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Clonal identity of *Candida albicans* in the oral cavity and the gastrointestinal tract of pre-school children

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The clonal relationship between oral and fecal *Candida albicans* isolated from children of pre-school age was examined using RAPD analysis. Significantly higher levels of *C. albicans* were found in saliva, dental plaque, carious specimens and stools of 56 patients with severe caries as compared to 52 healthy control subjects. The highest prevalence was found in carious specimens and a strong correlation was observed between its presence in saliva, dental plaque, carious specimen and feces. RAPD analysis of isolates from 23 patients with simultaneous oral and fecal *C. albicans* revealed clonal counterparts present in both oral and stool samples in 15 cases; five patients harbored closely related strains; and three patients harbored unrelated strains. Our results demonstrate a strong correlation between oral and gastrointestinal *C. albicans* colonization. We assume that carious teeth may constitute an ecologic niche for *C. albicans* potentially responsible for recurrent oral and non-oral candidiasis.

Key words: *Candida albicans*; candidiasis; caries; epidemiology; ecologic niche; biofilm

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Inflammatory periodontal diseases and dental caries are two of the most prevalent diseases worldwide. Childhood caries results in a considerable direct burden of pain and suffering as well as poorer general health (1, 18, 33). The overall prevalence of childhood caries in the United States is estimated at 1–5%, although among high-risk populations the prevalence has been reported to be as high as 60% (26). Treatment of children with caries can be expensive, often requiring extensive restorative treatment under general anesthesia.

It is now well established that caries is an infectious disease associated with the resident microorganisms of the dental plaque, and the demineralization of teeth is caused by organic acid produced by the bacterial fermentation of dietary carbohydrates. The frequent ingestion of carbohydrates may lead to the selection of bacteria that are acidogenic and aciduric, and concurrently to a low-pH environment. There is considerable evidence that mutans streptococci, particularly *Streptococcus mutans* and *Streptococcus sobrinus*, and lactobacilli are involved in the initiation and progress of dental caries (17). However, other species such as *Actinomyces* spp., *Streptococcus mitis*, and *Veillonella* spp. have also been associated with enamel caries (21).

Apart from bacteria, the importance of the presence of yeasts in the oral cavity (21) and the incidence of dental caries have been demonstrated for adults (28) and for children (23). Our own studies revealed that the oral cavity of children with healthy teeth is almost devoid of *Candida*. *Candida* species from healthy control subjects have been detected only in 2% of salival specimens examined. However, in children with carious teeth, yeasts were present in 67% of collected saliva and in 82% of carious specimens (32). The highest concentrations of yeasts were found in the carious cavity, providing a significant ecologic niche for the dissemination of the yeasts which occasionally result in serious medical diseases (17, 32).

Among members of the genus *Candida*, *Candida albicans* is the prevalent causative agent of candidiasis and constitutes the fourth most common nosocomial bloodstream isolate in industrial countries (25). It is generally believed that candidiasis arises from endogenous commensal strains inhabiting the oral cavity, gastrointestinal tract and genitourinary system (15, 29). Cannon and colleagues have proposed that *Candida* colonization of oral surfaces, including the denture-fitting surface, can

serve as a reservoir for disseminated infections such as aspirative pneumonia and gastrointestinal infections (7).

In this study, we have examined for the presence of *C. albicans* in carious lesions and provide evidence for clonal identity/relatedness between strains deriving from the oral cavity of children with carious teeth and from their feces. The clonal identity of *C. albicans* strains isolated from distant parts of the body permits the hypothesis that carious lesions may provide an ecologic niche for the release of planktonic *C. albicans* to distal sites of the body, occasionally causing non-oral candidiasis.

Material and methods

Study design

In 1999–2000, pre-school children were investigated with respect to the presence of *Candida* species in their oral cavities and feces. A total of 108 children comprising 52 control subjects with healthy teeth and 56 patients with carious teeth were selected on their DMF(T)/dmf(t) values. Diagnosis and evaluation of caries were performed according to general principles and nomenclature using DMF(T)/dmf(t) values defined for an international standardization of caries (5). Briefly, the DMF(T) value is calculated as the sum of decayed (D), missing (M) and filled (F) permanent teeth, corresponding to values with lowercase letters (dmf(t)) denoting primary dentition. The carious status of children with mixed dentition is described by the combined DMF(T)/dmf(t) value as a summation of the DMF(T) and the dmf(t) value and is a criterion for the carious status of the oral cavity depending on the age group. Thus, combined DMF(T)/dmf(t) values were determined and radiographs were performed to demonstrate osteolysis of the jawbones. Children with healthy teeth had a DMF(T)/dmf(t) value of zero and children with carious teeth a DMF(T)/dmf(t) value between 4.0 and 11.0. The control group consisted of 27 girls and 25 boys from nursery schools in the area of Giessen, Germany, with an average age of 53.3 months. The patient group consisted of 23 girls and 33 boys with an average age of 61.4 months, all visiting the Department of Paediatric Dentistry, University of Giessen, Germany. Participation in the study was voluntary.

General health status and dental treatment

Approximately 50% of the patients suffered from pain caused by abscesses and fistulae in the oral cavity. Most of the parents complained about a poor health status of their children exhibiting sub-febrile temperatures, fatigue and crotchety tempers. Severe or other chronic diseases were not reported. Neither the patients nor the healthy individuals were treated with antibiotics or antimycotics prior to or during the study. Pus in jaw bones was eliminated by trepanation of carious teeth. Complete dental treatment of carious teeth, including fillings, extractions and the sealing of fissures, was performed in regular settings or under general anesthesia.

Sample collection and processing

Saliva, dental plaque and a carious specimen, a mixture of carious dentin and debris, were collected simultaneously at the Department of Pediatric Dentistry, whereas the fecal specimen were collected by the parents themselves the day after the visit. Two hundred μl of nonstimulated saliva was collected using a pipette and immediately transferred into a sterile plastic cap. Dental plaque and carious specimens were collected with a spoon excavator. In general, 0.03 mg were collected, homogenized using a sterile mortar, diluted in 100 μl of PBS and stored in a sterile plastic cap. Two hundred milligrams of the provided stool specimen was diluted with 200 μl of PBS and homogenized. The collected samples were stored at 4°C until the end of the session at the Department of Pediatric Dentistry and inoculated at the proximate Institute for Medical Microbiology on the same day.

Identification of *Candida* species

One hundred microliters of each sample were plated on CHROMagar *Candida* (CHROMagar, Paris, France) and on Sabouraud agar (Merck KgaA, Darmstadt, Germany) and incubated at 37°C for 48 h. CHROMagar *Candida* is a differential medium used for isolation and presumptive identification of clinically important *Candida* species. After 24–48 h of incubation, contrasting colored colonies result from the cleavage of chromogenic substrates by species-characteristic enzymes (2, 4). *Candida* species were identified additionally according to their ability to produce germ tubes and by using Auxacolor Test (Sanofi Diagnostics Pasteur, Marnes-La Coquette, France) following protocols provided by the vendor. The yeast isolates were stored in 20% glycerine-Brain Heart Infusion (BHI) broth at –20°C. The quantity of yeasts was calculated as colony forming units (CFU) per sample and classified into the categories 0 = absent, 1 = very sparse (<10 CFU), 2 = sparse ($10–10^2$ CFU), 3 = moderate ($10^2–10^3$ CFU), and 4 = numerous ($>10^3$ CFU).

Randomly Amplified Polymorphic DNA (RAPD) analysis

Only isolates of patients with *Candida albicans* in both the oral cavity and feces were subjected to RAPD analysis. Strains obtained from stock cultures stored at –20°C were subcultured on Sabouraud agar and incubated as described above. Chromosomal DNA was extracted using the QIAamp tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations.

The clonality of the isolates was investigated using Ready-To-Go RAPD Analysis Beads (Amersham Pharmacia Biotech, Freiburg, Germany). The custom-synthesized primers used in the study were Primer 1 (5'-d[GGTGCGGGAA]-3'), Primer 2 (5'-d[GTTTCGCTCC]-3') and Primer 5 (5'-d[AACGCGCAAC]-3'). A 5 μl template containing 50 ng of yeast chromosomal DNA, 2.5 μl of one primer (20 pmol) and 17.5 μl pure water were mixed with the Ready-To-Go RAPD Analysis Bead containing PCR-buffer (30 mM KCl, 10 mM Tris-HCl pH 8.3, 2.5 μg BSA, 3 mM $MgCl_2$, 0.4 mM dNTP's) and 1 unit Amplitaq DNA polymerase for the amplification. The samples were covered with mineral oil (Sigma-Aldrich, Taufkirchen, Germany) to avoid evaporation. Thermocycling was performed in a PCR-Express cycler with tube control (Hybaid Limited, Ashford, UK) with initial denaturation at 95°C for 5 min, followed by 45 cycles of denaturation at 95°C for 1 min, annealing at 36°C for 1 min and extension at 72°C for 2 min with a final extension step at 72°C for 5 min.

The amplification products were analyzed by gel electrophoresis on a horizontal 2% agarose gel for 2.5 h at 150 V at room temperature in TBE running buffer. All experiments were carried out in duplicate to assess reproducibility.

Data analysis

All gels were stained with SYBR-Gold (Molecular Probes; Mo Bi Tec, Goettingen, Germany) and, in order to standardize the quality of images, documented under UV light using the Image Master VDS System (Amersham Pharmacia Biotech). Strain differentiation was achieved by comparison of band patterns using software program Gelcompar 4.0 (Applied

Maths, Kortrijk, Belgium). Patterns were normalized on the basis of the molecular weight marker to compensate for the differences in the run length of the gels. The similarity coefficients, S_{AB}s (equivalent to percentage similarities divided by 100), of DNA profiles were computed based on band positions by using the DICE coefficient. Similarity values were then clustered by the unweighted pair group method using arithmetic averages (UPGMA). Dendograms were generated to visualize relationships among the isolates. The cut-off in the dendrogram was calculated at a S_{AB} of 0.73 as a threshold for defining clusters of genetically similar isolates. The discriminatory power of the applied RAPD method was determined by the discriminatory index based on application of Simpson's index of diversity as described previously (13, 14).

Statistics

The differences in *C. albicans* colonization between patients and control group were evaluated using the Chi-squared test for qualitative and quantitative analysis. The Chi-squared test was also applied for demonstrating a correlation between *C. albicans* colonization of oral cavity and intestinal tract. The levels of significance were fixed at $P \leq 0.05$ (significant), $P \leq 0.01$ (very significant) and $P \leq 0.001$ (most significant). Statistical analysis of the data was accomplished in association with the Department of Medical Informatics at the University of Giessen, Germany, using the software package SPSSWin, version 8.0 (6).

Results

Clinical status of carious teeth and jawbones

A DMF(T)/dmf(t) value of 0 could be determined for all control subjects. Clinical assessment of the patients showed a combined DMF(T)/dmf(t) value of 10.84 with strong contribution from the decayed teeth which had a D(T)/d(t) ratio of 10.27. Only a few teeth were extracted (M/m) or filled (F/f). The DMF(T)/dmf/t) value is a criterion for the carious status of the oral cavity depending on the age group. For the examined population of children, the DMF(T) value can range from 0 to 28 and the dmf(t) value from 0 to 20. Apart from the carious teeth, 10 of the 23 patients with *C. albicans* simultaneously in their oral cavity and feces exhibited additional lesions such as fistulae (patient numbers 16 and 18), abscess (patient numbers 5, 14 and 20) and radiographic osteolysis (patient numbers 2, 3, 4, 5, 8, 14, 20 and 21). Figure 1(A,B) shows the clinical status and the radiograph of patient number 8 prior to treatment.

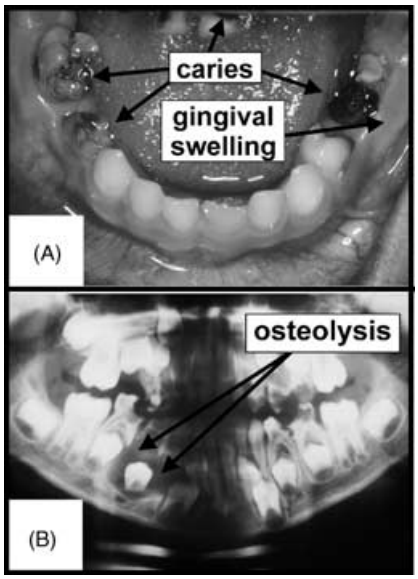

Fig. 1. Severe caries presented in patient eight providing an ecologic niche for *Candida albicans* able to release planktonic counterparts. (A) Photograph showing caries and gingival swelling. (B) Radiograph showing osteolyses.

Simultaneous colonization of *C. albicans* in the oro-intestinal tract

In all cases, the fungal differentiation of *Candida* isolates derived from saliva, dental plaque, carious specimen and feces revealed only *C. albicans;* other *Candida* species were not detected. To assess the level of colonization, the amounts of *C. albicans* in saliva, dental plaque, carious specimen, and feces were classified into five scores using quantity as a criterion (Table 1). *C. albicans* was absent (score 0) in 90.4% of saliva specimens, in 92.3% of dental plaque and in 98.1% of feces from control subjects and in 44.6%, 33.9% and 14.3% of the patients, respectively. On the other hand, the highest level of *C. albicans* (score 4) was found in 1.8% of feces, in 8.9% of dental plaque and in 41.1% of carious specimen derived from patients, whereas no control subject showed such high levels of colonization in any specimen. The qualitative and quantitative differences in *C. albicans* colonization of saliva, dental plaque and stool between patients and control group were statistically significant ($P \leq 0.001$). For all 108 subjects, a strong significant correlation between *C. albicans* colonization of saliva and feces as well as dental plaque and feces ($P \leq 0.001$) was observed. Only 2% of the fecal samples of healthy control subjects were contaminated with *C. albicans,* whereas the feces of the patients showed a very high contamination of 59%. The latter result correlates significantly with the high prevalence of *C. albicans* (85.7%) in the carious specimen of the patients.

Discrimination of *C. albicans* isolates by RAPD analysis

A total of 55 *C. albicans* isolates recovered from 23 patients with simultaneous colonization of oral cavity and intestinal tract were subjected to RAPD analysis using the three different primers 1, 2 and 5 (see Materials and methods). Reproducibility of RAPD-PCR patterns was assessed by carrying out the experiments in duplicate; no significant differences in profiles were observed. For a number of isolates, independent profiling experiments were carried out. All profiles had a S_{AB} of 1, indicating the robustness of the conditions used (data

Table 1. Quantities of *C. albicans* isolated from control subjects and patients

	Saliva		Dental Plaque		Carious specimen		Feces	
Score	%	(abs.)	%	(abs.)	%	(abs.)	%	(abs.)
Control subjects ($n = 52$)								
0	90.4	(47)	92.3	(48)	–	–	98.1	(51)
1	1.9	(1)	0	(0)	–	–	0	(0)
2	3.8	(2)	7.7	(4)	–	–	1.9	(1)
3	3.8	(2)	0	(0)	–	–	0	(0)
4	0	(0)	0	(0)	–	–	0	(0)
Patients ($n = 56$)								
0	44.6	(25)	33.9	(19)	14.3	(8)	41.0	(23)
1	30.4	(17)	21.4	(12)	1.8	(1)	16.1	(9)
2	21.4	(12)	16.1	(9)	8.9	(5)	26.8	(15)
3	3.6	(2)	19.6	(11)	33.9	(19)	14.3	(8)
4	0	(0)	8.9	(5)	41.1	(23)	1.8	(1)

Quantities are given in percentage whereas absolute numbers (abs.) are in parentheses.
Score: 0: absent, 1: very sparse (<10 CFU), 2: sparse (10–10^2 CFU), 3: moderate (10^2–10^3 CFU), 4: numerous (>10^3 CFU).

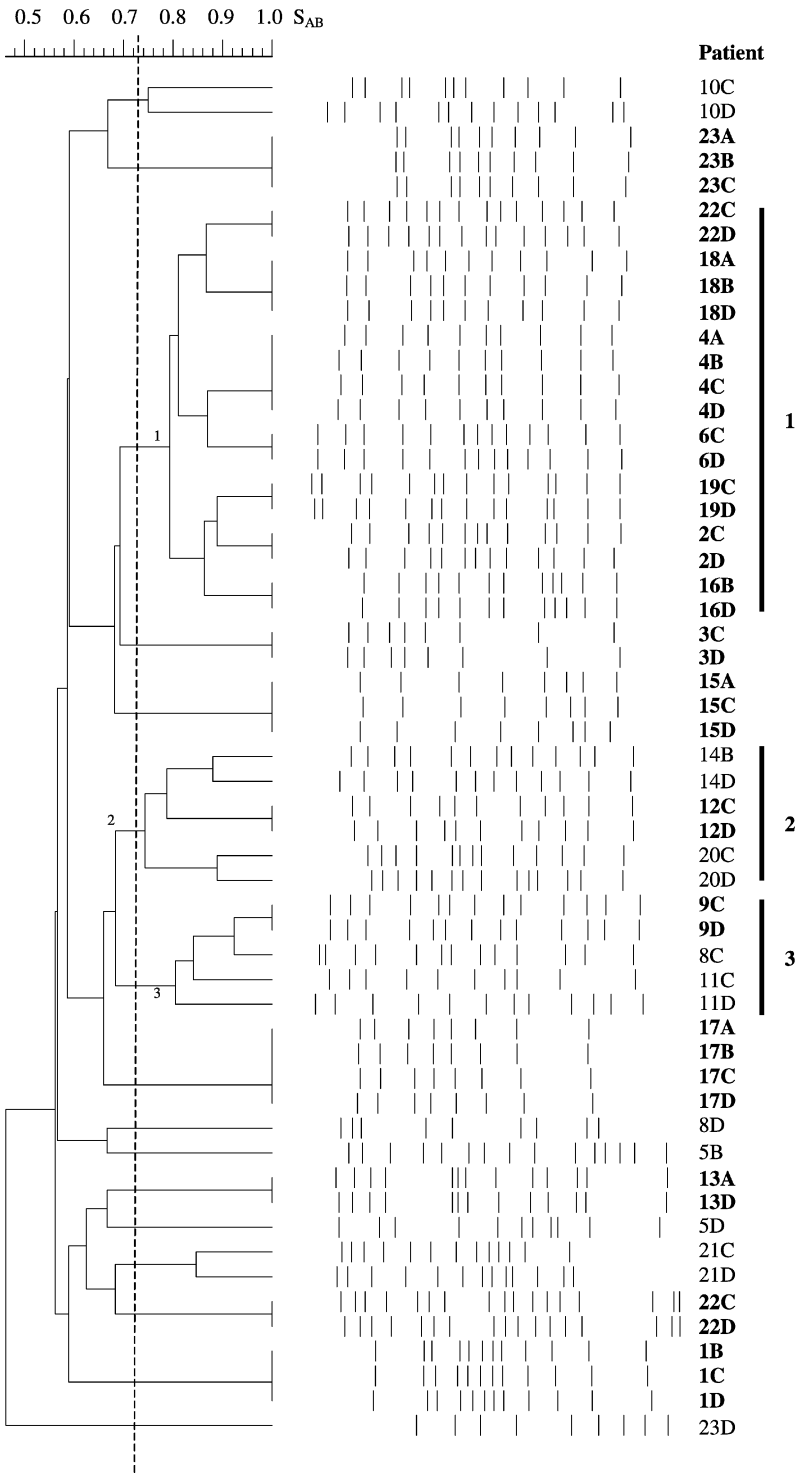

Fig. 2. UPGMA dendrogram generated from the similarity values (S_{AB}) showing intraindividual and interindividual similarities. S_{AB}s were determined by the DICE coefficient according to the RAPD profiles of 56 *C. albicans* strains isolated from saliva (A), dental plaque (B), carious specimen (C) and feces (D) of 23 examined patients. Identical strains are given in bold font. Dashed line at S_{AB} of 0.73 subdivides the dendrogram in three major clusters (indicated with bold vertical bars) comprising 28 strains.

not shown). The results of molecular typing of these subjects are given in Fig. 2.

The criterion used for genotyping was the difference in banding positions of each isolate in the RAPD assays. All three primers used in this study were successful in eliciting banding profiles for each isolate, although some were more discriminatory than others. In Fig. 3, the electrophoretic separation of RAPD analysis primer 1, 2 and 5 of *C. albicans* strains isolated from saliva and feces of patient number one is depicted, indicating identical amplicon patterns. The highest discriminatory power was encountered with primer 1, which produced up to 17 bands as demonstrated in RAPD profiles of isolates 22C and 22D in Fig. 2. Application of primer 1 resolved 55 isolates into 31 genotypes, whereby isolates with identical banding profiles (S_{AB} of 1) were pooled into a single genotype. For instance, the four strains isolated from saliva, dental plaque, carious specimen and feces of patient number 4 (Fig. 2, profiles 4A–D) show an identical band pattern and are considered as one single genotype. Determination of the discriminatory index revealed 0.977 for the applied RAPD method using primer 1. Primer 2 and 5 produced fewer bands, revealing a low discriminatory power, and were therefore not used for further analysis. Continuative data analyses were performed using only band patterns of primer 1. The genetic relatedness of the isolates determined by RAPD is illustrated by the dendrogram in Fig. 2.

Fifteen of the 23 patients (65.2%) with simultaneous colonization of oral cavity and intestinal tract harbored identical strains (S_{AB} of 1) in their oral cavity and feces. These included isolates 1B, C and D, 2C and D, 3C and D, 4A–D, 6C and D, 7C and D, 9C and D, 12C and D, 13A and D, 15A, C and D, 16B and D, 17A–D, 18 A, B and D, 19C and D, 22 C and D, which clearly exhibited identical profiles (see Fig. 2). Additionally, five patients harbored strains in their oral cavity and feces exhibiting strong similarities with S_{AB}s ranging from 0.75–0.89. These strains (strains 10C and D, 11C and D, 14B and D, 20 C and D and 21 C and D) showed differences either in number (e.g. strains 20C and D) or size of bands (e.g. strains 21C and D) or both number and size (e.g. strains 10C and D) (Fig. 2). Nonidentical strains isolated from oral cavity and feces were found in three patients (13%): patient numbers 5, 8 and 23. The corresponding fecal isolates comprising strains 5B and D, 8C and D and 23A-C and D showed explicitly different banding profiles, indicating distant relationships between these strains. Cluster analysis with the Gelcompar software showed that 28 of the 55 strains could be placed into three major clusters when a similarity level of S_{AB} of 0.73 was used as a calculated threshold (see dashed line in Fig. 2). The remaining strains could not be assigned to any of the three major clusters

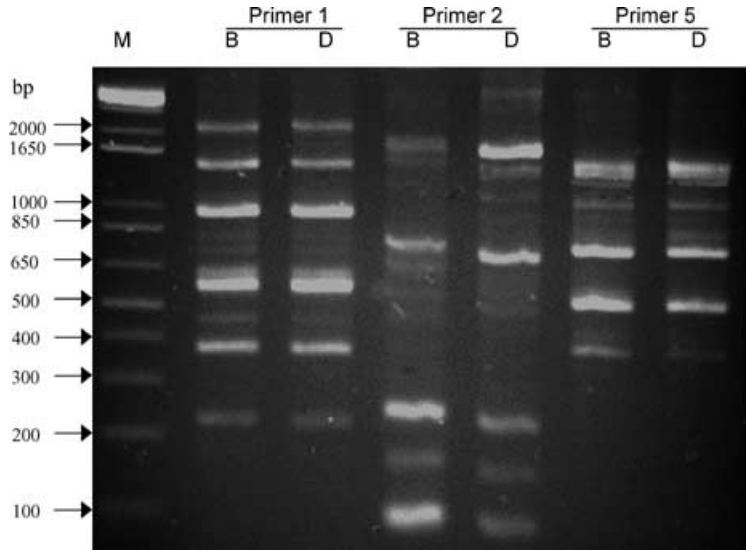

Fig. 3. Representative RAPD profiles of *C. albicans* using primers 1, 2 and 5 (see Materials and methods). Strains were isolated from dental plaque (B) and feces (D) of patient number one. Lane M, molecular weight standard: 1 kb Plus DNA Ladder (Invitrogen, Karlsruhe, Germany). Sizes (in base pairs) are indicated by arrowheads on the lefthand side.

but fell within the similarity index of S_{AB} of 0.58–0.7.

Discussion

Facultative pathogenic fungi are common residents of mucosal surfaces of the human oral cavity, the gastrointestinal and the urogenital tract. As a result of increasing numbers of immunocompromised individuals within the human population, the incidence of *Candida* infections (candidiasis) has increased dramatically in the last decade (10). Furthermore, *Candida* species are capable of biofilm formation, thus creating a protected niche for these opportunistic pathogens. Biofilms represent a safe haven against antibiotic/antimycotic treatment and can create a source of persistent infection by releasing planktonic clonal counterparts (8, 24). Among the known *Candida* species, *C. albicans* is the predominant causative agent of candidiasis (25).

In this study, we sought to obtain evidence that carious teeth constitute an ecologic niche for *C. albicans,* releasing genetically identical counterparts that colonize other mucosal surfaces within the body before finally emerging in fecal samples of infected patients. ''Randomly Amplified Polymorphic DNA'' (RAPD) analysis was used for molecular typing of *Candida* isolates since it is simple and robust, and provides a reliable discriminatory method for clinical epidemiologic studies of *Candida* infections (9, 22, 34).

Using this method we compared *C. albicans* isolates from the oral cavity and feces of 23 patients. A total of 55 isolates recovered from 23 patients could be differentiated into 31 genotypes by using primer 1, which showed the highest discriminatory power with a discriminatory index of 0.977. Our data support previous findings that the discriminatory power of RAPD analysis rests upon the primer used (11).

Of 23 patients, 65.2% had identical strains in their oral cavity and feces, indicating gastrointestinal colonization with *C. albicans* initiating from the carious lesion via saliva. Also, 21.4% of the patients had highly similar but nonidentical strains in their oral cavity and feces. These results suggest that such strains from different anatomic locations of the same individual may have evolved from a single progenitor strain that has adapted to the disparate ecologic conditions and is in the process of diverging genetically (12, 16). Interestingly, 28 of the 55 isolates could be placed into three major clusters with a similarity index higher than S_{AB} of 0.73. These 28 strains could be assigned to 12 patients who were neither related nor geographically located at a specific place but nevertheless harbored closely related *C. albicans* strains in their oral cavity and feces. This finding is consistent with earlier studies where genetic relatedness of *C. albicans* strains colonizing different body environments has been described (30). We assume that patients harboring closely related orotropic strains probably have a growth-specific advantage at this body location. Dissemination of single strains of *Candida* from one site to multiple body locations has also been described previously for *Candida lusitaniae* (19).

Screening of saliva, dental plaque, carious specimen and feces for *C. albicans* revealed that the carious specimen harbored the highest concentration of yeasts, which was in agreement with previous studies (Table 1) (32, 35). The ability of *C. albicans* to adhere to the surface of teeth, to form biofilms and to ferment carbohydrates enables this yeast to reside autonomously from resident bacteria. Our studies showed that the genetically identical counterparts apparently survived the acidic environment of the stomach, emerging viable in the feces of the patients. A recent study showed that *C. albicans* can colonize the gastric mucosa, where it is involved in gastric ulcer and chronic gastritis and that the yeast is able to survive and even divide at pH values of around 2.0 (20). Other *Candida* species require pH values above 3.0 for proliferation, indicating that adaptation to extreme acidic environments is part of the survival repertoire of *C. albicans* in the infected host (36). Indeed, a molecular basis for this phenotype has been postulated for the pH-regulated genes *PHR1* and *PHR2*. *PHR1/2* knock-out mutants exhibited pH-independent, constitutive growth and morphologic defects, indicating that the *PHR1/2* represents a pH-regulating system required for *C. albicans* to adapt to environments of diverse pH (27).

We found that only 2% of the feces of healthy control subjects harbored *C. albicans,* whereas 59% of the feces of the patients were contaminated with *C. albicans*. Statistical analysis of our results revealed a significant correlation between *C. albicans* colonization of saliva/dental plaque/carious specimen and feces. Thus, it can be assumed that if *C. albicans* is present in the lesions of carious teeth, there is a high probability that it will also be detectable in feces.

In 18 patients no *C. albicans* were found in feces despite intensive colonization of the oral cavity. Miscellaneous factors could affect transit of *C. albicans* into the gastrointestinal tract, explaining its absence in feces. The most likely assumption is that yeasts are not constantly emitted from carious lesions into saliva. The discontinuous contamination and deglutition of the saliva can also result in day-to-day variations in the detection of *C. albicans* in feces.

German children at the age of the examined group show usually a combined DMF (T)/dmf(t) value ranging between 0 and 4,

with an average of 0.2 (31). Patients in the present study had an overall poor oral health status comprising fistulae, abscesses and even osteolysis. The spectacular destruction of the teeth present in the oral cavity of these patients is indicated by a 50-fold increased DMF(T)/dmf(t) value of 10.84. Our patient group was therefore not representative of other patients with carious teeth. However, the patient group was suitable for the study design since we expected that patients with severe caries would harbor yeasts simultaneously in their oral cavity and feces more frequently than patients with mild carious.

To avoid overwhelming the body with *C. albicans* initiating from carious teeth a proper therapy is indispensable. Previous studies showed that antimycotic treatment transiently eradicates the yeasts from saliva but such treatment was unable to eliminate *C. albicans* permanently from within the carious lesions. Only thorough mechanical and surgical treatment of carious teeth resulted in a consistent elimination of fungi from the oral cavity in 90% of all cases. Mechanical dental treatment supplemented with antimycotic drug therapy eradicated *C. albicans* permanently from the oral cavity (32).

Recapitulating, we show that a large percentage of cariogenic patients harbor identical or closely related *C. albicans* strains in their oral cavity and feces. Our results indicate that carious teeth provide an ecologic niche for *C. albicans* that releases planktonic counterparts to occasionally cause infections and reinfections even of non-oral diseases and emphasize the multitudinousness of the colonization patterns of *C. albicans*. Orally located *C. albicans* strains are able to cause non-oral diseases by disseminating into various parts of the body (lung, stomach, intestine, vaginal and skin candidiasis) (3, 17). We recommend that a thorough dental treatment and oral hygiene are indispensable for the prevention of candidiasis, especially in patients at risk.

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References

1. Acs G, Shulman R, Ng MW, Chussid S. The effect of dental rehabilitation on the body weight of children with early childhood caries. Pediatr Dent 1999: **21**: 109–113.
2. Ainscough S, Kibbler CC. An evaluation of the cost-effectiveness of using CHROMagar for yeast identification in a routine microbiology laboratory. J Med Microbiol 1998: **47**: 623–628.
3. Andrutis KA, Riggle PJ, Kumamoto CA, Tzipori S. . Intestinal lesions associated with disseminated candidiasis in an experimental animal model. J Clin Microbiol 2000: **38**: 2317–2323.
4. Anson JJ, Allen KD. Evaluation of CHROMagar *Candida* medium for the isolation and direct identification of yeast species from the female genital tract. Br J Biomed Sci 1997: **54**: 237–239.
5. Baume LJ. Allgemeine Grundsätze für eine internationale Normung der Karies-Statistiken (Fédération Dentaire Internationale). J Int Dent 1962: **12**: 279–280.
6. Bühl A, Zöfel P. SPSS Version 8. Einführung in die moderne Datenanalyse unter Windows, 5th edn. Bonn: Addison-Wesley, 1999.
7. Cannon RD, Holmes AR, Mason AB, Monk BC. Oral *Candida*: clearance, colonization, or candidiasis? J Dent Res 1995 **74**: 1152–1161.
8. Chandra J, Kuhn DM, Mukherjee PK, Hoyer LL, McCormick T, Channoum MA. Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance. J Bacteriol 2001: **183**: 5385–5394.
9. 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**: 1332–1336.
10. Coleman DC, Bennett DE, Sullivan D, Gallagher PJ, Henman MC, Shanley DB. Oral *Candida* in HIV infection and AIDS: new perspectives/new approaches. Crit Rev Microbiol 1993: **19**: 61–82.
11. Dassanayake RS, Samaranayake YH, Samaranayake LP. Genomic diversity of oral *Candida krusei* isolates as revealed by DNA fingerprinting and electrophoretic karyotyping. APMIS 2000: **108**: 697–704.
12. Flaitz CM, Hicks MJ. Oral candidiasis in children with immune suppression: clinical appearance and therapeutic considerations. J Dent Child 1999: **66**: 161–166.
13. Hunter PR, Fraser CAM. Application of a numerical index of discriminatory power to a comparison of four physiochemical typing methods for *Candida albicans*. J Clin Microbiol 1989: **27**: 2156–2169.
14. Hunter PR, Gaston MA. Numerical Index of the discriminatory ability of typing systems: an application of Simpson Index of diversity. J Clin Microbiol 1988: **26**: 2465–2466.
15. Jacob LS, Flaitz CM, Nichols CM, Hicks MJ. Role of dental carious lesions in the pathogenesis of oral candidiasis in HIV infection. J Am Dent Assoc 1998: **129**: 187–194.
16. Leung WK, Dassanayake RS, Yau JY, Jin LJ, Yam WC, Samaranayake LP. Oral colonization, phenotypic, and genotypic profiles of *Candida* species in irradiated, dentate, xerostomic nasopharyngeal carcinoma survivors. J Clin Microbiol 2000: **38**: 2219–2226.
17. Loesche WJ. Association of the oral flora with important medical diseases. Curr Opin Periodontol 1997: **4**: 21–28.
18. Low W, Tan S, Schwartz S. The effect of severe caries on the quality of life in young children. Pediatr Dent 1999: **21**: 325.
19. Merz WG, Khazan U, Jabra-Rizk MA, Wu LC, Osterhout GJ, Lehmann PF. Strain delineation and epidemiology of *Candida* (*Clavispora*) *lusitaniae*. J Clin Microbiol 1992: **30**: 449–454.
20. Mühlschlegel FA, Fonzi WA. PHR2 of *Candida albicans* encodes a functional homolog of the pH-regulated gene PHR1 with an inverted pattern of pH-dependent expression. Mol Cell Biol 1997: **17**: 5960–5967.
21. Nyvad B. Microbial colonization of human tooth surfaces. APMIS 1993: **32**: 1–45.
22. 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**: 2348–2358.
23. Raitio M, Pienihakkinen K, Scheinin A. Assessment of single risk indicators in relation to caries increment in adolescents. Acta Odontol Scand 1996: **54**: 113–117.
24. Ramage G, Walle KV, Wickes BL, Lopez-Ribot JL. Biofilm formation by *Candida dubliniensis*. J Clin Microbiol 2001: **39**: 3234–3240.
25. Richardson MD, Warnock DW. Fungal infection, diagnosis and management. Oxford: Blackwell Scientific Publications, 1993.
26. Ripa LW. Nursing caries: a comprehensive review. Pediatr Dent 1988: **10**: 268–282.
27. Saporito-Irvin SM, Birse CE, Sypherd PS, Fonzi WA. PHR1, a pH-regulated gene of *Candida albicans*, is required for morphogenesis. Mol Cell Biol 1995: **15**: 601–613.
28. Scheinin A, Pienihakkinen K, Tiekso J, Holmberg S. Multifactorial modeling for root caries prediction. Community Dent Oral Epidemiol 1992: **20**: 35–37.
29. Sobel JD. Epidemiology and pathogenesis of recurrent vulvovaginal candidiasis. Am J Obstet Gynecol 1985: **152**: 924–935.
30. Soll DR, Galask R, Schmid J, Hanna C, Mac K, Morrow B. Genetic dissimilarity of commensal strains of *Candida* spp. carried in different anatomical locations of the same healthy women. J Clin Microbiol 1991: **29**: 1702–1710.
31. Sziegoleit F, Ansari C, Wleklinski C, Wetzel WE. Kariesprävalenz und Selbsteinschätzung der Mundgesundheit. Oralprophylaxe 2001: **23**: 41–47.

32. Sziegoleit F, Sziegoleit A, Wetzel WE. Effect of dental treatment and/or local application of amphotericin B to carious teeth on oral colonization by *Candida*. Med Mycol 1999: **37**: 345–350.
33. U.S. Department of Health and Human Services. Oral health in America: a report of the Surgeon general-executive summary. Bethesda, MD: National Institute of Dental and Craniofacial Research, National Institutes of Health, 2000.
34. Waltimo TMT, Cassanyake RS, Orstavik D, Haapasalo MP, Samaraanayake LP. Phenotypes and randomly amplified polymorphic DNA profiles of *Candida albicans* isolates from root canal infections in a Finnish population. Oral Microbiol Immunol 2001: **16**: 106–112.
35. Wetzel WE, Hanisch S, Sziegoleit A. Keimbesiedelung der Mundhöhle bei Kleinkindern mit Nursing-Bottle-Syndrom. Schweiz Monatsschr Zahnmed 1993: **103**: 1107–1112.
36. Zwolinska-Wcislo M, Budak A, Bogdal J, Trojanowska D, Stachura J. Fungal colonization of gastric mucosa and its clinical relevance. Med Sci Monit 2001: **7**: 982–988.