ISSN 1807-5274
Rev. Clín. Pesq. Odontol., Curitiba, v. 5, n. 3, p. 203-224, set./dez. 2009
Licenciado sob uma Licença Creative Commons

MOLECULAR FINGERPRINTING METHODS FOR STUDIES INVOLVING ORAL *Candida albicans*

Métodos de caracterização molecular para estudos envolvendo Candida albicans *de origem bucal*

Marcelo Fabiano Gomes Boriollo[a], José Francisco Höfling[b], Denise Madalena Palomari Spolidori[c], Ana Maria Trindade Grégio[d], Alinne Ulbrich Mores Rymovicz[d], Alessandra de Paula e Carvalho[d], Luiz Eduardo Nunes Ferreira[d], Lakshman Perera Samaranayake[e], Edvaldo Antonio Ribeiro Rosa[d]

[a]Department of Genetics, José do Rosário Vellano University, Alfenas, MG - Brazil.
[b]Laboratory of Microbiology and Immunology, Faculty of Dentistry of Piracicaba, Universidade Estadual de Campinas (Unicamp), Piracicaba, SP - Brazil.
[c]Laboratory of Oral Microbiology, Department of Physiology and Pathology, Universidade de São Paulo (USP), Araraquara, SP - Brazil.
[d]Laboratory of Stomatology, Center of Biological and Health Sciences, Pontifícia Universidade Católica do Paraná (PUCPR), Curitiba, PR - Brazil, e-mail: edvaldo.rosa@pucpr.br
[e]Oral Biosciences, Faculty of Dentistry, University of Hong Kong, Hong Kong, HKSAR.

Abstract

OBJECTIVE: This review was aimed to discuss the literature concerning the fingerprint methods for epidemiological studies of oral-borne *Candida albicans*. **DISCUSSION**: Interest in obtaining a better understanding of the pathogenesis, epidemiology, genetics and evolution of *Candida albicans* has led to the development of innumerable investigations. These studies have employed fingerprinting systems, such as multilocus enzyme electrophoresis, electrophoretic karyotyping, randomly amplified polymorphic DNA, and restriction length fragment polymorphism, with and without hybridization. The efficacy of these systems has been examined at different levels of discrimination. A validation strategy has been delineated which compares two or more unrelated methods. Moreover, the different fingerprinting patterns produced could be registered in database programs and submitted to comparison with parameters of the host and characteristics of the pathogen. These procedures permit urrent and retrospective comparison of a selection of clinical and epidemiologically important strains, which could show one or several characteristics of the host or pathogen. Additionally, the sum of this growing amount of information could contribute even more to the understanding of the dynamics of infectious organisms in human populations, the complex relationship between commensalism and infection, and genetic and evolutionary mechanisms. **CONCLUSIONS**: Multiple molecular systems are available for studies involving *C. albicans*. This growing amount of information contributes to the understanding of the dynamics of this fungus in human populations.

Keywords: *Candida albicans*. Molecular epidemiology. Molecular fingerprinting.

Resumo

OBJETIVO*: Esta revisão discute as informações existentes acerca dos métodos de caracterização para estudos epidemiológicos envolvendo Candida albicans de origem bucal.* ***DISCUSSÃO****: O interesse no melhor entendimento da patogênese, epidemiologia, genética e evolução de C. albicans tem levado os pesquisadores à condução de inúmeras investigações. Esses estudos empregam sistemas de caracterização molecular como eletroforese de enzimas multilocus, cariotipagem por eletroforese, amplificação do DNA polimórfico ao acaso e polimorfismo dos fragmentos de restrição com e sem hidridização. A eficácia desses sistemas tem sido avaliada nos seus diferentes níveis de discriminação. Uma estratégia de validação foi delineada, a qual compara dois ou mais métodos não relacionados. Ainda, os diferentes padrões de caracterização molecular produzem dados que podem ser avaliados por programas computacionais e permite a comparação comparâmetros do hospedeiro e características do patógeno. Tais procedimentos permitem comparações correntes e retrospectivas de cepas clínicas e epidemiologicamente importantes, que podem mostrar uma ou mais características do hospedeiro ou do patógeno. A somatória do montante de informação pode contribuir para o entendimento da dinâmica dos organismos infecciosos em populações humanas, as relações complexas entre comensalismo e infecção, e mecanismos genéticos e evolutivos.* ***CONCLUSÕES****: Vários sistemas de caracterização molecular estão disponíveis para estudos envolvendo C. albicans. Este aumento de informação contribui na compreensão da dinâmica deste fungo em populações humanas.*

Palavras-chave*:* Candida albicans. *Epidemiologia molecular. Caracterização molecular.*

INTRODUCTION

Candida albicans and related species are found ubiquitously and commensally in the body cavities (rectal, buccal, vaginal, urethral, nasal, and aural) and skin microbiota of humans (1). These species are considered opportunistic pathogens capable of causing infections varying from mucocutaneous disorders, not compromising to the individual, to invasive diseases involving almost all of the organs. The frequency of infections caused by *Candida* spp. have been increasing worldwide due to a multiplicity of predisposing factors that facilitate its conversion from a commensal to a parasitic form (2, 3). The rise of these infections has been associated with immunological deficiencies, as observed in innumerable cases of oropharyngeal candidiasis in patients with AIDS (4). The progression of colonization toward infection in mucosal membranes has been related to a process dependent on the defense mechanisms of the host and on the ability of *Candida* spp. to overcome such mechanisms (5). Different clinical manifestations of buccal candidiases have been described, corresponding to excessive growth of yeasts and their penetration through the epithelial mucosa (6).

To understand the dynamics of the infectious organisms in human populations, to interpret the complex relationship between commensalism and infection, to identify the origin of an infection or monitor the emergence of drug-resistant strains, molecular methods based on genotypic fingerprinting systems (molecular markers) should be available to determine the genetic relationship of these organisms of epidemiological importance (7, 8). By definition, an isolate corresponds to a clone collected independently from other isolates. Two isolates collected independently could be completely unrelated or genetically indistinguishable. On the contrary, a strain is referred to as a collection of isolates from the same species, which are highly related or genetically indistinguishable.

To classify two isolates as belonging to the same strain, or as members of different strains, sensitive molecular genotyping methods need to be adequately applied. This necessity is especially important when diseases arise from commensal organisms, when strains become specialized in particular locations of the body or under compromising conditions, when strains undergo microevolution for rapid adaptation, or when strains

are transmitted among individuals. Therefore, genotypic fingerprinting systems should be examined with respect to their efficacy at different levels of discrimination: (*i*) identifying the same strain in independent isolates, (*ii*) identifying microevolutionary changes in a strain (highly related isolates, however not identical), (*iii*) grouping moderately related isolates, and (*iv*) identifying completely unrelated isolates. In addition, such systems should furnish a database to estimate the probability of a particular strain being isolated twice by chance in a particular geographic location (8).

Until the moment, no genotypic fingerprinting method has been able to furnish a definitive measure of the genetic distance between two isolates. Tibayrenc has been mentioning the inexistence of means to determine completely the identity of two microbial genotypes, objection to the sequencing of the entire genome (9). Different molecular markers could show different molecular clocks (i.e., the evolutionary speed at which they change). Thereby, an optimal genotypic fingerprinting method would be based on a number of molecular markers. Such a method would also be resistant to homoplasy (i.e., common characteristics among isolates that do not have the same ancestry) and provide quantitative data that reflects genetic distance.

The comparison of data generated by unrelated genotypic fingerprinting methods, employing the same group of test isolates, becomes a means of evaluating such requirements, mentioned previously (10, 11). In addition, the inclusion of identical isolates, highly related (but not identical) isolates, and independent isolates with unknown relationships (including moderately related and completely unrelated isolates) were suggested in groups of test isolates in comparative analyses of genotypic fingerprinting methods (12). According to Soll, when two methods identify the identical isolates and nonidentical but highly related isolates as such, generally cluster less closely related isolates in a similar fashion, and distinguish among completely unrelated isolates, then, in essence, the two methods have cross-verified each other for all of the levels of resolution (*i*, *ii*, *iii* and i*v*). However, these verifications can be only for species with a predominantly clonal population structure (8).

The data generated by a particular genotypic fingerprinting method, for one strain (i.e., gel electrophoretic patterns), must be relatively stable over many generations. Therefore, this fact requires the occurrence of little recombination between the sequences selected for study. In addition, the populations of isolates in study must undergo primarily clonal reproduction. On the other hand, when panmixia occurs at a high frequency, due to sexual reproduction and recombination, the results of epidemiological studies, obtained by genotypic fingerprinting methods, make interpretation difficult (8).

Fortunately, many fungal agents undergo recombination or gene changes at extremely low frequencies (13). Some studies have shown a primarily clonal mode of reproduction in *C. albicans*, justifying the applicability of the standard molecular markers in epidemiological studies (14, 15). In addition to the stability resulting from clonal reproduction, a genotypic fingerprinting method should access mainly sequences that are not highly reorganizational (i.e., sequences that are reasonably stable over time). For example, a complex DNA fingerprinting probe such as the Ca3 probe (11kb) of *C. albicans* (16, 17) contains both repetitive sequences dispersed throughout the genome (RPS repetitive element) and unique sequences represented at only one locus (18, 19). Because sequences in the genome containing clusters of full-lengh RPS units (20, 21) undergo frequent reorganization (duplication and deletion) (19), frequent changes occur in Ca3 hybridization patterns containing full-length RPS sequences. Bands containing such sequences represent, on average, 20% of the patterns generated by hybridization with the Ca3 probe. The remaining 80% represents less variable sequences that tend to stabilize the patterns (1). In contrast, a probe that consists entirely of RPS elements tends to generate an additional, less stable fingerprinting pattern, when compared to the Ca3 probe (19), and consequently the pattern shows low efficacy during the grouping of moderately related isolates. Both full-length Ca3 and restricted RPS probes can be employed in studies that assess rapid changes due to microevolution. However, only the full-length Ca3 probe can be employed in studies in which moderately related isolates are to be analyzed (8).

The typeability, reproducibility and discriminatory power have been developed and suggested during in the evaluation of the efficiency of various genomic fingerprinting methods (22, 23). Typeability and reproducibility represent quantitative

systems that are frequently expressed as percentage measures. The typeability of a method corresponds to the percentage of distinct strains obtained. The percentage of strains that show the same results for repeated assays corresponds to reproducibility, that is, the ability of a method to produce the same result when a particular strain is repeatedly tested. Reproducibility is especially important for the construction of a reliable database, which shows all the known strains within a species, to which unknown organisms can be compared for classification. Therefore, the discriminatory power of a method corresponds to its ability to clearly differentiate unrelated strains, such as those that are geographically distinct from one source of organisms, and demonstrate at the same time the relationship of all the organisms isolated from individuals infected directly from the same source (22, 23).

There has been a strong interest in acquiring a better understanding of the pathogenesis, epidemiology, genetics and evolution of infections caused by *C. albicans*. This has led to the development of extensive research, employing fingerprinting methods such as MLEE (multilocus enzyme electrophoresis), EK (electrophoretic karyotyping), RAPD (randomly amplified polymorphic DNA), RFLP (restriction length fragment polymorphism) without hybridization or REA (restriction enzyme analysis) and RFLP with hybridization, which will be examined.

Multilocus enzyme electrophoresis

The method of multilocus enzyme electrophoresis (MLEE or MEE), also known as isoenzyme typing, has been employed during various decades as the standard method for the genetic analysis of populations in eukaryotes (24-29). Pioneer work in the 1980s, employing MLEE for the genetic analysis of *Escherichia coli* and *Shigella*, stirred enormous interest among medical microbiologists (30, 31). Henceforth, numerous studies were conducted producing immeasurable findings toward the understanding of the natural history of infectious diseases. MLEE has been considered the gold standard in the study of the population genetics of microorganisms (32). Its analytical capacity allows the study of codominant markers for each locus in diploid organisms, an essential requirement for evolution biologists that is not achieved by a few of the popular DNA fingerprinting methods (8).

In the field of medical mycology, isoenzyme typing has shown great potential in studies of taxonomic, systematics, genetics, evolutive, and epidemiologic characterization, especially for the yeast *C. albicans* (33-56). An enzyme reaction can be demonstrated by electrophoretic bands of enzymatic activity in gels, indicating the existence of isoenzymes or isozymes (57). Isoenzymes constitute multiple molecular structures of the same enzyme with individual affinity for the same substrate, catalyzing the same cellular reaction (58). Its control occurs genetically through one or several alleles or genes, situated at one or several loci (59-61). Isoenzymes controlled through alleles from a single locus are known as alloenzymes or allozymes (62, 63). Its electrophoretic migration is determined by its net electrical charge based on amino acid sequence, and consequently, mobile variants (electromorphs or alloenzymes) can be directly compared with alleles of the corresponding structural gene locus (31).

Isoenzyme analysis in the distinction of fungal species depends on intrapopulational genetic variation. Organisms with high genetic variability can express highly variable phenotypes, and thereby mask inter- or intraspecies differences. This is evident mainly with different non-metabolic enzymes that exhibit great structural variation by virtue of the intensity of environmental selective pressure (64-68). On the contrary, metabolic enzymes have a low vulnerability to environmental selection, and are usually employed as isoenzyme markers (69).

The patterns of isoenzyme electrophoretic bands are frequently predictable, since they depend on genetic and nuclear conditions of each organism. Nevertheless, many mycologists limit interpretations of electrophoretic results to simply counting of the bands (51, 52, 54, 70, 71). The genetic interpretation, when possible, furnishes abundant additional information about the nuclear condition, genetics and taxonomy of a group of organisms (72-74). In this context, different criteria of interpretation have been employed for haploid or diploid organisms (31, 72, 75, 76). Based on these criteria, allelic composition has been determined based on a group comprised of ten to thirty metabolic enzymes considered representative of the whole genome of an organism, which support the study of the population genetics in bacteria, fungi and protozoa (8, 32).

The genetic interpretation of results obtained by MLEE has been applied for a variety of purposes in the field of medical microbiology. In terms of specific alleles, allelic proportions have inferred the degree of genetic recombination in natural populations. These proportions have also been utilized in determining the degrees of genetic isolation among natural populations, caused by geographic and ecological regions or biological barriers. Its use has shown taxonomic and systematic implications, since for clonal microorganisms, the degrees of inter- and intrapopulational relationships have been studied. Species-specific clones identified by MLEE were also associated with clinical patterns and re-incidence, and high indices of pathogenicity. Such results have been employed in epidemiologic delineations permitting better understanding of the development of epidemics, in which it represents an immeasurable complement to current methods of molecular typing, particularly in large-scale epidemiologic studies. Additionally, MLEE has been considered a typing method with high discriminatory power and reproducibility (12, 38, 75-77).

Comparative studies among MLEE, RAPD and Southern blot hybridization with Ca3 probe show strong concordance in results, with groups of *C. albicans* isolates, oral and from other anatomical sites, originating from three geographic regions of the United States [HIV-positive patients and cancer patients (El Paso, TX.), vaginitis (Ann Arbor, MI; Iowa City, IA), sexual partners (Ann Arbor, MI; Iowa City, IA) and healthy individuals (Iowa City, IA)]. Three main groups containing isolates of unrelated origin were generated by the above methods, without any indication that some loci analyzed by MLEE corresponded to amplification sequences by RAPD or by the Ca3 probe. In addition, microevolutionary changes in strains of related origin were revealed by Southern blot hybridization with Ca3 probe (high resolving power), MLEE (moderate resolving power) and RAPD (moderate resolving power) (12). However, MLEE can be considered an optimal genotypic fingerprinting method for fungal infections, since it provides information on all levels of resolution (*i*, *ii*, *iii* and *iv*). Nevertheless, the one disadvantage of this method is that it is time-consuming, in view of the laborious intensive enzyme assays and the careful attention required in the processing of the data and the genetic interpretation (8).

Electrophoretic karyotyping

With the invention of pulsed-field gel electrophoresis (PFGE) (78), and its respective systems OFAGE (orthogonal-field-alternative gel electrophoresis), FIGE (field-inversion gel electrophoresis), CHEF (contour-clamped homogeneous electric field) or TAFE (transverse alternate field electrophoresis), chromosomal fragments of the genome of yeasts have been easily separated in gels, thereby establishing electrophoretic karyotyping (EK) (8). These innovations appear to be ideal to study the epidemiology of fungi, since this technology was found to be reliable. In general, cells immersed in gelled agarose plugs must be mixed directly with enzyme solution to remove the cell wall. Protease and detergent can also be added, following cell incubation to remove membranes and proteins. Shortly thereafter, the DNA-containing plugs are placed inside wells on agarose gels, and electrophoresis is carried out according to the specifications of the particular system for chromosome separation. The chomosomal fragments of yeasts are separated according to size, which can be visualized after staining with ethidium bromide. In addition, specific chromosomes can be identified using Southern blot hybridization with chromosome-specific DNA probes (i.e., rDNA) (8).

The first applications of PFGE demonstrated variations in karyotype patterns among unrelated isolates of *C. albicans* (79-86), and thereby provided a potential genotypic fingerprinting method (8). Despite the karyotypic variability among *C. albicans* strains, Thrash-Bingham and Gorman employed Southern blot hybridization with probes of cloned genes and demonstrated that (*i*) genomic organization was maintained and that (*ii*) translocation processes contributed to such variability (87). Essentially important was the finding reported by Sangeorzan and coworkers, who demonstrated patterns of electrophoretic karyotyping that were highly reproducible between experiments, relatively intensive in the preparation of methods within the same laboratory, and not influenced by high-frequency phenotypic switching in strains of *C. albicans* (88). However, Holmberg and Feroze demonstrated technical variability with the CHEF system due to reagents, sample preparation and conditions of electrophoretic runs (89).

Due to the excellent discriminatory power and reproducibility (90-94), electrophoretic karyotyping has been employed extensively as a molecular marker for *C. albicans* (95-112). This method showed greater discriminatory power compared to RFLP without hybridization, in assays of independent isolates (90, 92, 94). Nevertheless, its efficiency in grouping moderately related strains has not been carefully evaluated. Other indications suggest that electrophoretic karyotyping cannot fulfill the requirements necessary for DNA fingerprinting analysis in *C. albicans*, since these yeasts can undergo chromosomal reorganizations at high or low frequency that, consequently, induce increased or reduced karyotypic alterations, respectively (113). Cells of *C. albicans* 3153A strains, which express varying phenotypes (switching), undergo highly frequent alterations in the size of two chromosomes that harbor rDNA cistrons (113). The capacity of a cell to express two frequencies of chromosomal reorganization consequently leads to two frequencies of karyotypic alterations as a result of reversible differentiation, regardless of the use of electrophoretic karyotyping as a genotypic fingerprinting method effective for the study of groups of moderately related isolates (8). In addition to the studies on karyotypic alterations with increased or reduced frequency in *C. albicans*, karyotypic patterns in variant strains (switching) have been demonstrated to diverge and on other occasions converge (113-116). The convergence of karyotypic patterns leads to homoplasy, which is inconsistent with the aims of a genotypic fingerprinting method. When divergence is followed by convergence of a DNA fingerprinting pattern, two similar or identical karyotypes can be interpreted as the same strain, when in reality the isolates being compared may be unrelated (8). The high-frequency karyotypic alterations in *C. albicans* can interfere with the capacity to discriminate moderately related and unrelated strains. However, electrophoretic karyotyping can be employed effectively to determine microevolution within an infectious strain (117). Nevertheless, demonstrations of high-frequency reorganization and homoplasy reduce the efficacy of karyotyping as a general DNA fingerprinting method for *C. albicans* (8). Other factors that have limited the use of PFGE are the high cost and relatively long time to carry out the assays, which consequently reduce laboratory capacity in analyzing large numbers of samples (7, 93). Moreover, a modified version of electrophoretic karyotyping has furnished the method with better efficacy, with the aim of fulfilling the necessary requirements. The resolving power of karyotyping has improved by digesting the chromosomal DNA of *Candida* spp. with restriction enzymes, such as *SfiI, NotI* and *BssHII,* previously for assays using pulsed-field gel electrophoresis (93, 106, 118-124).

Randomly Amplified Polymorphic DNA

Randomly amplified polymorphic DNA (RAPD) was first described by Williams and collaborators (1990) (125) and Welsh & McClelland (1990) (126). Although a variety of strategies based on the polymerase chain reaction (PCR) have been developed (127), RAPD has emerged as the DNA fingerprinting method most commonly used for fungal infections (125-127). Employing randomly primers, of approximately 10 bases (oligonucleotide), amplicons throughout the genome are subject to being recognized and amplified. The amplified products are commonly separated in agarose gels and stained with ethidium bromide (7, 8). In the development of a RAPD system for a particular species, a certain number of primers need to be tested to select those oligonucleotides capable of providing the most variability among independent isolates. A single primer can generate a relatively complex pattern that varies among isolates. However, in many cases its use produces one to three intense electrophoretic bands capable of differentiating isolates. Therefore, the primers should be selected and tested independently for each isolate in question, and the information obtained must be combined (8). This strategy has been the objective of Pujol and coworkers, who tested forty randomly primers (each a length of 10 bases) on a limited number of test isolates of *C. albicans*. Eight of these primers were selected based on providing maximum variability. However, only patterns showing intense and reproducible electrophoretic bands (one to six bands for each primer) were employed in the studies of grouping. In addition, these patterns demonstrated parity with those obtained by MLEE and Southern blot hybridization with Ca3 probe (12). These investigators also demonstrated that RAPD, MLEE and Ca3 fingerprinting of *C. albicans*

not only can group moderately related isolates, but also furnish similar levels of resolution of micro-evolution within a clonal population. Nevertheless, these microevolutionary changes were independent, and consequently the three methods do not identify alterations in the same highly related isolates. More accurately, these methods measure similar frequencies of variants within the same strain (12).

RAPD has demonstrated great success in studies of fungal infections, especially for *C. albicans* (12, 45, 90, 97, 128-136). Despite that the technique is rapid and simple and has a high discriminatory power, various limitations of its procedures have been pointed out. The lack of inter- and intra-laboratory reproducibility, although not insurmountable, has resulted in the generation of a complex and difficult to interpret database (7, 8, 91, 93, 137). Variations in results due to artifacts can also occur due to small differences in the concentration of primers, in temperature of amplification and in magnesium concentration of the reaction mixture (138). Alterations of these parameters can notably affect the presence of low intensity bands, and the position and clarity of high intensity bands (8). In addition, various investigators have demonstrated significant variations in RAPD methodology due to different sources of *Taq* polymerase, which were capable of producing pseudoclusters in grouping analyses (139-141).

Restriction Length Fragment Polymorphism, without hybridization

One of the first genotypic fingerprinting methods employed to study the relationship of strains in fungal infections was restriction enzyme analysis (REA), or the comparison of restriction length fragment polymorphism (RFLP) without the use of hybridization probes (8, 91, 93). This methodology has been applied in a series of epidemiologic studies of various fungal infections, especially for *C. albicans* (77, 92, 94, 98, 112, 129, 142-148). This technique has been considered safe and reliable. Generally, cell DNA has been extracted from spheroplasts, digested with one or more restriction enzymes (i.e., *EcoRI* and *HinfI*), separated in agarose gels and visualized by staining with ethidium bromide. The resolution of this method depends on agarose concentration, time of electrophoresis, voltage and the endonuclease employed. Nevertheless, all the experimental conditions must be determined empirically. The patterns result from different lengths of DNA fragments, which are determined by restriction sites identified by specific endonucleases. The variations among strains can occur based on alterations or secondary modifications in restriction site sequences, deletion of recognition sites or deletion and insertion at DNA sequences located between recognition sites (8, 91, 93).

In fungal infections, the enhanced complexity of the eukaryotic genome increases the number of bands obtained with conventional endonucleases. This diminishes the resolution of band patterns that represent unique sequences, since the complexity of banding patterns generated by RFLP often makes the interpretation of the relationship of the strains difficult (8, 93). In a manner similar to all eukaryotic genomes, fungal genomes contain repetitive rDNA genes with relatively homologous sequences and intergenic regions. Eukaryotic ribosomal cistrons are normally grouped in one or two chromosomes (8). The genome of *C. albicans* contains approximately 50 to 130 rDNA cistrons per diploid genome (149). Fungi also have many copies of the mitochondrial genome (150). Sequences of rDNA (145) and mitochondrial DNA (151, 152), the latter less extensively, represent the majority of intense bands in RFLP patterns (8). Various studies of *C. albicans* by RFLP have pointed out the utility of this method in the identification of identical strains from independent isolates, as well as in the discrimination between unrelated isolates (142, 144-146, 153). RFLP without hybridization probe has been considered a legitimate method to deal with selective epidemiologic questions related to fungal infections. However, this methodology has a disadvantage in analyzing groups of isolates that are moderately related. For this reason, RFLP has not been well suited for large epidemiologic studies. In addition, this method has not been critically validated through comparison with other methods at different levels of resolution (*i*, *ii*, *iii* and i*v)* (175).

Restriction length fragment polymorphism, with hybridization

A general RFLP pattern of eukaryotic cellular DNA is considered insufficiently resolved

because all the restriction fragments are revealed (8). Nevertheless, the RFLP methodology can be enhanced by transferring the DNA fragments to nitrocellulose or nylon membranes, which can be hybridized with specific probes (9, 154, 155). This procedure permits the selective visualization of a limited number of fragments, and still furnishes a high-resolution fingerprinting pattern (8). This methodology, referred to as Southern blot hybridization, is based on DNA probes comprised of sequences (i.e., radiolabelled) capable of recognizing one or more corresponding fragments, present in RFLP gels (8, 91, 154, 155). The stringency of this hybridization can be controlled by varying the salt concentration and/or temperature (156). Specific band patterns are seen when a particular probe recognizes a single specific DNA sequence (i.e., a single gene). The existence of a relatively intact, single sequence within a restriction fragment in the gel has been exclusively identified by hybridization using a specific DNA probe. In haploid organisms only one band appears in RFLP-Southern blot hybridization patterns, while in diploid organisms such as *C. albicans*, one or two bands are present in these patterns. Nonetheless, the site action of a particular endonuclease could be contained within a single sequence of DNA corresponding to the site of hybridization with a specific probe, which consequently could result in the appearance of more than two bands in RFLP-Southern blot hybridization patterns (8).

Various fungus-specific and species-specific probes have been developed based on evolutionarily conserved areas of fungal genomes and of particular fungal species, respectively (157-159). Probes that hybridize with single sequences or a single gene (single-gene probes) can discriminate some fungal isolates based on allelic polymorphism. These probes tend to generate patterns with one or two bands, which consequently do not provide the level of complexity of the data necessary to determine genetic distance. Nevertheless, several single-gene probes employed in combination can furnish results similar to those obtained with MLEE and generate data complex enough to estimate genetic distance. Thereby, probes that hybridize with repetitive sequences dispersed throughout the genome have been targets of numerous investigations, since these sequences can vary among strains (8). rDNA probes employed in Southern blot hybridization analysis using total cellular DNA of *C. albicans* demonstrated a certain resolution in unrelated strains, whose complexity in hybridization patterns were compared with those using single-gene probes (160, 161). Stein and coworkers employed the rDNA probe and demonstrated five different patterns in eighteen isolates of *C. albicans* (162). Hybridization with the rDNA probe of *C. albicans* digested with *Eco*RI showed a pattern with a maximum of three bands, which was relatively very common for apparently unrelated strains, suggesting the existence of homoplasy (162, 163). rDNA and rDNA spacer regions were tested as DNA fingerprinting probes in other fungal infections besides *C. albicans*; however, in no case were patterns generated complex enough to consider such probes in an effective fingerprinting system (164-168). Eukaryotic ribosomal cistrons constitute tandem repetitive sequences arranged in groups and are normally separated by spacer regions (169-172), and when digested by endonucleases, they generate fragments of relatively similar size, resulting in simpler Southern blot hybridization patterns (i.e., patterns with few bands). Consequently, rDNA probes have not been very effective for genotypic fingerprinting systems in fungi (8).

Some investigators have demonstrated a circular structure for the mitochondrial genome of *C. albicans*, with a size of 41kb comprising extensive repetitive sequences in fewer numbers of copies than those of rDNA (152, 173, 174). Total cellular DNA of *C. albicans* digested with *Eco*RI has revealed Southern blot hybridization patterns with the mitochondrial DNA probe that are apparently more complex than those obtained with an rDNA probe (8). Studies have demonstrated five distinct bands, whose patterns varied among isolates of *C. albicans*, suggesting that Southern blot hybridization with the mitochondrial DNA probe is effective in identifying identical strains among independent isolates and in distinguishing unrelated strains (151, 152). In the study of type I and type II of *Candida stellatoidea*, a species phylogenetically analogous to *C. albicans*, the hybridization patterns of type I with the mitochondrial DNA probe were identical, while those of type II varied (175). In addition, the patterns of some isolates of type II *C. stellatoidea* were indistinguishable from some isolates of *C. albicans*. This demonstrates the lack of species specificity or low resolving power of the method, if *C. stellatoidea* is accepted as an

independent species (175). In this study, it was also postulated that type II *C. stellatoidea* represents a subgroup of *C. albicans* (175), as suggested by other studies (176, 177). Consequently, methods with mitochondrial DNA and rDNA probes have not been commonly employed in wide epidemiologic studies of fungal infections, and none of them have been validated for the different levels of genetic resolution (8).

The DNA probes 27A (178) and Ca3 (16, 17), for specific hybridization with the *C. albicans* genome, were cloned almost at the same time during the late 1980s, and subsequently, a certain analogy was demonstrated between the two probes (19). These probes contain sequences of RPS repetitive elements and non-RPS sequences of *C. albicans* (20, 21), which consequently hybridize with a large part of the same bands in Southern blot hybridization assays (8). However, these two probes are not identical. The 27A probe contain sequences downstream from the RPS groups that hybridize with single bands, while the Ca3 probe contains sequences upstream from the RPS groups that hybridize with single bands (19). For comparison, the Ca3 probe shows greater complexity than does 27A, and moreover, contains an additional repetitive sequence called sequence B. In general, its pattern also shows more complexity and satisfies the four requirements necessary for an effective DNA fingerprinting method (8, 12). The Ca3 and 27A probes were also employed in various epidemiologic studies of *C. albicans* and the phylogenetically related species *C. dubliniensis* (178-195).

The strategy and the methods employed to clone and characterize the complex probes 27A and Ca3 were relatively reliable. Thereby, a Southern blot hybridization assay of DNA fragments digested with endonuclease, using probes capable of identifying repetitive sequences dispersed throughout the genome, could determine the variability among isolates by means of the diversity of spread out loci. Nevertheless, such probes could hybridize with (*i*) additional less variable sequences, including sequences that vary as a result of allelic polymorphisms, and (*ii*) some hypervariable sequences, revealing micro-evolutionary changes within a strain. All this information has been provided by a single Southern blot hybridization pattern, which represents the virtue of these complex probes. A complex probe must generate a sufficiently complex pattern in order to provide correct and sensitive measurements capable of reflecting the relationship of the isolates. The main part of the pattern produced should be relatively stable over time for any particular strain. In addition, the probe could contain one or more sequences that hybridize with monomorphic fragments (i.e., fragments that exhibit the same size among all or most of strains within a species). These monomorphic bands could facilitate normalization in a universal pattern, whose objective would be the computational storage of data (i.e., a computer-aided construction of a database) for subsequent comparative and retrospective studies (8).

Sherer and Stevens demonstrated that the probe 27A of 6.7kb, cloned from a clinical strain of *C. albicans* 616, shows a repetitive sequence dispersed throughout the genome. They examined two clones by Southern blot hybridization using probe 27A, of fragments after digestion with *Sau*3A and demonstrated that there is a common sequence between the two clones and 27A (17), which was subsequently identified as a RPS repetitive element by Iwaguchi and collaborators (21). From an epidemiologic and taxonomic point of view, Southern blot hybridization patterns with the probe 27A, of atypical isolates of *C. albicans* derived from HIV-positive patients, demonstrated genetic characteristics identical to those of *C. dubliniensis*, suggesting also a widespread geographic distribution for this species(196). In addition, Southern blot hybridization with probe 27A also shows greater discriminatory power in clinical strains of *C. albicans* when compared to results from EK, REA with *Not*I and PCR fingerprinting (197). The probe Ca3 of 11 kb (198), primarily referred to as JH3 in assays of phenotypic switching among isolates de *C. albicans* causing vaginitis (17), was cloned from the laboratory strain 3153A and found to be dispersed among seven of the eight chromosomes of *C. albicans* (16). Anderson and collaborators digested the probe Ca3 with *Eco*RI and obtained seven fragments, which were classified in decreasing order of size as A, B, C, D1, D2, E and F (199). Later, these fragments were mapped in the 5' to 3' direction (19). When DNA of the *C. albicans* 3153A strain was digested with *Eco*RI and analyzed by fragments A (~4.2 kb), B (~3 kb) and C (~2.9 kb) of probe Ca3, three distinct patterns were obtained. Nevertheless, the combination of these patterns

resulted in the identification of all the principal bands obtained in the hybridization patterns with intact probe Ca3 (199). Fragment A produced three distinct patterns (~5.8 kb; ~4.5 kb; ~5.8 and 4.5 kb) when analyzed by Southern blot hybridization of DNA of clinical isolates of *C. albicans* that was digested with *Eco*RI. In this case, fragment A identified only one gene, whose patterns represented allelic variations on only one gene locus. Fragment B generated a pattern that included more than the half of the bands obtained in hybridization patterns with intact probe Ca3. The majority of B pattern was polymorphic and representative of many moderately variable bands, which are necessary for grouping studies (8), or were even used in demonstrating parity among MLEE, RAPD and Ca3 fingerprinting (12). Finally, fragment C generated patterns that included bands of highly variable molecular weight, which have demonstrated immense value in microevolutionary studies of infectious strains (19, 117, 183, 189, 191).

In the determination genomic distribution of the sequences that hybridize with the three main fragments of probe Ca3 (A, B and C), Southern blot hybridization assays of *C. albicans* chromosomes, separated electrophoretically, were investigated (199). Seven distinct chromosomal bands of the *C. albicans* strain 3153A were obtained by transverse alternating-field electrophoresis (TAFE) and numbered in decreasing order of size. Since *C. albicans* has eight chromosomes (200), the overlapping of bands possibly occurred at least in one position (199). Intact probe Ca3 showed strong hybridization with bands 1, 3, 5, 6 and 7. Subfragment B showed strong hybridization with bands 5 and 7, and weak hybridization with band 6. Subfragment C showed strong hybridization with bands 1, 3 and 6, and weak hybridization with bands 5 and 7. These results demonstrated that subfragments B and C have sequences dispersed in more than one chromosome, in which subfragment C, containing the RPS sequences, was more disseminated than subfragment B (199).

Southern blot hybridization with probe Ca3 (16, 17, 199) has been employed in various epidemiologic and microevolutionary studies of clinical isolates of *C. albicans* (201-205). The probe Ca3 utilized in assays of DNA digested with *Eco*RI has identified more than 20 bands, which included monomorphic patterns, both moderately variable and hypervariable (188). All these patterns were employed to determine the relationship of clinical isolates, while hypervariable patterns were utilized to monitor microevolution in infectious and commensal strains of *C. albicans* (72, 117, 183, 191, 192). Analyses with RAPD, MLEE and Southern blot hybridization using probe Ca3 showed strong agreement in results, in grouping studies of *C. albicans* isolates of oral or other anatomical sites, derived from three geographic regions of the United States (HIV-positive and cancer patients, vaginitis sexual partners and healthy individuals). Three main groups, containing isolates of unrelated origin, were constructed by the above methods without any indication of some locus analyzed by MLEE corresponding to amplification sequences identified by RAPD or probing with Ca3. In addition, microevolutionary changes within a strain (related origin) were revealed by Southern blot hybridization with Ca3 probe, MLEE and RAPD (12). These microevolutionary changes can also involve reorganizations of the insertion type or intrachromosomal deletion of the RPS elements dispersed specifically throughout all the genome of *C. albicans* (19). Isolates of *C. albicans* from various anatomical sites originating from healthy women were compared by Southern blot hybridization the Ca3 probe. Of eleven women who harbored commensal *C. albicans* in both oral and vaginal cavities, four had highly similar strains, but not identical, in alternative sites. This finding suggests the occurrence of microevolution among those strains that are highly similar, from one progenitor that adapted in two different niches of the body. In addition, such divergent populations could be distinguished genetically (192).

Schroeppel and coworkers employed the same technique to assess the genetic relationship of *C. albicans* strains isolated from patients (strains maintained and substituted) and their sexual partners. In the patient in whom infectious strains were maintained, there were minimal genetic changes in successive episodes of vaginal candidiasis. In the patient in whom infectious strains were substituted by others, a transition infection developed an infectious population that was genetically mixed, and the substitution of strains apparently were derived from the oral cavity of sexual partners. These results demonstrated that the strains of recurrent infections of vaginal candidiasis are genetically unstable, and that

treatment with antifungals can result in the selection of previously infectious variants, or substitution by strains genetically unrelated (191).

The physical relationships among the Ca3 probe (16), the large genomic *Eco*RI fragments that hybridize with the C fragment of Ca3 (19, 199), the 27A probe (17), the HOK fragment (206), and RPS elements (20, 21) were determined (206, 207). The Ca3, 27A and HOK probes showed sequences of RPS elements and the upstream end of C2 of the RPS group. Therefore, all these three probes identify a group of common bands in a Southern blot of genomic DNA digested with *Eco*RI. Thus, the RPS elements used exclusively as a DNA fingerprinting probe tend to generate patterns similar to those obtained with the C fragment of the Ca3 probe (19, 21). Although the C pattern shows substantial utility in microevolution studies, based on the hypervariability in the length of their RPS sequences localized in tandem at particular locations of the genome (19), their use as an effective method of DNA fingerprinting provides for an equivocal interpretation of isolates that are not highly related (19). The problem in the utilization of a probe that contains repetitive elements dispersed in the genome was demonstrated in *C. albicans*. Lasker and collaborators cloned the species-specific repetitive element CARE-2 (1.06 kb) from the genome of *C. albicans*. The Southern blot hybridization patterns demonstrated different numbers of CARE-2 copies on different chromosomes of *C. albicans* (208). Complex patterns were also produced by Southern blot hybridization with the CARE-2 probe, in DNA digested with *Eco*RI for various clinical isolates of *C. albicans*, which showed approximately the same number of bands in patterns obtained by Southern blot hybridization with the Ca3 probe. However, in contrast to Ca3, each CARE-2 band pattern was variable (12). The CARE-2 probe distinguished unrelated isolates and identified the same strain independent isolates. However, while MLEE, RAPD and Ca3 fingerprinting demonstrated parity in grouping clinical isolates within the three highly similar groups, two of the three groups fragmented forming groups of minor and unrelated isolates in the CARE-2 dendrogram (12). In this manner, the patterns of the CARE-2 probe identified only hypervariable fragments that, consequently, become less effective in grouping moderately related isolates (8).

The results obtained with the CARE-2 probe (12) pointed to an erroneous concept in relation to the use of a DNA fingerprinting probe. There is a certain tendency to reduce a complex DNA fingerprinting probe to a simple repetition element. Such is the case in reducing the Ca3 probe to a RPS element, whose basis is supported by the mistaken notion that most variable pattern corresponds to the better DNA fingerprinting methods for strains. On the other hand, Ca3 band patterns, analyzed individually by their subfragments with regard to variability, revealed subgroups with hypervariability, moderate variability, low variability and no variability. In this manner, this information tends to strengthen the value of a probe in regard to the various levels of resolution proposed for an effective DNA fingerprinting method (8). In addition, the reduction of the Ca3 probe to an RPS results in a convenient probe for microevolution studies of strains (i.e., analysis of hypervariability) (19).

CONCLUSION

The evolution in molecular biology has dramatically augmented the capacity of investigators to study innumerable fungal infections; especially those caused by the opportunistic pathogen *C. albicans* and other *Candida* species. Multiple molecular systems are available for scientific studies into the epidemiology, evolution, taxonomy and systematics of infectious fungi. Nonetheless, the use of an adequately standardized system, encompassing various genotypic fingerprinting methods associated with a universal database program, has been the worry of many molecular mycologists. To determine the levels of resolution furnished by a genotypic fingerprinting method, a validation strategy has been delineated which compares two or more unrelated methods. For *C. albicans*, this strategy has validated the use of MLEE, RAPD and Ca3, since these methods were capable of achieving all levels of resolution. Within the context of epidemiology, all isolates have a history, both for the host and the pathogen, which could be further explored. Each strain submitted to analysis by the different genotypic fingerprinting methods could be registered in database programs and subjected to comparison with host parameters (i.e., age, sex, weight, medical characteristics, predisposing conditions, prosthetic devices, geographic location, socio-economic

factors, association with other individuals, etc.) and the characteristics of the pathogen (i.e., carbohydrate assimilation profiles, antigenicity, secretion of proteases, patterns of drug sensitivity, formation of hyphae, phenotypic switching, etc.). These procedures would permit current and retrospective comparison of a selection of clinical and epidemiologically important strains, which could show one or several characteristics of the host or pathogen. In addition, all of this growing amount of information could contribute still more to the understanding of the dynamics of infectious organisms in human populations, the complex relationship between commensalism and infection, and genetic and evolutionary mechanisms. It could even help to identify the origin of an infection and to monitor the emergence of strains resistant to unsuitable environmental factors.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support of FAPESP – Fundação de Amparo à Pesquisa do Estado de São Paulo (Proc. 00/03045-5). We also thank Dr. Albert Leyva for his help in the English language editing.

REFERENCES

1. Segal E, Baum GL. Pathogenic yeasts and yeast infections. Boca Raton: CRC Press; 1994.
2. Samaranayake LP. Oral candidosis: an old disease in new guises. Dent Update. 1990;17(1):36-8.
3. Samaranayake YH, Samaranayake LP, Pow EH, Beena VT, Yeung KW. Antifungal effects of lysozyme and lactoferrin against genetically similar sequential *Candida albicans* isolates from a human immunodeficiency virus-infected southern Chinese cohort. J Clin Microbiol. 2001;39(9):3296-02.
4. Greenspan D, Greenspan JS. HIV related oral disease. Lancet. 1996;348:729-33.
5. Cannon RD, Chaffin WL. Oral colonization by *Candida albicans*. Crit Rev Oral Biol Med. 1999;10(3):359-83.
6. Holmstrup P, Axéll T. Classification and clinical manifestations of oral yeast infections. Acta Odontol Scand. 1990;48(1):57-9.
7. Olive DM, Bean P. Principles and applications of methods for DNA-based typing of microbial organisms. J Clin Microbiol. 1999;37(6):1661-9.
8. Soll DR. The ins and outs of DNA fingerprinting the infectious fungi. Clin Microbiol Rev. 2000;13(5):322-70.
9. Tibayrenc M. Genetic epidemiology of parasitic protozoa and other infectious agents: the need for an integrated approach. Int J Parasitol. 1998;28(1):85-104.
10. Tibayrenc M, Neubauer K, Barnabé C, Guerrini F, Skarecky D, Ayala FJ. Genetic characterization of six parasitic protozoa: parity between randomly-primer DNA typing and multilocus enzyme electrophoresis. Proc Natl Acad Sci USA. 1993;90(4):1335-9.
11. Van Belkum A, Melchers W, de Pauw BE, Scherer S, Quint W, Meis JF. Genotypic characterization of sequential *Candida albicans* isolates from fluconazole-treated neutropenic patients. J Infect Dis. 1994;169(5):1062-70.
12. Pujol C, Joly S, Lockhart SR, Noel S, Tibayrenc M, Soll DR. Parity among the randomly amplified polymorphic DNA method multilocus enzyme electrophoresis and Southern blot hybridization with the moderately repetitive DNA probe Ca3 for fingerprinting *Candida albicans*. J Clin Microbiol. 1997;35(9):2348-58.
13. Taylor JW, Geiser DM, Burt A, Koufopanou V. The evolutionary biology and population genetics underlying fungal strain typing. Clin Microbiol Rev. 1999;12(1):126-46.
14. Gräser Y, Volovsek M, Arrington J, Schönian G, Presber W, Mitchell TG, et al. Molecular markers reveal that population structure of the human pathogen *Candida albicans* exhibits both clonality and recombination. Proc Natl Acad Sci USA. 1996;93(22):12473-7.
15. Pujol C, Reynes J, Renaud F, Mallie M, Bastide JM. Genetic analysis of *Candida albicans* strains studies by isoenzyme electrophoresis. J Mycol Med. 1993;3:14-9.

16. Sadhu C, McEachern MJ, Rustchenko-Bulgac EP, Schmid J, Soll DR, Hicks JB. Telomeric and dispersed repeat sequences in *Candida* yeasts and their use in strain identification. J Bacteriol. 1991;173(2):842-50.

17. Scherer S, Stevens DA. A *Candida albicans* dispersed repeated gene family and its epidemiologic applications. Proc Natl Acad Sci USA. 1988;85(5):1452-6.

18. Joly S, Pujol C, Schröppel K, Soll DR. Development and verification of two species fingerprinting probes for *Candida tropicalis* amenable to computer analysis. J Clin Microbiol. 1996;34(12):3063-71.

19. Pujol C, Joly S, Notan B, Srikantha T, Soll DR. Microevolutionary changes in *Candida albicans* identified by the complex Ca3 fingerprinting probe involve insertions and deletions of the full-length repetitive RPS at specific genomic sites. Microbiology. 1999;145(10):2635-46.

20. Chibana H, Iwaguchi SI, Homma M, Chindamporn A, Nakagawa Y, Tanaka K. Diversity of tandemly repetitive sequences due to short periodic repetitions in the chromosomes of *Candida albicans*. J Bacteriol. 1994;176(13):3851-8.

21. Iwaguchi SL, Homma M, Chibana H, Tanaka K. Isolation and characterization of a repeated sequence (RPS1) of *Candida albicans*. J Gen Microbiol. 1992;138(9):1893-900.

22. Arbeit DR. Laboratory procedures for the epidemiologic analysis of microorganisms. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, editors. Washington: ASM; 1995. p. 190-208.

23. Hunter PR, Gaston MA. Numerical index of the discriminatory ability of typing systems an application of Simpson´s index of diversity. J Clin Microbiol. 1988;26(11):2465-6.

24. Ayala FJ. Molecular evolution. Sinauer Associates. Sunderland: Mass; 1976.

25. Lewontin RC. The genetic basis of evolutionary change. New York: Columbia University Press; 1974. p. 346.

26. Nei M. Molecular polymorphism and evolution. Amsterdam: North-Holland Publishing Co; 1975.

27. Nevo E, Beiles A, Ben-Shlomo R. The evolutionary significance of genetic diversity: ecological demographic and life history correlates. Lect Notes Biomath. 1980;53(4)13-213.

28. Rattazzi MC, Scandalios JG, Whitt GS. Isozymes: current topics in biological and medical research. New York: Alan R Liss; 1983.

29. Selander RK, Whittam TS. Protein polymorphism and the genetic structure of populations. In: Nei M, Koehn RK, editors. Evolution of genes and proteins. Sinauer Associates. Sunderland; 1983. p. 89-114.

30. Selander RK, Lewin BR. Genetic diversity and structure in *Escherichia coli* populations. Science. 1980;210(4469):545-7.

31. Selander RK, Caugant DA, Ochman H, Musser JM, Gilmour MN, Whittam TS. Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. Appl Environ Microbiol. 1986;51(5):873-84.

32. Boerlin P. Applications of multilocus enzyme electrophoresis in medical microbiology. J Microbiol Meth. 1997;28(8):221-31.

33. Arnavielhe S, Blancark A, Mallié M, Gouin F, Ottomani A, Manalli JC, et al. Suivi mycologique d´infections à *Candida albicans* dans divers services hospitaliers. Path Biol. 1996;44(7):447-51.

34. Arnavielhe S, Blancark A, Mallié M, Quilici M, Bastide JM. Multilocus enzyme electrophoresis analysis of *Candida albicans* isolates from three intensive care units. An Epidemiological Study. Mycoses. 1997;40(5):159-67.

35. Barchiesi F, Arzeni D, Del Prete MS, Sinicco A, Falconi Di Francesco L, et al. Fluconazole susceptibility and strain variation of *Candida albicans* isolates from HIV-infected patients with oropharyngeal candidosis. J Antimicrob Chemother. 1998;41(5):541-8.

36. Bertout S, Renaud F, Swinne D, Mallie M, Bastide JM. Genetic multilocus studies of different strains of *Cryptococcus neoformans*: taxonomy and genetic structure. J Clin Microbiol. 1999;37(3):715-20.

37. Boerlin P, Boerlin-Petzold F, Durussel C, Addo M, Pagani J-L, Chave J-P, et al. Cluster of oral atypical *Candida albicans* isolates in a group of human immunodeficiency virus-positive drug users. J Clin Microbiol. 1995;33(5):1129-35.

38. Boerlin P, Boerlin-Petzold F, Goudet J, Durussel C, Pagani JL, Chave J-P, et al. Typing *Candida albicans* oral isolates from human immunodeficiency virus-infected patients by multilocus enzyme electrophoresis and DNA fingerprinting. J Clin Microbiol. 1996;34 (5):1235-48.

39. Brandt ME, Hutwagner LC, Klug LA, Baughman WS, Rimland D, Graviss EA, et al. Molecular subtype distribution of *Cryptococcus neoformans* in four areas of the United States Cryptococcal Disease Active Surveillance Group. J Clin Microbiol. 1996;34(4):912-7.

40. Brandt ME, Hutwagner LC, Kuykendall RJ, Pinner RW. Comparison of multilocus enzyme electrophoresis and random amplified polymorphic DNA analysis for molecular subtyping of *Cryptococcus neoformans*. J Clin Microbiol. 1995;33(7):1890-5.

41. Brandt ME, Bragg SL, Pinner RW. Multilocus enzyme typing of *Cryptococcus neoformans*. J Clin Microbiol. 1993;31(10):2819-23.

42. Caugant DA, Sandven P. Epidemiological analysis of *Candida albicans* strains by multilocus enzyme electrophoresis. J Clin Microbiol. 1993;31(2):215-220.

43. Doebbeling BN, Lehmann PF, Hollis RJ, Wu LC, Widmer AF, Voss A, et al. Comparison of pulsed-field gel electrophoresis with isoenzyme profiles as a typing system for *Candida tropicalis*. Clin Infect Dis. 1993;16(3):377-83.

44. Lehmann PF, Kemker BJ, Hsiao CB, Dev S. Isoenzyme biotypes of *Candida* species. J Clin Microbiol. 1989;27(11):2514-21.

45. Lehmann PF, Lin D, Lasker BA. Genotypic identification and characterization of species and strains within the genus *Candida* by using random amplified polymorphic DNA. J Clin Microbiol. 1992;30(12):3249-54.

46. Lehmann PF, Wu L C, Pruitt WR, Meyer SA, Ahearn DG. Unrelatedness of groups of yeasts within the *Candida haemulonii* complex. J Clin Microbiol. 1993;31(7):1683-7.

47. Lehmann PF, Wu LC, Mackenzie DWR. Isozyme changes in *Candida albicans* domestication. J Clin Microbiol. 1991;29(11):2623-5.

48. Lin D, Wu LC, Rinaldi MG, Lehmann PF. Three distinct genotypes within *Candida parapsilosis* from clinical sources. J Clin Microbiol. 1995;33(7)1815-1821.

49. Mata AL, Rosa RT, Rosa EAR, Gonçalves RB, Höfling JF. Clonal variability among oral *Candida albicans* assessed by allozyme electrophoresis analysis. Oral Microbiol Immunol. 2000;15(6):350-4.

50. Pujol C, Reynes J, Renaud F, Raymond M, Tibayrenc M, Ayala FJ, et al. The yeast *Candida albicans* has a clonal mode of reproduction in a population of infected human immunodeficiency virus-positive patients Proc Natl Acad Sci USA. 1993;90(20):9456-9.

51. Rosa EAR, Pereira CV, Rosa RT, Höfling JF. Evaluation of different dehydrogenases to recognize *Candida* species commonly isolated from human oral cavities. Rev Argent Microbiol. 1999;31(4):165-72.

52. Rosa EAR, Rosa RT, Pereira CV, Höfling JF. Grouping oral *Candida* species by multilocus enzyme electrophoresis. Int J Syst Evol Microbiol. 2000;50(3):1343-9.

53. Rosa EAR, Rosa RT, Pereira CV, Höfling JF. Inter and Intra-specific genetic variability of oral *Candida* species. Rev Iberoam Micol. 2001;18(2):60-4.

54. Rosa EAR, Rosa RT, Pereira CV, Boriollo MFG, Höfling JF. Analysis of parity between protein-based electrophoretic methods for characterization of oral *Candida* species. Mem Inst Oswaldo Cruz. 2000;95(6):801-6.

55. Rosa EAR, Rosa RT, Boriollo MFG, Bernardo WLC, Höfling JF. Oral *Candida albicans* and *Candida dubliniensis* differentiation by multilocus enzyme electrophoresis and sodium dodecylsulphate-polyacrylamide gel electrophoresis. Rev Argentina Microbiol. 2003;35(6):24-8.

56. San Millan RM, Wu LC, Salkin IF, Lehmann PF. Clinical isolates of *Candida guilliermondii* include *Candida fermentati*. Int J Syst Bacteriol. 1997;47:385-93.

57. Markert CL, Moller F. Multiple forms of enzymes: tissue ontogenic and species-specific patterns. Proc Natl Acad Sci USA. 1959;45(5):753-63.

58. Dixon H, Webb EC. Enzymes. NewYork: Academic Press; 1979.

59. Harris H. Isoenzymes. NewYork: Academic Press; 1975.

60. Markert CL. Biology of isoenzymes. In: Markert CL. Isoenzymes. New York: Academic Press; 1975. p. 1-9.

61. Scandalios JG. Genetic control of multiple molecular forms of enzymes in plants, a review. Biochem Genet. 1969;3(4):37-79.

62. Conkle MT, Hodgskiss PD, Nunnally LB, Hunter SC. Starch gel electrophoresis of conifer seeds, a laboratory manual. Berkeley Pacific Southwest Forest and Range Experiment Station. US Forest Service. US Department of Agriculture. (Gen tech rep PSW-64) Washington: US Department of Agriculture; 1982.

63. Prakash S, Lewontin RC, Hubby JL. A molecular approach to the study of genic heterozygosity in natural populations IV patterns of genetic variation in central marginal and isolated populations of *Drosophila pseudobscura*. Genetics. 1969;61(4):841-58.

64. Brown AJL, Langley CH. Re-evaluation of level of genic heterozygosity in natural populations of *Drosophila melanogaster* by two-dimensional electrophoresis. Proc Natl Acad Sci. 1979;76(5):2381-4.

65. Huettermann A, Volger C, Schorn R, Ahnert G, Ganser HG. Studies on isoenzyme polymorphism in *Fomes annosus*. Eur J For Pathol. 1979;9(3):265-74.

66. Johnson GB. Enzyme polymorphism and metabolism. Science. 1974;184(132):28-37.

67. Newman P. Variation amongst isozymes of *Rhynchosporium secalis*. Plant Pathol. 1985;34(6):329-37.

68. Racine RR, Langley CH. Genetic heterozygosity in a natural population of *Mus musculus* assessed using two-dimensional electrophoresis. Nature. 1980;283(5750):855-7.

69. Whittam TS, Ochman H, Selander RK. Multilocus genetic structure in natural populations of *Escherichia coli*. Proc Natl Acad Sci. 1983;80(6):1751-5.

70. Shannon MC, Ballal SK, Harris JW. Starch gel electrophoresis of enzyme from nine species of *Polyporus*. Amer J Bot. 1973;60(5):96-100.

71. Shecter Y. Symposium on the use of electophoresis in the taxonomy of algae and fungi. Bull Torrey Bot Club. 1973;100(12):253-312.

72. Harris H, Hopkinson DA. Handbook of enzyme electrophoresis in human genetics. Amsterdam: North-Holland Publishing Co; 1976.

73. Micales JA, Alfenas AC, Bonde MR. Izoenzimas na taxonomia e na genética de fungos In: Alfenas AC, editor. Eletroforese de isoenzimas e proteínas afins. Fundamentos e aplicações em plantas e microrganismos. Viçosa: Ed. da UFV; 1998. p. 477-512.

74. Siciliano MJ, Shaw CR. Separation and visualization of enzymes on gels. In: Smith I. Chromatographic and electrophoretic techniques. London: AW Heinemann Medical Books; 1976. p. 185-209.

75. Murphy RW, Sites JW, Buth DG, Haufler CH. Proteins I: isoenzyme electrophoresis. In: Hillis DM, Moritz C, editors. Molecular systematics. Sunderland, Mass: Sinauer Associates Inc Publishers; 1990. p. 45-126.

76. Pasteur N, Pasteur G, Bonbomme F, Catalan J, Britton-Davidian J. Manuel technique de génétique par électrophorèse dês proteins. Technique et documentation. Paris: Lavoisier; 1987. p. 217.

77. Hunter PR. A critical review of typing methods for *Candida albicans* and their applications. Crit Rev Microbiol. 1991;17(6):417-34.

78. Schwartz DC, Cantor CR. Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. Cell. 1984; 37(1):67-75.

79. Kwon-Chung KJ, Wickes BL, Merz WG. Association of electrophoretic karyotype of *Candida stellatoidea* with virulence for mice. Infect Immun. 1988;56(7):1814-9.

80. Lasker BA, Carle GF, Kobayashi GS, Medoff G. Comparison of the separation of *Candida albicans* chromosome-sized DNA by pulsed-field gel electrophoresis technique. Nucleic Acids Res. 1989;17(10):3783-93.

81. Lott TJ, Boiron P, Reiss E. An electrophoretic karyotype for *Candida albicans* reveals large chromosomes in multiples. Mol Gen Genet. 1987;209(1):170-4.

82. Magee BB, Magee PT. Electrophoretic karyotypes and chromosome numbers in *Candida* species. J Gen Microbiol. 1987;133(2):425-30.

83. Merz WG, Connelly C, Hieter P. Variation of electrophoretic karyotypes among clinical isolates of *Candida albicans*. J Clin Microbiol. 1988;26(5):842-5.

84. Snell RG, Wilkins RJ. Separation of chromosomal DNA molecules from *Candida albicans* by pulsed field gel electrophoresis. Nucleic Acids Res. 1986;24(22):4401-6.

85. Suzuki T, Kobayashi I, Mizuguchi I, Banno I, Tanaka K. Electrophoretic karyotypes in medically important *Candida* species. J Gen Appl Microbiol. 1988;34(9):409-16.

86. Vollrath D, Davis RW. Resolution of DNA molecules greater than 5 megabases by contour-clamped homogeneous electric fields. Nucleic Acids Res. 1987;15(19):7865-76.

87. Thrash-Bingham C, Gorman JA. DNA translocations contribute to chromosome length polymorphisms in *Candida albicans*. Curr Genet. 1992;22(2):93-100.

88. Sangeorzan JA, Zervos MJ, Donabedian S, Kauffman CA. Validity of contour-clamped homogeneous electric field electrophoresis as a typing system for *Candida albicans*. Mycoses. 1995;38(2):29-36.

89. Holmberg K, Feroze F. Comparative study of the GenePath group 4 reagent system and other CHEF systems for karyotype analysis of *Candida* spp. J Clin Lab Anal. 1995;9(3):184-92.

90. Bostock A, Khattak MN, Matthews R, Burnie J. Comparison of PCR fingerprinting by random amplification of polymorphic DNA with other molecular typing methods for *Candida albicans*. J Gen Microbiol. 1993;139(9):2179-84.

91. Gottfredsson M, Cox GM, Perfect JR. Molecular methods for epidemiological and diagnostic studies of fungal infections. Pathology. 1998;30(4):405-18.

92. Magee PT, Bowdin L, Staudinger J. Comparison of molecular typing methods for *Candida albicans*. J Clin Microbiol. 1992;30(10):2674-9.

93. Reiss E, Tanaka K, Bruker G, Chazalet V, Coleman D, Debeaupuis P, et al. Molecular diagnosis and epidemiology of fungal infections. Med Mycol. 1998;36(1):249-57.

94. Vazquez JA, Beckley A, Sobel JD, Zervos MJ. Comparison of restriction enzyme analysis versus pulsed-field gradient gel electrophoresis as a typing system for *Candida albicans*. J Clin Microbiol. 1991;29(5):962-7.

95. Asakura K, Iwaguchi S, Homma M, Sukai T, Higashide K, Tanaka K. Electrophoretic karyotypes of clinically isolated yeasts of *Candida albicans* and C glabrata. J Gen Microbiol. 1991;137(11):2531-8.

96. Barchiesi F, Hollis RJ, Del Poeta M, McGough DA, Scalise G, Rinaldi MG, et al. Transmission of fluconazole-resistent *Candida albicans* between patients with AIDS and oropharyngeal candidiasis documented by pulsed-field gel electrophoresis. Clin Infect Dis. 1995;21(3):561-4.

97. Bart-Delabesse E, Boiron P, Carlotti A, Dupont B. *Candida albicans* genotyping in studies with patients with AIDS developing resistance to fluconazole. J Clin Microbiol. 1993;31(11):2933-7.

98. Bart-Delabesse E, van Deventer H, Goessens W, Poirot JL, Lioret N, van Belkum A, et al. Contribution of molecular typing methods and antifungal susceptibility testing to the study of a candidemia cluster in a burn care unit. J Clin Microbiol. 1995;33 (12):3278-83.

99. Barton RC, van Belkum A, Scherer S. Stability of karyotype in serial isolates of *Candida albicans* from neutropenic patients. J Clin Microbiol. 1995;33(4):794-6.

100. Berenguer J, Diaz-Guerra TM, Ruiz-Diez B, de Quiros JCB, Rodriguez-Tudela JO, Martinez-Suarez JV. Genetic dissimilarity of two fluconazole-resistant *Candida albicans* strains causing meningitis and oral candidiasis in the same AIDS patients. J Clin Microbiol. 1996;34(6):1542-5.

101. Branchini ML, Geiger DC, Fischman O, Pignatari AC. Molecular typing of *Candida albicans* strains isolated from nosocomial candidemia. Rev Inst Med Trop. 1995; 37(8):483-7.

102. Doi M, Mizuguchi I, Homma M, Tanaka K. Electrophoretic karyotypes of isolates of *Candida albicans* from hospitalized patients. J Med Vet Mycol. 1994;32(2):133-40.

103. Doi M, Homma M, Iwaguchi SI, Horibe K, Tanaka K. Strain relatedness of *Candida albicans* strains isolated from children with leukemia and their bedside parent. J Clin Microbiol. 1994;32(9):2253-9.

104. Espinel-Ingroff A, Quart A, Steele-Moore L, Metcheva I, Buck GA, Bruzzese VL, et al. Molecular karyotyping of multiple yeast species isolated from nine patients with AIDS during prolonged fluconazole therapy. J Med Vet Mycol. 1996;34(2):111-6.

105. Iwaguchi S, Homma M, Tanaka K. Variation in the electrophoretic karyotype analysed by the assignment of DNA probes in *Candida albicans*. J Gen Microbiol. 1990;136(12):2433-42.

106. Lischewski A, Ruhnke M, Tennagen I, Schönian G, Morschhauser J, Hacker J. Molecular epidemiology of *Candida* isolates from AIDS patients showing different fluconazole resistance profile. J Clin Microbiol. 1995;33(3):769-71.

107. Lupetti A, Guzzi G, Paladini A, Swart K, Campa M, Senesi S. Molecular typing of *Candida albicans* in oral candidiasis: karyotype epidemiology with human immunodeficiency virus-seropositive patients in comparison with that with healthy carriers. J Clin Microbiol. 1995;33(5):1238-42.

108. Mahrous M, Lott TJ, Meyer SA, Awant AD, Ahearn DG. Electrophoretic kariotyping of typical and atypical *Candida albicans* J Clin Microbiol. 1990;28(5):876-81.

109. Millon L, Manteaux A, Reboux G, Drobacheff C, Monod M, Barale T, et al. Fluconazole-resistant recurrent oral candidiasis in human immunodeficiency virus-positive patients: persistence of *Candida albicans* strains with the same genotype. J Clin Microbiol. 1994;32 (4):1115-8.

110. Pfaller MA, Rhine-Chalberg J, Redding SW, Smith J, Farinacci G, Fothergill AW, et al. Variations in fluconazole susceptibility and electrophoretic karyotype among oral isolates of *Candida albicans* from patients with AIDS and oral candidíases J Clin Microbiol. 1994;32(1):59-64.

111. Vazquez JA, Sobel JD, Demitriou R, Vaishampayan J, Lynch M, Zervos M. Karyotyping of *Candida albicans* isolates obtained longitudinally in women with recurrent vulvovaginal candidiasis. J Infect Dis. 1994;170(6):1566-9.

112. Voss A, Pfaller MA, Hollis RJ, Rhine-Chalberg J, Doebbeling BN. Investigation of *Candida albicans* transmission in a surgical intensive care unit cluster by using genomic DNA typing methods. J Clin Microbiol. 1995;33(3):576-80.

113. Ramsey H, Morrow B, Soll DR. An increase in switching frequency correlates with an increase in recombination of the ribosomal chromosomes of *Candida albicans* strain 3153A. Microbiology. 1994;140(7):1525-31.

114. Rustchenko-Bulgac EP. Variations of *Candida albicans* electrophoretic karyotypes. J Bacteriol. 1991;173(20):6586-96.

115. Rustchenko-Bulgac EP, Howard DH. Multiple chromosomal and phenotypic changes in spontaneous mutants of *Candida albicans*. J Gen Microbiol. 1993;139(6):1195-207.

116. Rustchenko-Bulgac EP, Sherman F, Hicks JB. Chromosomal rearrangements associated with morphological mutants provide a means for genetic variation of *Candida albicans*. J Bacteriol. 1990;172(3):1276-83.

117. Lockhart S, Fritch JJ, Meier AS, Schroeppel K, Srikantha T, Galask R, et al. Colonizing populations of *Candida albicans* are clonal in origin but undergo microevolution through C1 fragment reorganization as demonstrated by DNA fingerprinting and C1 sequencing. J Clin Microbiol. 1995;33(6):1501-9.

118. Branchini ML, Pfaller MA, Rhine-Chalberg J, Frempong T, Isenberg HD. Genotypic variation and slime production among blood and catheter isolates of *Candida parapsilosis*. J Clin Microbiol. 1994;32(2):452-6.

119. Cormican MG, Hollis RJ, Pfaller MA. DNA macrorestriction profiles and antifungal susceptibility of *Candida* (*Torulopsis*) *grablata*. Diagn Microbiol Infect Dis. 1996;25(2):83-7.

120. Defontain A, Coarer M, Bouchara JP. Contribution of various techniques of molecular analysis to strain identification of *Candida grablata*. Microb Ecol H Dis. 1996;9(4):27-33.

121. Dib JC, Dube M, Kelly C, Rinaldi MG, Patterson JE. Evaluation of pulsed-field gel electrophoresis as a typing system for *Candida rugosa*. Comparison of karyotype and restriction fragment length polymorphisms. J Clin Microbiol. 1996;36(5):1494-6.

122. Diekema DJ, Messer SA, Hollis RJ, Wenzel RP, Pfaller MA. An outbreak of *Candida parapsilosis* prosthetic valve endocarditis. Diagn Microbiol Infect Dis. 1997;29(3):147-53.

123. King D, Rhine-Chalberg J, Pfaller MA, Moser SA, Merz WG. Comparison of four DNA-based methods for strain delineation of *Candida lusitaniae*. J Clin Microbiol. 1995;33(6):1467-70.

124. Pontieri E, Gregori L, Gennarelli M, Ceddia T, Novelli G, Dallapiccola B, et al. Correlation of SfiI macrorestriction endonuclease fingerprinting analysis of *Candida parapsilosis* isolates with source of isolation. J Med Microbiol. 1996;45(3):173-8.

125. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 1990;18(22):6531-5.

126. Welsh J, McClelland M. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 1990;18(24):7213-8.

127. Caetano-Anollés G. Amplifying DNA with arbitrary oligonucleotide primers. Genome Res. 1993;3(7):85-94.

128. Bonacorsi SP, Munck A, Gerardin M, Doit C, Brahimi N, Navarro J, Bingen E. In situ management and molecular analysis of candidaemia related to a totally implantable vascular access in a cystic fibrosis patient. J Infect. 1996;33(1):49-51.

129. Clemons KV, Feroze F, Holmberg K, Stevens DA. Comparative analysis of genetic variability among *Candida albicans* isolates from different geographic locales by three genotypic methods. J Clin Microbiol. 1997;35(6):1332-6.

130. Del Castilo L, Bikandi J, Nieto A, Quindos G, Sentandreu R, Ponton J. Comparison of morphotypic and genotypic methods for strain delineation in *Candida*. Mycoses. 1997;40(12):445-50.

131. Gyanchandani A, Khan ZK, Farooqui N, Goswami M, Ranade SA. RAPD analysis of *Candida albicans* strains recovered from immunocompromised patients (ICP) reveals an apparently non-random infectivity of the strains. Biochem Mol Biol Int. 1998;44(15):19-27.

132. Holmberg K, Feroze F. Evaluation of an optimized system for random amplified polymorfism DNA (RAPD)-analysis for genotypic mapping of *Candida albicans* strains. J Clin Lab Anal. 1996;10(2):59-69.

133. Howell SA, Anthony RM, Power E. Application of RAPD and restriction enzyme analysis to the study of oral carriage of *Candida albicans*. Lett Appl Microbiol. 1996;22(2):125-8.

134. Robert F, Lebreton F, Bougnoux ME, Paugam A, Wassermann D, Schlotterer M, et al. Use of random amplified polymorphic DNA as a typing method for *Candida albicans* in epidemiological surveillance of a burn unit. J Clin Microbiol. 1995;33 (9):2366-71.

135. Steffan P, Vazquez JA, Boikov D, Xu C, Sobel JD, Akins RA. Identification of *Candida* species by randomly amplified polymorphic DNA fingerprinting of colony lysates. J Clin Microbiol. 1997;35(8):2031-9.

136. Tietz HJ, Kussner A, Thanos M, De Andrade MP, Presber W, Schonian G. Phenotypic and genotypic characterization of unusual vaginal isolates of *Candida albicans* from Africa. J Clin Microbiol. 1995;33(9):2462-5.

137. Cobb BD, Clarkson JM. A simple procedure for optimizing the polymerase chain reaction (PCR) using modified Taguchi methods. Nucleic Acids Res. 1994;22(18):3801-5.

138. Ellsworth DL, Rittenhouse KD, Honeycutt RL. Artifactual variation in randomly amplified polymerase DNA banding patterns. Bio-Techniques. 1993;14(2):214-7.

139. Loudon KW, Coke AP, Burnie JP. "Pseudoclusters" and typing by random amplification of polymorphic DNA of *Aspergillus fumigatus*. J Clin Pathol. 1995;48(2):183-4.

140. Loudon KW, Coke AP, Burnie JP, Lucas GS, Liu Yin JA. Invasive aspergillosis: clusters and sources? J Med Vet Mycol. 1994;32(3):217-24.

141. Meunier JR, Grimont PA. Factors affecting reproducibility of random amplified polymorphic DNA fingerprinting. Res Microbiol. 1993;144(5):373-9.

142. Clemons KV, Shankland GS, Richardson MD, Stevens DA. Epidemiologic study by DNA typing of a *Candida albicans* outbreak in heroin addicts. J Clin Microbiol. 1991;29(5):205-7.

143. Khatib R, Thirumoorthi MC, Riederer KM, Sturm L, Oney L A, Baran Jr J. Clustering of *Candida* infections in the neonatal intensive care unit: concurrent emergence of multiple strains simulating intermittent outbreaks. Pediatr Infect Dis J. 1998;17(2):130-4.

144. Pfaller MA, Cabezudo I, Hollis R, Huston B, Wenzel RP. The use of biotyping and DNA fingerprinting in typing *Candida albicans* from hospitalized patients. Diag Microbiol Infect Dis. 1990;13(6):481-9.

145. Scherer S, Stevens DA. Application of DNA typing methods to epidemiology and taxonomy of *Candida* species. J Clin Microbiol. 1987;25(4):675-9.

146. Smith RA, Hitchcock CA, Evans EG, Lacey CJ, Adams DJ. The identification of *Candida albicans* strains by restriction fragment length polymorphism analysis of DNA. J Med Vet Micol. 1989;27(6):431-4.

147. Swanson H, Hughes PA, Messer SA, Lepow ML, Pfaller MA. *Candida albicans* arthritis one year after successful treatment of fungemia in a healthy infant. J Pediatr. 1996;129(5):688-94.

148. Tanaka K. Strain-relatedness among different populations of the pathogenic yeast *Candida albicans* analyzed by DNA-based methods. Nagoya J Med Sci. 1997;60(1):1-14.

149. Iwaguchi S Homma M, Tanaka K. Clonal variation of chromosome size derived from the rDNA cluster region in *Candida albicans*. J Gen Microbiol. 1992;138(6):1177-84.

150. Su CS, Meyer SA. Characterization of mitochondrial DNA in various *Candida* species: isolation restriction endonucleases reaction size and base composition. Int J Syst Bacteriol. 1991;41(1):6-14.

151. Olivo PD, McManus EJ, Riggsby WS, Jones JM. Mitochondrial DNA polymorphism in *Candida albicans*. J Infect Dis. 1987; 156(1):214-5.

152. Wills JW, Lasker BA, Sirotkin K, Riggsby WS. Repetitive DNA of *Candida albicans*: nuclear and mitochondrial components. J Bacteriol. 1984;157(3):918-24.

153. Pfaller MA, Messer SA, Houston A, Rangel-Frausto MS, Wiblin T, Blumberg HM, et al. National epidemiology of mycoses survey: a multicenter study of strain variation and antifungal susceptibility among isolates of *Candida* species. Diag Microbiol Infect Dis. 1998;31(11):289-96.

154. Fox BC, Mobley HL, Wade JC. The use of a DNA probe for epidemiology studies of candidíases in immunocompromised hosts. J Infect Dis. 1989;159(3):488-94.

155. Wilkinson BM, Morris L, Adams DJ, Evans EG, Lacey CJ, Walmsley RM. A new sensitive polynucleotide probe for distinguishing *Candida albicans* strains and its use with a computer assisted archiving and pattern comparison system. J Med Vet Mycol. 1992;30(2):123-31.

156. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. New York: Cold Spring Harbor; 1989.

157. Mitchell TG, Sandin RL, Bowman BH, Meyer W, Merz WG. Molecular mycology: DNA probes and applications of PCR technology. J Med Vet Mycol. 1994;32(1):351-66.

158. Oren I, Manavathu EK, Lerner SA. Isolation and characterization of a species-specific DNA probe for *Candida albicans*. Nucleic Acids Res. 1991;19(25):7113-6.

159. Weissman Z, Berdicevsky I, Cavari B. Molecular identification of *Candida albicans*. J Med Vet Mycol. 1995;33(3):205-7.

160. Magee BB, D'Souza TM, Magee PT. Strain and species identification by restriction length polymorphisms in the ribosomal DNA repeat of *Candida* species. J Bacteriol. 1987;169(4):1639-43.

161. Mercure S, Montplaisir S, Lemay G. Correlation between the presence of a self-splicing intron in the 25S rDNA of *Candida albicans* and strain susceptibility to 5-fluorocytosine. Nucleic Acids Res. 1993;21(25):6020-7.

162. Stein GE, Sheridan VL, Magee BB, Magee PT. Use of rDNA restriction fragment length polymorphisms to differentiate strains of *Candida albicans* in women with vulvovaginal candidíases. Diagn Microbiol Infect Dis. 1991;14(6):459-64.

163. Whelan WL, Kirsch DR, Kwon-Chung KJ, Wahl SM, Smith PD. *Candida albicans* in patients with the acquired immunodeficiency syndrome: absence of a novel or hypervirulent strain. J Infect Dis. 1990;162 (2):513-8.

164. Carlotti A, Grillot R, Couble A, Villard J. Typing of *Candida krusei* clinical isolates by restriction endonuclease analysis and hybridization with the CkF1 2 DNA probe. J Clin Microbiol. 1994;32(7):1691-9.

165. Fan M, Currie BP, Gutell RR, Ragan MA, Casadevall A. The 16S-like 58S and 28S-like rRNAs of the two varieties of *Cryptococcus neoformans*: sequence secondary structure phylogenetic analysis and restriction fragment polymorphism. J Med Vet Mycol. 1994;32(3):163-80.

166. Fraser VJ, Keath EJ, Powderly WG. Two cases of blastomycosis from a common source: use of DNA restriction analysis to identify strains. J Infect Dis. 1991;163(6):1378-81.

167. Spitzer ED, Lasker BA, Travis SJ, Kobayashi GS, Medoff G. Use of mitochondrial and ribosomal DNA polymorphisms to classify clinical and soil isolates of *Histoplasma capsulatum*. Infect Immun. 1989;57(5):1409-12.

168. Spreadbury CL, Bainbridge BW, Cohen J. Restriction fragment length polymorphisms in isolates of *Aspergillus fumigatus* probed with part of the intergenic spacer region from the ribosomal RNA gene complex of *Aspergillus nidulans*. J Gen Microbiol. 1990;136(10):1991-4.

169. Glover DM. Genes for ribosomal DNA. In: Maclean M, Gregory SP, Flavell RA editors. Eukaryotic genes: their structure and regulation. United Kingdom: Butterworth & Co. Cambridge; 1983. p. 207-224.

170. Hatlan LE, Attardi G. Preparation of the HeLa cell genome complementary to tRNA and 5SRNA. J Mol Biol. 1971;56(6):535-54.

171. Schweizer E, MacKechnie C, Halvorson HD. The redundancy of ribosomal and transfer RNA genes in Saccharomyces cerevisiae. J Mol Biol. 1969;40(2):261-78.

172. Vlad M. Quantitative studies of rDNA in amphibians. J Cell Sci. 1997;24:109-118.

173. Scherer S, Magee RT. Genetics of *Candida albicans*. Microbiol Rev. 1990;54(3):226-41.

174. Wills JW, Troutman WB, Riggsby WS. Circular mitochondrial genome of *Candida albicans* contains a large inverted duplication. J Bacteriol. 1985;164(1):7-13.

175. Kwon-Chung KJ, Riggsby WS, Uphoff RA, Hicks JB, Whelan WL, Reiss E, et al. Genetic differences between type I and tipy II *Candida stellatoidea*. Infect Immun. 1989;57(2):527-32.

176. Lacher DA, Lehmann PF. Application of multidimensional scaling in numerical taxonomy: analysis of isoenzyme types of *Candida* species. Ann Clin Lab Sci. 1991; 21(2):94-103.

177. Pujol C, Renaud F, Mallie M, Meeus T, Bastide JM. Atypical strains of *Candida albicans* recovered from AIDS patients. J Med Vet Mycol. 1997;35(2):115-21.

178. Anthony RM, Midgley J, Sweet SP, Howell SA. Multiple strains of *Candida albicans* in the oral cavity of HIV positive and HIV negative patients. Microbiol Ecol Health Dis. 1995;8(5):23-30.

179. Coleman DC, Sullivan DJ, Bennett DE, Moran GP, Barry HJ, Shanley DB. Candidiasis: the emergence of a novel species *Candida dubliniensis*. AIDS. 1997;11(5):557-67.

180. Hellstein J, Vawter-Hugart H, Fotos P, Schmid J, Soll DR. Genetic similarity and phenotypic diversity of commensal and pathogenic strains of *Candida albicans* isolated from the oral cavity. J Clin Microbiol. 1993;31(12):3190-9.

181. Kleinegger C, Lockhart SR, Vargas K, Soll DR. Frequency intensity species and strains of oral yeast vary as a function of host age. J Clin Microbiol. 1996;34(9):2246-54.

182. Kuehnert MJ, Clark E, Lockhart SR, Soll DR, Chia J, Jarvis WR. *Candida albicans* endocarditis traced to a contaminated aortic valve allograft: implications for regulation of allograft processing. Clin Infect Dis. 1998;27(4):688-91.

183. Lockhart SR, Reed BD, Pierson CL, Soll DR. Most frequent scenario for recurrent *Candida* vaginitis is strains maintenance with "substrain shuffling": demonstration by sequential DNA fingerprinting with probes Ca3 C1 and CARE2. J Clin Microbiol. 1996;34(4):767-77.

184. McCullough MJ, Ross BC, Dwyer BD, Reade PC. Genotype and phenotype of oral *Candida albicans* from patients infected with the human immunodeficiency virus. Microbiology. 1994;140(5):1195-202.

185. Mercure S, Poirier S, Lemay G, Auger P, Montplaisic S, Repentigny L. Application of biotyping and DNA typing of *Candida albicans* to the epidemiology of recurrent vulvovaginal candidiasis. J Infect Dis. 1993;168(2):502-7.

186. Pfaller MA, Lockhart SR, Pujol C, Swails-Wenger JA, Messer SA, Edmond MB, et al. Hospital specificity region specificity and fluconazole-resistance of *Candida albicans* bloodstream isolates. J Clin Microbiol. 1998;36(6):1518-29.

187. Pierson DL, Mehta SK, Magge BB, Mishra SK. Person-to-person transfer of *Candida albicans* in the spacecraft environment. J Med Vet Mycol. 1995;33(3):145-50.

188. Schmid J, Voss E, Soll DR. Computer-assisted methods for assessing *Candida albicans* strain relatedness by Southern blot hybridization with repetitive sequence Ca3. J Clin Microbiol. 1990;28(6):1236-43.

189. Schmid J, Rotman M, Reed B, Pierson CL, Soll DR. Genetic similarity of *Candida albicans* strains from vaginitis patients and their partners. J Clin Microbiol. 1993;31 (1):39-46.

190. Schoofs A, Odds FC, Colebunders R, Ieven M, Wouters L, Goossens H. Isolation of *Candida* species on media with and without added fluconazole reveals high variability in relative growth susceptibility phenotypes. Antimicrob Agents Chemother. 1997;41(8):1625-35.

191. Schroeppel K, Rotman M, Galask R, Mac K, Soll DR. The evolution and replacement of *Candida albicans* strains during recurrent vaginitis demonstrated by DNA fingerprinting. J Clin Microbiol. 1994;32(11):2646-54.

192. Soll DR, Galask R, Schmid J, Hanna C, Mac K, Morrow B. Genetic dissimilarity of commensal strains of *Candida* spp. carried in different anatomical localizations of the same healthy women. J Clin Microbiol. 1991;29(8):1702-10.

193. Sullivan D, Bennett D, Henman M, Harwood P, Flint S, Mulcahy F, et al. Oligonucleotide fingerprinting of isolates of *Candida* species other than *Candida albicans* and of atypical *Candida* species from human immunodeficiency virus-positive and AIDS patients. J Clin Microbiol. 1993;31(8):2124-33.

194. Sullivan DJ, Westerneng TJ, Haynes KA, Bennett DE, Coleman D. *Candida dubliniensis* sp nov: phenotypic and molecular characterization of a novel species associated with oral candidosis in HIV infected individuals. Microbiology. 1995;141(7):1507-21.

195. White TC, Pfaller MA, Rinaldi MC, Smith J, Redding S. Stable azole drug resistance associated with a substrain of *Candida albicans* from an HIV-infected patient. Oral Dis. 1997;3(5):102-9.

196. Sullivan D, Haynes K, Bille J, Boerlin P, Rodero L, Lloyd S, et al. Widespread geographic distribution of oral *Candida dubliniensis* strains in human immunodeficiency virus-infected individuals. J Clin Microbiol. 1997;35(4):960-4.

197. Soll DR, Staebell M, Langtimm CJ, Pfaller M, Hicks J, Rao TVG. Multiple *Candida* strains in the course of a single systemic infection. J Clin Microbiol. 1988;26(8):1448-59.

198. Diaz-Guerra TM, Martinez-Suarez JV, Laguna F, Rodriguez-Tudela JL. Comparison of four molecular typing methods for evaluating genetic diversity among *Candida albicans* isolates from human immunodeficiency virus-positive patients with oral candidíases. J Clin Microbiol. 1997;35(4):856-61.

199. Anderson J, Srikantha T, Morrow B, Miyasaki SH, White TC, Agabian N, et al. Characterization and partial nucleotide sequence of the DNA fingerprinting probe Ca3 of *Candida albicans*. J Clin Microbiol. 1993;31(6):1472-80.

200. Wickes B, Staudinger J, Magee BB, Kwon-Chung K-J, Magee PT, Scherer S. Physical and genetic map of *Candida albicans*: several genes previously assigned to chromosome 1 map to chromosome R the rDNA-containing linkage group. Infect Immun. 1991;59(7):2480-4.

201. Lockhart SR, Joly S, Vargas K, Swails-Wenger J, Enger L, Soll DR. Defenses against oral *Candida* carriage break down in the elderly. J Dent Res. 1999;78(4):857-68.

202. Odds F, Schmid J, Soll DR. Epidemiology of *Candida* infections in AIDS. In: Bossche HV, editor. Mycoses in AIDS patients. New York: Plenum; 1990. p. 67-74.

203. Marco F, Lockhart SR, Pfaller MA, Pujol C, Rangel-Frausto MS, Wiblin T, et al. Elucidating the origins of nosocomial infections with *Candida albicans* by DNA fingerprinting with the complex probe Ca3. J Clin Microbiol. 1999;37(9):2817-28.

204. Schmid J, Tay YP, Wan L, Carr M, Parr D, McKinney W. Evidence for nosocomial transmission of *Candida albicans* obtained by Ca3 fingerprinting. J Clin Microbiol. 1995;33(5):1223-30.

205. Soll DR. DNA fingerprinting of *Candida albicans*. J Mycol Med. 1993;3(6):37-44.

206. Chindamporn A, Nakagawa Y, Mizuguchi I, Chibana H, Doi M, Tanaka K. Repetitive sequences in (RPSs) in the chromosomes of *Candida albicans* are sandwiched between two novel stretches HOK and RB2 common to each chromosome. Microbiology. 1998;144(4):849-57.

207. Chibana H, Magee BB, Grindle S, Ran Y, Scherer S, Magee PT. A physical map of chromosome 7 of *Candida albicans*. Genetics. 1998;149(4):1739-52.

208. Lasker BA, Page LS, Lott TJ, Kobayashi GS. Isolation characterization and sequencing of *Candida albicans* repetitive element 2. Gene. 1992;116(1):51-7.

Received: 6/15/2009
Recebido: 15/6/2009

Accepted: 7/28/2009
Aceito: 28/7/2009

Reviewed: 11/25/2009
Revisado: 25/11/2009