to be the major pillars on which the application of protein electrophoresis in microbes was based. Moreover, the comparison of electrophoretic protein patterns has been considered a technique having satisfactory taxonomic resolution, which may be applicable to the level of species, subspecies and biotypes[24]. The aim of the present investigation was the evaluation of the protein patterns and numerical analysis of *C. albicans* strains from healthy children oral cavities according to their socioeconomic base and/or school in order to establish their similarities and possible dissemination routes.

MATERIALS AND METHODS

Yeast isolation. A total of seventy-five *C. albicans* isolates from the oral cavities (saliva) of seventy-five healthy students (6-8 year-old), living in Piracicaba city, São Paulo State, Brazil, having a socioeconomic category (A, B, C, D, or E) and/or from schools (AV, CS, FE, FMC, HF, LAE, MA, or MEP)[33] were studied. These isolates were identified by morphological criteria by germ tube test, chlamydospore test, growth in chromogenic medium CHROMagar Candida[34], and associated to carbohydrate assimilation and fermentation tests[33,44] (Table 1).

Cell cultivation and whole-cell protein extraction. All strains were grown in 50 mL of YEPD medium (2% dextrose, 2% peptone, 1% yeast extract) in a shaker table at 150 rpm, at 30 ºC, overnight (late log phase – approx 10^8 cells/mL)[4,8]. After growth, the cells were harvested by centrifugation at 3,000 *g* for 5 min, and the pellets were washed three times in cold sterile 0.9% NaCl in order to remove either culture medium traces or extra-cellular metabolites[60,61]. The last washed pellets were transferred to micro-centrifuge tubes (2 mL) plus glass beads (v/v) and 500 µL of cold sterile water were added[3,40]. Cells were lysed using a Mini-Bead Beater cell disrupter (Biospec Products, Inc.) at 4,200 rpm, repeating four times of 30 sec each, at 5 min intervals, and placed in an ice bath[3,40]. After cell disruption, tubes were centrifuged at 10,000 *g* for 5 min, and the supernatant protein concentration was determined, according to BRADFORD (1976)[6,15] and adjusted to 1.6 µg.µL^{-1} [2]. Equal volumes of supernatant and loading buffer (5 mM Tris, 2.5% 2-mercaptoethanol, 1.5% SDS, 0.025% bromophenol blue, 15% glycerol) were mixed and heated in a boiling water bath for 10 min[7].

Polyacrylamide gel electrophoresis (PAGE). SDS-PAGE protein profiles were obtained after electrophoresis of 50 µL of denatured protein solution in polyacrylamide slab gels[56] with SDS (sodium dodecylsulfate) in a discontinuous buffer system[25] with 4.5% stacking gel and 12.5% running gel. The electrophoresis was performed at 125 volts in a cold chamber and the protein bands present in the gels were fixed in a solution of 12.5% sulfosalicylic acid for 20 min and stained with 0.025% Coomassie Blue G-250 for 12h. The gels were destained by successive washings in acetic acid:methanol:water (1:2.5:6.5) solution [1].

Table 1
Relation of *C. albicans* samples collected from the oral cavities of healthy students having a socioeconomic base

	Schools							
	AV	CS	FE	FMC	HF	LAE	MA	MEP
Sample code	A1	D21	A7	B18	A24	A23	A17	B31
	A20	D23	A9	C9	B21	A30	B24	C32
	B4	E7	A10	C47	B33	A33	B56	C33
	B5	E8	A15	D5	C12	A40	B54	C36
	B32	E10	A21	D6	C16	A48	B46	C40
	C4	E25	B11	D13	C17	A50	B23	D19
		E30	B13	D14	C20	A52		D20
		E32	B15	D25	C23	A55		
		E39	B16	D28	C44	A61		
			C5	D32	C48	A64		
				D36		B38		
				E17				
				E43				
				E48				
				E52				
				E57				
n	6	9	10	16	10	11	6	7

n corresponds to the number of isolates in students population (one isolate per each healthy student). The letters A, B, C, D, and E correspond to isolates deriving from healthy students classified in socioeconomic categories A (19 isolates), B (17 isolates), C (15 isolates), D (12 isolates), and E (12 isolates), respectively. The abbreviations AV, CS, FE, FMC, HF, LAE, MA, and MEP correspond to the schools Alphaville (private), E.E.P.G. "Prof. Carlos Sodero" (public), E.E.P.G. "Profa. Francisca Elisa" (public), E.E.P.G. "Prof. Francisco Mariano da Costa" (public), E.E.P.S.G. "Prof. Honorato Faustino" (public), Liceu Colégio Albert Einstein (private), E.E.P.S.G. "Prof. Elias de Melo Ayres" (public), and E.E.P.S.G. "Prof. Manasses Ephraim Pereira" (public), respectively.

Numerical analysis. The images of the gels were captured using an HP 4C scanner (Hewlett Packard Co.) and the relative mobility (Rm values and/or molecular weights) of each protein band was determined by SigmaGel software (Jandel Co.). Matches and mismatches among the bands (originated from presence/absence of protein bands) had the representations 1 and 0, respectively, considering a confidence interval of ± 1.245[5]. These data made it possible to construct a binary value matrix that was analyzed using the statistics package NTSYS-pc version 1.70 (Applied Biostatistics, Inc.). The Dice similarity coefficient (1945), S_D = 2a/(2a + u) = 2a/(2a + b + c), was used to obtain the matrix of similarity (S_D)[14]. Dendrogram, represented by non-rooted trees, based on S_D values was generated by the unweighted pair-group arithmetic average (UPGMA) clustering method[16,50,51]. Matrix of cophenetic values (S_C) was derived from the UPGMA dendrogram and the Pearson product-moment correlation coefficient (r_{CS}),

$$r_{ki} = \frac{s_{ki}}{\sqrt{s_k^2 s_i^2}} = \frac{\sum_{j=1}^{p}(y_{ij} - \bar{y}_i)(y_{kj} - \bar{y}_k)}{\sqrt{\sum_{j=1}^{p}(y_{ij} - \bar{y}_i)^2}\sqrt{\sum_{j=1}^{p}(y_{kj} - \bar{y}_k)^2}},$$

was then computed between the elements S_{ij} of the original similarity matrix

(S_D) and cophenetic values C_{jk} of the matrix S_C[16,23,26,51,53,54]. The type-strain of *C. albicans* CBS562 (Centralbureau voor Schimmelcultures, Delft, The Netherlands) and molecular weight markers (Bovine Serum Albumin 66,000 Da, Bovine Pancreas Trypsinogen 24,000 Da, Bovine Milk β-Lactoglobulin 18,400 Da – Sigma-Aldrich Co.) were included in this experiment, in order to establish the degree of similarity among the *C. albicans* strains and to determine reproducibility[7,12,13,59].

RESULTS

Reproducibility. The strain protein profiles on different gels were reproducible after three repetitions of each electrophoretic running. Protein extracts of *C. albicans* CBS562 and molecular weight markers were applied in all gels, providing mean value $S_D = 0.897$.

Strain clustering. The electrophoretic whole-cell protein patterns of *C. albicans* made possible the observation of 20 major bands per lane, within a molecular range weight varying between 18,400 Da and 66,000 Da (Fig. 1). The application of UPGMA clustering method made it possible to build a similarity dendrogram (Fig. 2), in which thirteen major clusters (13 of the 36 78.8-*phenons*) may be distinguished with $0.788^{78.8\%} < S_D \leq 1.0^{100\%}$. These clusters have the following compositions:

Cluster 1 (78.8-*phenon* I): 4 (5.3%) isolates (D19^{MEP}, E32^{CS}, E39^{CS}, and E43^{FMC}) with $0.788 < S_D < 1.0$. Their composition was heterogeneous in regards to both schools (CS, FMC, and MEP) and socioeconomic categories (D and E).

Cluster 2 (78.8-*phenon* II): 4 (5.3%) isolates (E8^{CS}, E10^{CS}, E25^{CS},

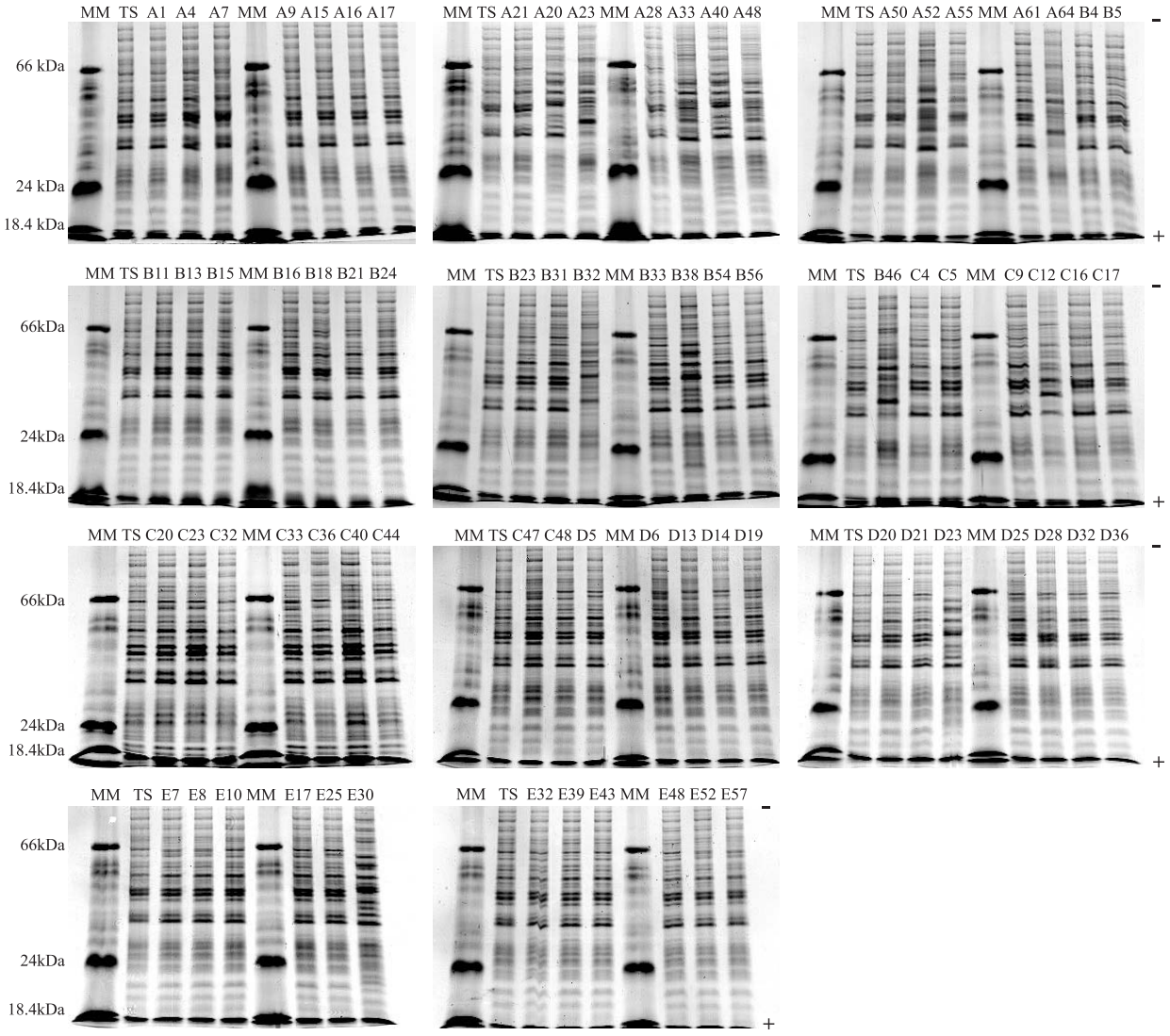

Fig. 1 - Electrophoregrams of whole-cell protein profiles from the seventy-five *C. albicans* strains isolated from healthy students' oral cavities classified in five socioeconomic categories. MM: Molecular weight markers (Bovine Serum Albumin 66,000Da, Bovine Pancreas Trypsinogen 24,000Da, Bovine Milk b-Lactoglobulin 18,400Da – Sigma-Aldrich Co.). TS: *C. albicans* CBS562 (type-strain).

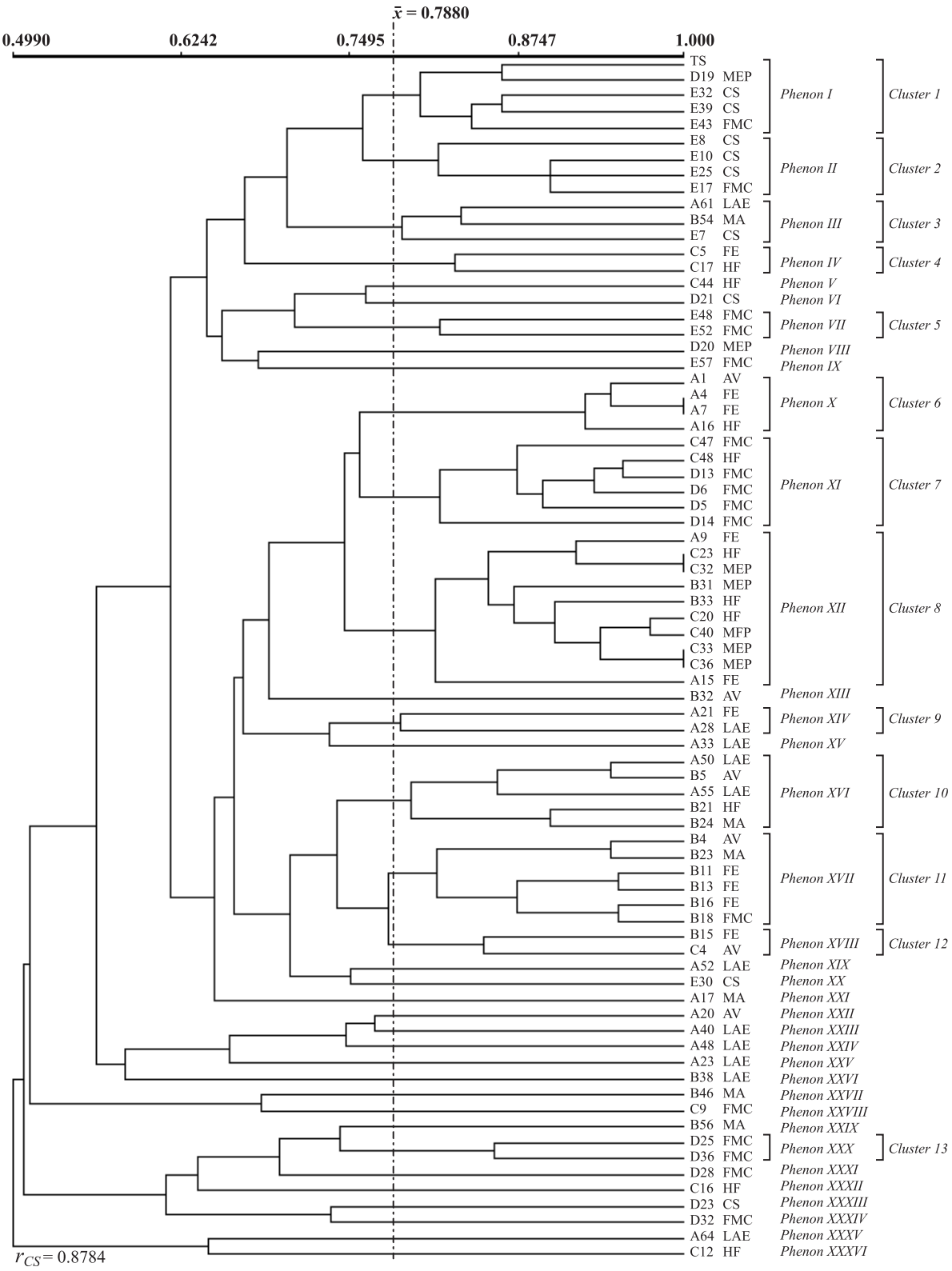

Fig. 2 - UPGMA dendrogram showing the similarity among *C. albicans* strains isolated from healthy students' oral cavities from five socioeconomic categories, based on their protein profiles obtained by SDS-PAGE.

and E17^{FMC}) with $0.788 < S_D < 1.0$. Their composition was heterogeneous and homogeneous in regards to schools (CS and FMC) and socioeconomic category (E), respectively.

Cluster 3 (78.8-*phenon* III): 3 (4%) isolates (A61^{LAE}, B54^{MA}, and E7^{CS}) with $0.788 < S_D < 1.0$. Their composition was heterogeneous in regards to both schools (CS, LAE, and MA) and socioeconomic categories (A, B, and E).

Cluster 4 (78.8-*phenon* IV): 2 (2.7%) isolates (C5^{FE} and C17^{HF}) with $0.788 < S_D < 1.0$. Their composition was heterogeneous and homogeneous in regards to schools (FE and HF) and socioeconomic category (C), respectively.

Cluster 5 (78.8-*phenon* VII): 2 (2.7%) isolates (E48^{FMC} and E52^{FMC}) with $0.788 < S_D < 1.0$. Their composition was homogeneous in regards to both school (FMC) and socioeconomic category (E).

Cluster 6 (78.8-*phenon* X): 4 (5.3%) isolates (A1^{AV} , A4^{FE}, A7^{FE}, and A16^{HF}) with $0.788 < S_D \leq 1.0$. Their composition was heterogeneous and homogeneous in regards to schools (AV, FE, and HF) and socioeconomic category (A), respectively.

Cluster 7 (78.8-*phenon* XI): 6 (8%) isolates (C47^{FMC}, C48^{HF}, D13^{FMC}, D6^{FMC}, D5^{FMC}, and D14^{FMC}) with $0.788 < S_D < 1.0$. Their composition was heterogeneous in regards to both schools (HF and FMC) and socioeconomic categories (C and D).

Cluster 8 (78.8-*phenon* XII): 10 (13.3%) isolates (A9^{FE}, C23^{HF}, C32^{MEP}, B31^{MEP}, B33^{HF}, C20^{HF}, C40^{MEP}, C33^{MEP}, C36^{MEP} and A15^{FE}) with $0.788 < S_D \leq 1.0$. Their composition was heterogeneous in regards to both schools (FE, HF, and MEP) and socioeconomic categories (A, B, and C).

Cluster 9 (78.8-*phenon* XIV): 2 (2.7%) isolates (A21^{FE} and A28^{LAE}) with $0.788 < S_D < 1.0$. Their composition was heterogeneous and homogeneous in regards to schools (FE and LAE) and socioeconomic category (A), respectively.

Cluster 10 (78.8-*phenon* XVI): 5 (6.7%) isolates (A50^{LAE}, B5^{AV}, A55^{LAE}, B21^{HF}, and B24^{MA}) with $0.788 < S_D < 1.0$. Their composition was heterogeneous in regards to both schools (AV, HF, LAE, and MA) and socioeconomic categories (A and B).

Cluster 11 78.8-(*phenon* XVII): 6 (8%) isolates (B4^{AV}, B23^{MA}, B11^{FE}, B13^{FE}, B16^{FE}, and B18^{FMC}) with $0.788 < S_D < 1.0$. Their composition was heterogeneous and homogeneous in regards to schools (AV, FE, FMC, and MA) and socioeconomic category (B), respectively.

Cluster 12 (78.8-*phenon* XVIII): 2 (2.7%) isolates (B15^{FE} and C4^{AV}) with $0.788 < S_D < 1.0$. Their composition was heterogeneous in regards to both schools (AV and FE) and socioeconomic categories (B and C).

Cluster 13 (78.8-*phenon* XXX): 2 (2.7%) isolates (D25^{FMC} and D36^{FMC}) with $0.788 < S_D < 1.0$. Their composition was homogeneous in regards to both school (FMC) and socioeconomic category (D).

Such compositions showed higher similarities among *C. albicans* strains collected from healthy children classified in identical or different socioeconomic categories. In contrast to these strongly related strains, such behavior was not observed among isolates from healthy children classified in socioeconomic categories A and D, B and D, or C and E. Geographically, some healthy children from identical and different schools also are carriers of highly similar *C. albicans*, but such similarity was not present among other isolates from children from certain schools (i.e. absence of high similarity among isolates from children from schools AV and CS-MEP, CS and AV-FE-HF, FE and CS, FMC and LAE, HF and CS, LAE and FMC-MEP, MA and MEP, MEP and AV-LAE-MA). These data may reflect a restricted dissemination route of such microorganisms in certain population groups in terms of its socioeconomic categories and geographic site (Fig. 3).

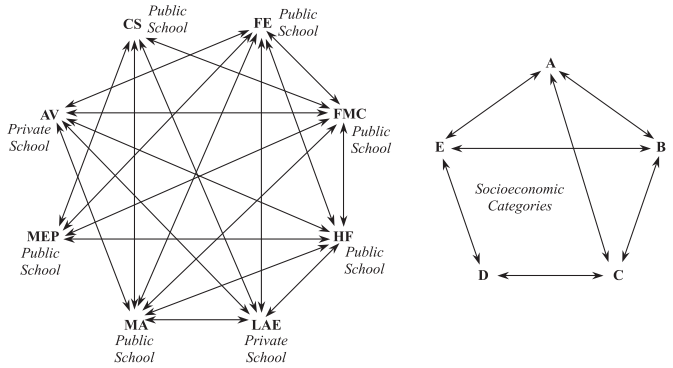

Fig. 3 - A possible dissemination route of *C. albicans* oral strains from healthy students, based on group compositions generated by UPGMA dendrogram analysis.

A total of 23 (30.6%) isolates (C44^{HF}, D21^{CS}, D20^{MEP}, E57^{FMC}, B32^{AV}, A33^{LAE}, A52^{LAE}, E30^{CS}, A17^{MA}, A20^{AV}, A40^{LAE}, A48^{LAE}, A23^{LAE}, B38^{LAE}, B46^{MA}, C9^{FMC}, B56^{MA}, D28^{FMC}, C16^{HF}, D23^{CS}, D32^{FMC}, A64^{LAE}, and C12^{HF}) showed similar values of $0.499^{49.9\%} \leq S_D < 0.788^{78.8\%}$. These results suggest a higher protein polymorphism degree among some *C. albicans* strains from the socioeconomic categories A ($8^{42.1\%}$ /19 isolates) and D ($5^{41.7\%}$ / 12 isolates), followed by C ($4^{26.7\%}$ /15 isolates), B ($4^{23.5\%}$ /17 isolates), and E ($2^{16.7\%}$ /12 isolates) categories. The same behavior was also detected between the LAE ($7^{63.6\%}$ /11 isolates) and MA ($3^{50\%}$ /6 isolates), followed by CS ($3^{33.3\%}$ /9 isolates), AV ($2^{33.3\%}$ /6 isolates), HF ($3^{30\%}$ /10 isolates), FMC ($4^{25\%}$ /16 isolates), and MEP ($1^{14.3\%}$ /7 isolates) schools.

DISCUSSION

The analysis of the electrophoretic protein profiles combining computer-statistics program enabled the identification, classification, and even the reclassification of numerous strains, species and genera of bacteria and yeasts in taxonomic and epidemiological studies[11,20,23,28,39,57,58,59]. In the present investigation, seventy-five *C. albicans* strains isolated from the oral cavities of healthy students, according to their socioeconomic categories and/or schools, were analyzed by SDS-PAGE and numerical analysis.

The reproducibility of electrophoretic protein profiles on different slab gels, by the inclusion of molecular weight markers and *C. albicans* CBS562 protein extract, gave the mean value of $S_D = 0.897$ in agreement with the minimum acceptable value obtained in previous studies[50]. The

electrophoregrams of *C. albicans* strains (Fig. 1) showed several protein profiles within a range of molecular weights varying between 18.4 kDa and 66 kDa, showing relative similarity when visually compared. The protein electrophoretic fingerprinting showed that protein bands greater than 45 kDa are repeated in the majority of *Candida* species, suggesting that it may be representative of the genus[40,58,59]. In an attempt to find a region of the lanes with a number of bands great enough to be able to provide an interpretation in terms of intraspecific polymorphism, the protein bands between 18.8 kDa and 66 kDa were analyzed. Such procedure was suggestive enough to express this variability making it possible to determinate several polymorphism degrees or similarities ($0.788^{78.8\%} < S_D \leq 1.0^{100\%}$) among the isolates. During numerical analysis, the confidence interval of ± 1.25 was used to build a binary matrix, followed by their treatment with the Dice similarity coefficient in order to have a similarity matrix (S_D). Such criterion allowed the maximization of minimal similarity values in dendrograms, with emphasis on the numerical analysis and protein fingerprinting of yeasts[5,17]. In order to ensure whether or not the UPGMA algorithm assess resemblance between two OTUs in the dendrogram construction, the Pearson product-moment correlation coefficient (r_{CS}) was computed among the elements S_{ij} (similarity matrix S_D) and C_{jk} (cophenetic matrix S_C) derived from the dendrogram. It is a measure of the agreement between similarity values present in the dendrogram and those of the matrix S_D[16,23,26,51,53,54]. This coefficient showed a value of r_{CS} = 0.8784 ranging between 0.6 and 0.95[51,54], according to the findings of FARRIS (1969) who pointed out that UPGMA algorithm always maximizes the r_{CS} values. Drawing a vertical line across the dendrogram at a similarity value of $0.7880^{78.8\%}$ (average similarity), 36 phenons (36 78.8-phenons) were detected. The average similarity value has been employed in several epidemiological studies of *Candida* species using proteins and DNA fingerprinting techniques associated to numerical analysis, allowing better discriminatory power during analysis of the compositions phenons (clusters) and their relationships[19,36,37,39,40]. Besides, this the vertical line that crosses the dendrogram arbitrarily (i.e. 0.7, 0.8, 0.9) does not correspond to criteria for the establishment of different taxonomic and epidemiological positions[52].

A total of 13 major phenons (clusters) were evaluated, according to their homogeneous (identical socioeconomic category and/or school) and heterogeneous (distinct socioeconomic categories and/or schools) characteristics in order to identify *C. albicans* subspecies and their similarities in healthy infantile populations and to establish their possible dissemination routes. Regarding social epidemiological aspects, the composition of the clusters showed higher similarities ($0.788^{78.8\%} < S_D \leq 1.0^{100\%}$) among some *C. albicans* strains isolated from healthy children independent of their socioeconomic strata (high, medium, or low). Interestingly, similar higher isolates were not found in oral cavities from healthy children from social stratum A and D, B and D, or C and E. This may be explained by the absence of a dissemination route among the children of those strata. According to MOREIRA *et al*. (2001) the prevalence of *C. albicans* and other *Candida* species is not determined by social factors. Nevertheless, the lack of research on the prevalence of such species in different social strata in our country limits further speculations concerning this matter[33]. Geographically, some healthy children from identical and different schools (private and public) also are carriers of *C. albicans* highly similar, but such similarity was not found among other isolates from children from certain schools (i.e. absence of higher similarity among isolates from children from schools AV and CS-MEP, CS and AV-FE-HF, FE and CS, FMC and LAE, HF and CS, LAE and FMC-MEP, MA and MEP, MEP and AV-LAE-MA). These data also may reflect a restricted dissemination route of *C. albicans* oral strains in certain population groups of healthy students, occurring because of either socioeconomic categories and geographic sites of each child (Fig. 3). Common and frequent mechanisms involved in the diversity of *Candida* species could explain this similarity. These include chromosomal rearrangements, chromosomal alterations, and complex and unknown gene regulations[41,42]. Moreover, tandemly repetitive sequences and telomeric and subtelomeric sequences have been described previously, and it has been postulated that these sequences may be involved in chromosome organization and rearrangements[10,43]. Furthermore, these *C. albicans* strains could be derived from a unique strain as a consequence of the loss of one allele by recombinations or chromosomal rearrangements *sensu lato*[38]. In contrast to higher similarity, the lower similarity or higher polymorphism degree ($0.499^{49.9\%} \leq S_D < 0.788^{78.8\%}$) of the protein profiles was shown in 23 (30.6%) *C. albicans* oral isolates. Of these, regarding social epidemiological aspects, 42.1%, 41.7%, 26.6%, 23.5%, and 16.7% were isolates from children of socioeconomic categories A (8/19 isolates), D (5/12 isolates), C (4/15 isolates), B (4/17 isolates), and E (2/12 isolates), respectively, and geographical categories, 63.6%, 50%, 33.3%, 33.3%, 30%, 25%, and 14.3% were isolates from children from schools LAE (7/11 isolates), MA (3/6 isolates), CS (3/9 isolates), AV (2/6 isolates), HF (3/10 isolates), FMC (4/16 isolates), and MEP (1/7 isolates), respectively. These results suggest a higher protein polymorphism degree among some *C. albicans* strains from healthy children, independent of their socioeconomic strata or geographic sites. Recently, multilocus enzyme electrophoresis analysis showed that healthy children could harbor just one or more genetic subtypes[29], as well as immunocompromised patients with predominance of one strain, which could result from intraspecies competition[38]. MATEE *et al*. (1996) showed the predominance of several *C. albicans* biotypes in African infant populations, suggesting an inexistent correlation between biotypes and exogenous factors (i.e. age, nutrition, and gender)[30]. Southern Blot hybridization analysis with Ca3 probe showed a higher genetic similarity among *C. albicans* clinical strains collected from patients independent of their geographic regions[46] according to our results. Research, employing isoenzyme and DNA fingerprinting analysis (MLEE, RAPD, RFLP using hybridization with Ca3 and CARE2 probes) also showed strictly related *C. albicans* clinical strains (i.e. sexual pairs, different anatomical sites of the same individual, and phenotypic variants of the same isolate) and some non-related isolates (i.e. different geographic regions, immunocompromised and immunocompetent non-related individuals) clustered in phenons with similarity of $S_{AB} \geq 0.94$[36]. The higher and lower similarity among *C. albicans* strains may be explained by the relationship and non-relationship or existent and non-existent dissemination routes, respectively, among healthy-children groups. In this context, the genetic similarity among *C. albicans* strains, harvested from women with vulvovaginitis and their male partners[47,55], as well as the genetic similarity among isolates from family members having the same activities has been reported[31]. Complementary studies involving healthy students and their families, teachers, servants, hygiene and nutritional habits must be done in order to establish specific sources of colonization patterns in population groups of healthy children. In general terms, the whole-cell protein profiles obtained by SDS-PAGE associated with computer-assisted numerical analysis, showed to be an important resource for taxonomic and epidemiological studies of yeasts.

RESUMO

Padrões eletroforéticos de proteínas e análise numérica de *Candida albicans* isoladas da cavidade oral de crianças saudáveis

O objetivo da presente pesquisa foi avaliar os graus de polimorfismos protéicos entre setenta e cinco linhagens de *C. albicans* isoladas da cavidade oral de crianças saudáveis provenientes de cinco categorias socioeconômicas e oito escolas (particulares e públicas) do município de Piracicaba, Estado de São Paulo, a fim de identificar subespécies de *C. albicans* e suas similaridades em grupos de populações infantis e estabelecer suas possíveis rotas de disseminação. Culturas celulares foram desenvolvidas em meio YEPD, coletadas por centrifugação e lavadas com solução salina gelada. As proteínas celulares totais foram extraídas por rompimento celular usando pérolas de vidro e submetidas à técnica de SDS-PAGE. Após a eletroforese, as bandas de proteínas foram coradas com Coomassie-blue e analisadas pelo conjunto de programas estatísticos NTSYS-pc versão 1.70. Matriz de similaridade e dendrograma foram gerados, pela aplicação do coeficiente de similaridade de Dice e do algoritmo UPGMA, respectivamente, os quais permitiram avaliar os graus de polimorfismo ou similaridade intra-específico, baseados nos padrões eletroforéticos de proteínas totais de isolados orais de *C. albicans*. Um total de 13 principais fenons (grupos) foi analisado de acordo com suas características homogêneas (categoria socioeconômica e/ou escola idênticas) e heterogêneas (categorias socioeconômicas e/ou escolas diferentes). Com relação ao aspecto epidemiológico socioeconômico, as composições dos grupos mostraram alta similaridade ($0.788 < S_D \leq 1.0$) entre algumas linhagens de *C. albicans* isoladas de crianças saudáveis independentemente de suas camadas socioeconômicas (alta, média e baixa). Isolados de alta similaridade não foram encontrados nas cavidades orais de crianças saudáveis pertencentes às camadas sociais A e D, B e C, ou C e E. Isto pode ser explicado pela ausência de uma rota de disseminação entre estas crianças. Geograficamente, algumas crianças saudáveis entre escolas idênticas e diferentes (particulares e públicas) também são portadoras de linhagens semelhantes, mas tal similaridade não foi encontrada entre outros de determinadas escolas. Esses dados podem refletir uma rota de disseminação restrita destes microrganismos em alguns grupos de escolares saudáveis, a qual pode ser dependente da categoria socioeconômica ou local geográfico de cada criança. Em contraste à alta similaridade, a baixa similaridade ou alto grau de polimorfismo ($0.499 \leq S_D < 0.788$) dos perfis protéicos foi demonstrado em 23 (30,6%) isolados orais de *C. albicans*. Considerando o aspecto epidemiológico social, 42,1%, 41,7%, 26,6%, 23,5% e 16,7% foram isolados de crianças provenientes das categorias socioeconômicas A, D, C, B e E, respectivamente, e geograficamente, 63,6%, 50%, 33,3%, 33,3%, 30%, 25% e 14,3% foram isolados de crianças provenientes das escolas LAE, MA, CS, AV, HF, FMC e MEP, respectivamente. Tais resultados sugerem um maior grau de polimorfismo entre algumas linhagens isoladas de crianças saudáveis independentemente de suas camadas sociais ou locais geográficos. Estudos complementares envolvendo escolares saudáveis e seus familiares, professores, serventes, hábitos nutricionais e de higiene deverão ser realizados a fim de estabelecer as fontes de tais padrões de colonização em grupos de populações de crianças saudáveis. Os perfis de proteínas totais obtidos por SDS-PAGE associados com análise numérica computadorizada podem proporcionar critérios adicionais para os estudos epidemiológicos e taxonômicos de *C. albicans*.

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