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ORAL MICROBIOLOGY
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Genotypic relatedness of yeasts in thrush and denture stomatitis

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Background/aim: *Candida* is an opportunistic pathogen. Understanding its genetic characters might increase our understanding of the pathogenesis of candidosis. We examined the genetic relationships of yeasts from the most common forms of oral candidosis: thrush and denture stomatitis.
Methods: Yeasts were sampled from palate, buccal mucosa, gingival sulci/periodontal pockets and/or denture fitting surface of 19 thrush patients and 22 denture stomatitis patients. Random amplified polymorphic DNA and the Dendron computer-assisted program were used to determine the genotypic relatedness of the yeasts.
Results: A dendrogram generated from 105 thrush isolates had similarity coefficients (S_{AB}) ranging from 0.58 to 1 with four clusters derived at S_{AB} 68%. Another dendrogram was generated from 91 isolates from denture stomatitis, with S_{AB} ranging from 0.59 to 1. Three clusters were established at S_{AB} 71%. In a composite dendrogram incorporating the thrush and denture stomatitis data and orally healthy data compiled from a previous study, five genotypic clusters were generated at S_{AB} 68%. Cluster II, the most dominant, comprised isolates from thrush, denture stomatitis and healthy conditions, while clusters III and IV contained yeasts mainly from thrush.
Conclusions: Palatal yeast carriage was significantly increased in thrush and denture stomatitis, also after radiation, chemotherapy and denture wearing. The buccal mucosa was favorable for yeast colonization regardless of oral condition. Yeasts in thrush were more diverse than in conditions of oral health. The common clone (II) of infecting yeasts and commensals suggested that commensals could induce thrush and denture stomatitis, whereas the unique clones in thrush (III, IV) might have been established through strain replacement or maintenance with minor genetic variation.

Key words: denture stomatitis; genotypic relatedness; thrush; yeasts

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Oral candidosis is an opportunistic infection caused by *Candida* species. Clinically, oral candidosis has been classified in several ways. Lehner (13) provided the most used classification in which the designations acute pseudomembranous candidosis (thrush), acute atrophic candidosis, chronic hyperplastic candidosis and chronic atrophic candidosis (denture stomatitis) are used. Axell et al. (1) divided oral candidosis into primary and secondary forms. Among primary candidosis, acute and chronic forms, *Candida*-induced lesions (including denture stomatitis) and keratinized primary lesions superinfected with *Candida* were listed. In the present study the clinical entities thrush and denture stomatitis were examined. Thrush is the most common form of oral candidosis and occurs especially after chemotherapy and radiation. It is characterized by soft, cream-colored, elevated plaques that can easily be wiped off the buccal mucosa, tongue dorsum, or palate. Denture stomatitis affects 28–65% of denture wearers (4, 8) and is a common *Candida* infection secondary to long-standing occlusion of the oral mucosa by a denture. The characteristic clinical feature is a uniform bright erythema, usually of the upper denture-bearing area, limited by the denture margin. *Candida* species are causative agents of oral candidosis. *Candida albicans* is the most commonly isolated species, but *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis* and *Candida krusei* are also recovered (9). A recently discovered species, *Candida dubliniensis*, is isolated particularly from mucosal lesions in patients infected with human immunodeficiency virus (HIV) (6). Classification and identification of *Candida* in oral

candidosis are important for diagnosis, investigations of epidemiology and pathogenesis, and treatment of the disease. Serotyping, biotyping and genotyping, including multilocus enzyme electrophoresis, restriction fragment length polymorphism, ribotyping, random amplified polymorphic DNA (RAPD), karyotyping and 18S rRNA sequencing have evolved as effective and popular typing methods for yeasts. A number of studies have used these techniques for typing of yeasts from thrush in HIV/acquired immunodeficiency syndrome (AIDS) patients and from healthy carriers (3, 17, 27), while we have found only a single report from denture stomatitis (19). In the present study we have focused on genotyping because this is claimed to be more reliable than phenotyping (20, 30) and on the genotypic diversity of yeasts in thrush and denture stomatitis because very little is known about this for our most common forms of oral candidosis.

The aims of the present study were:

- to assess the occurrence of yeasts in the oral cavity of patients with thrush and denture stomatitis,
- to identify genotypes and genotype clusters of yeasts from thrush and denture stomatitis,
- to determine the genotypic relatedness of the yeasts within and between these two most common forms of oral candidosis, and
- to study the distribution of yeasts and yeast genotype clusters of thrush and denture stomatitis in different oral sites, such as buccal mucosa, palate, gingival sulci/periodontal pockets, and in addition, the denture-fitting surface of denture stomatitis patients.

Material and methods

Participants

The thrush group comprised 19 patients (13 male, 6 female) with thrush seen at the Section for Maxillo-Facial Surgery, ENT Department, Rikshospitalet, University Hospital and at the Head & Neck Cancer Group, Radiumhospitalet, Oslo, Norway. The age of the patients varied from 9 to 84 years (mean 57.7 ± 20.3). All the patients received head/neck radiation and chemotherapy because of neck or head cancers.

The denture stomatitis group comprised 22 patients (9 male, 13 female) with denture stomatitis who were examined at the Faculty of Dentistry, University of Oslo and at two homes for the elderly in Hvittingfoss, Norway. The age of the patients varied from 50 to 94 years (mean 70.4 ± 10.8). All the patients wore a complete or three-quarter partial removable denture. The exclusion criteria were use of corticosteroids, immunosuppressive agents, cytotoxic chemotherapy or irradiation therapy in the previous 3 months, pregnancy, endocrine disorders, malignancies, immunodeficiencies, or malnutrition.

The orally healthy subjects were 19 oral yeast-positive individuals chosen from 45 orally healthy subjects (19 male, 26 female) in a previous study (33); they were included for comparison. The age of the healthy subjects varied from 16 to 74 years (mean 32.9 ± 14.1).

Yeast isolation, cultivation and identification

Yeasts were sampled from the palate, buccal mucosa and gingival sulci/periodontal pockets of each participant, and also from the fitting surface of the dentures of the denture stomatitis patients. Palate, buccal mucosa and denture were sampled by streaking these locales with sterile cotton-tipped swabs (Selefatrade, Spanga, Sweden). When teeth were present, subgingival plaque samples were collected by inserting two sterile paper points (Roeko, Munich, Germany) into two to four gingival sulci/periodontal pockets for 15 s after removing the supragingival plaque. All samples were streaked directly onto Sabouraud dextrose agar plates at the chairside. The plates were incubated aerobically at 37°C for 3 days. Colonies exhibiting distinct morphologies were picked, subcultured, transferred to Todd Hewitt broth with dimethyl sulfoxide, and stored at −80°C for further use. All colonies exhibited some of the following criteria: circular or filamentous form, entire or curled margins, smooth or rough surface, flat or raised elevation, and white or brown color. Sample collection, cultivation and isolation were standardized.

After subculture the isolates were identified with the commonly used commercial kit ID 32C (bioMérieux, Marcy-l'Etoile, France). The preparation and incubation of the kits were carried out according to the manufacturer's recommendations. Reading of the kits was automatic in an ATB reader (API, bioMérieux). The results of the reactions, transferred into a numerical code, were treated in a database for identification (API Plus, bioMérieux).

Yeast DNA preparation

Yeasts were seeded from frozen stocks onto Sabouraud dextrose agar plates and recultured aerobically at 30°C for 24 h. After plating, yeast cells were incubated in 1 ml SPPZ buffer (1 M sorbitol, 50 mM potassium phosphate, 1 mg Zymolase 20T) and 3 µl 0.1% β-mercaptoethanol (Sigma, St Louis, MO) at 37°C for 90 min. The pellets were then incubated in 500 µl GES (60% guanidine thiocyanate, 0.1 M EDTA pH 8, 0.5% lauroylsarcosine) for 30 min at room temperature, thereafter in 100 µl of 5 M potassium acetate (Sigma) for 30 min on ice, and then washed with 500 µl chloroform:pentanol (24:1, v/v) (Sigma). After centrifugation, the upper aqueous phase was transferred to a new Eppendorf tube and an equal volume of cold ethanol was added. After centrifugation and drying, the DNA was treated with RNase (Sigma) in 100 µl TE buffer (10 mM Tris–HCl, 1 mM EDTA pH 8) at 37°C for 30 min, and washed with 200 µl isopropanol. DNA was precipitated by centrifugation, dried, and dissolved in 100 µl TE buffer. DNA purity was determined with a spectrophotometer (MBA 2000, Perkin-Elmer, Norwalk, CT).

RAPD

The polymerase chain reaction (PCR) mix (25 µl) contained approximately 50 ng yeast DNA as template, 1× PCR buffer (20 mM Tris–HCl pH 8.4, 50 mM KCl), 200 µm stock dNTPs, 2.5 mM $MgCl_2$, 0.1 µM primer and 1.5 U *Taq* polymerase (Applied Biosystems, Foster City, CA). The primer GC10/1 (7) (5′-CGGTGC-GACG-3′) (Invitrogen, Carlsbad, CA) was selected from two primers GC10/1 and M13 (5′-GAGGGTGGCGGTTCT-3′) (Invitrogen) (35). Amplification was performed in a GeneAmp PCR system 2700 (Applied Biosystem) with initial denaturation at 94°C for 5 min, followed by 45 cycles of denaturation at 94°C for 1 min, annealing at 30°C for 1 min, extension at 72°C for 2 min, and final extension at 72°C for 10 min.

The PCR products were electrophoresed on 1.5% agarose gels at 150 V for 70 min. The gels were stained with ethidium bromide and the bands were visualized under UV light. The images of the DNA fingerprints were captured with a Kodak DC 120 digital camera (Eastman Kodak Company, New Haven, CT) and transferred to a computer.

Dendron computer-assisted gel analysis

DNA fingerprints of the yeast isolates were analyzed by the Dendron computer-assisted program version 2.4 (Solltech Inc.,

Oakdale, IA) (27, 28, 30). A similarity coefficient (S_{AB}) was applied to determine the genotypic relatedness among strains by comparing band positions and intensities with the unweighted pair-group method (29) with arithmetic average (UPGMA). The dendrograms were generated based on the S_{AB} value computed between all pairs of isolates (23, 28).

Nine type species strains of yeasts, *C. albicans* Centraalbureau Voor Schimmelcultures (CBS) 562, *C. dubliniensis* CBS 7987, *C. parapsilosis* CBS 604, *C. tropicalis* CBS 94, *C. glabrata* CBS 138, *C. krusei* CBS 573, *Candida norvegensis* CBS 1922, *Candida inconspicua* CBS 180, and *Saccharomyces cerevisiae* CBS 1171 were used as references. The DNA fingerprints of the yeasts of the orally healthy group (32), retrieved through exactly the same carefully standardized methods as used here and by the same investigator, were included for comparison.

Statistical analysis

The chi-squared test was applied to analyze the relationship among yeasts from different oral sites, i.e. the buccal mucosa, palate and gingival sulci/periodontal pockets of thrush, denture stomatitis and oral health subjects. A significance level of $P < 0.05$ was chosen.

Results

Yeast occurrence and yeast species

All 19 thrush patients (100%) were yeast positive, while 20 of 22 subjects (90.9%) with denture stomatitis were yeast carriers. The corresponding figure in subjects with oral health was 42.1% (33). The average number of isolates, selected from colony morphology, was 5.53 per thrush patient, 4.55 per denture stomatitis patient, and 3.16 per healthy subject (33). The frequency of yeasts from the buccal mucosa, palate, and gingival sulci/periodontal pockets of the thrush and denture stomatitis patients as well as from the denture-fitting surface of the denture stomatitis patients is shown in Fig. 1.

The yeast species isolated from the thrush, denture stomatitis and orally healthy subjects are listed in Table 1. *C. albicans* was by far the most prevalent species in both disease and health. Six other *Candida* species were detected in disease, together with one species of *Saccharomyces*, while one additional *Candida* species was recovered from healthy subjects.

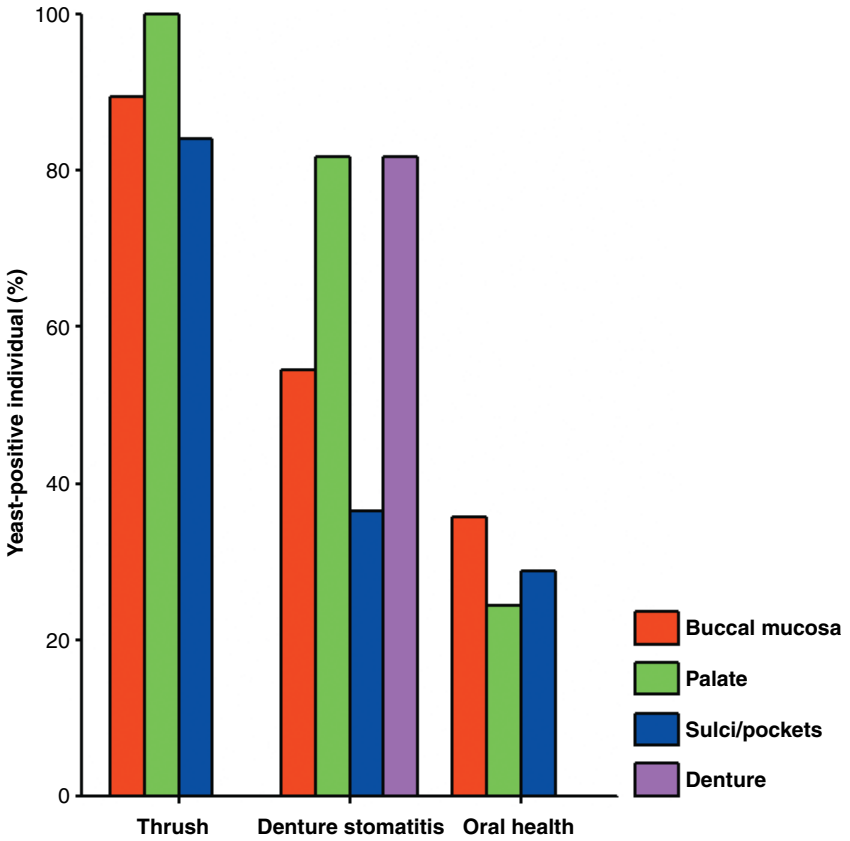

Fig. 1. Occurrence (%) of yeasts from oral sites in patients with thrush or denture stomatitis and in orally healthy subjects.

Genotypes, genotypic clusters and genotypic relatedness of yeasts from thrush and denture stomatitis

RAPD fingerprints were obtained from the 19 thrush patients and the 20 yeast-positive denture stomatitis patients using the primer GC10/1. The fingerprints of each strain contained one to six intense bands ranging from 154 to 1033 kilobases (Fig. 2).

The dendrogram from the 19 thrush patients (Fig. 3) was generated from RAPD fingerprints of 105 yeast isolates. The S_{AB} ranged from 0.58 to 1 with an average of 0.68 ± 0.09. Eighty-three genotypes and four major genotypic clusters (i, ii, iii and iv) were derived at a threshold S_{AB} of 68%. The four genotypic clusters comprised 99 (94%) yeast isolates and 77 (93%) genotypes. Cluster ii was the most dominant, accounting for 50 (48%) isolates from 17 (89%) patients.

The dendrogram of the yeasts from the 20 yeast-positive patients with denture stomatitis (Fig. 4) was generated from the RAPD fingerprints of 91 yeast isolates. The S_{AB} ranged from 0.59 to 1 with an average of 0.71 ± 0.07. Eighty genotypes and three major genotypic clusters (j, jj and jjj) were derived at a threshold S_{AB} of 71%. The three genotypic clusters comprised 78 (86%) yeast isolates and 67 (84%) genotypes. Cluster jj was most dominant and included 66 (73%) isolates from 20 patients.

A composite dendrogram (Fig. 5) was established by pooling the fingerprints of the 256 yeast isolates from the thrush, denture stomatitis, healthy group and nine type species strains of yeasts. The S_{AB} ranged from 0.57 to 1 with an average of 0.68 ± 0.08. Two hundred and twenty-six genotypes and five major genotypic clusters (I, II, III, IV and V) were generated at a threshold S_{AB} of 68%, which comprised 251 (98%) isolates from 58 individuals and 212 (94%) genotypes. Cluster II was most dominant, containing 197 (74%) yeast isolates from 57 individuals with thrush, denture stomatitis or oral health. Table 2 shows the occurrence of yeast genotypic clusters in the thrush, denture stomatitis and orally healthy subjects. Clusters III and IV appeared only in disease, i.e. thrush and denture stomatitis, while cluster V was seen exclusively in oral health. For cluster III, the frequency of

Table 1. Occurrence of yeast species in thrush, denture stomatitis and oral health[1] by ID 32C

Species	% of all individuals			% of yeast-positive individuals			% of total isolates		
	Thrush ($n = 19$)[2]	Denture stomatitis ($n = 22$)	Oral health ($n = 45$)	Thrush ($n = 19$)[3]	Denture stomatitis ($n = 20$)	Oral health ($n = 19$)	Thrush ($n = 105$)[4]	Denture stomatitis ($n = 91$)	Oral health ($n = 60$)
C. albicans	89.5	90.9	35.6	89.5	100	84.2	17	20	16
C. tropicalis	21.1	13.6	0	21.1	15	0	4	3	0
C. glabrata	10.5	9.1	0	10.5	10	0	2	2	0
C. dubliniensis	10.5	4.5	6.7	10.5	5	15.8	2	1	3
C. utilis	5.3	0	0	5.3	0	0	1	0	0
C. guilliermondii	5.3	0	0	5.3	0	0	1	0	0
C. parapsilosis	0	4.5	0	0	5	0	0	1	0
C. inconspicua	0	4.5	0	0	5	0	0	1	0
S. cerevisiae	0	4.5	0	0	5	0	0	1	0

[1]From Song et al. (33).
[2]n = number of all individuals.
[3]n = number of yeast-positive individuals.
[4]n = number of total yeast isolates.

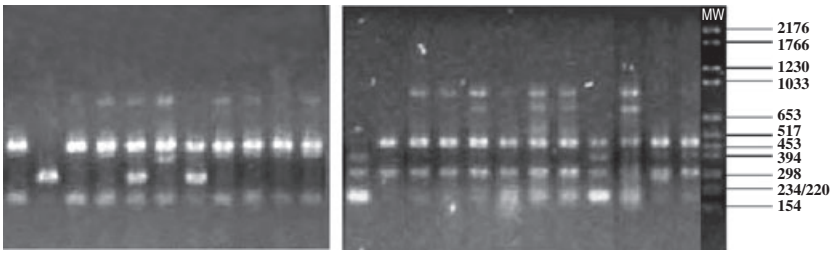

Fig. 2. Typical RAPD fingerprints of thrush and denture stomatitis isolates. MW = molecular weight marker VI.

yeasts in thrush was higher than in denture stomatitis ($P = 0.02$). The type strains of *C. albicans* and *S. cerevisiae* fell in cluster II, those of *C. parapsilosis*, *C. tropicalis*, *C. krusei* and *C. dubliniensis* appeared in cluster III. *C. glabrata*, *C. norvegensis* and *C. inconspicua* did not fall within any oral yeast cluster.

Distribution of yeasts and yeast genotype clusters of thrush and denture stomatitis in oral sites

The occurrence of yeasts isolated from the various oral sites in thrush, denture stomatitis and oral health is shown in Fig. 1. Yeasts recovered from the buccal mucosa were frequently found in individuals of all the groups. Regarding yeast-positive subjects, yeasts from the palate were more frequently found in the thrush and denture stomatitis groups than in the orally healthy group ($P = 0.000$ and 0.002, respectively). In the denture stomatitis group the frequency of yeasts was lower than in the thrush group for yeasts isolated from gingival sulci/periodontal pockets ($P = 0.005$).

No clear association was found between genotypic clusters and oral sites in disease or health. In cluster II, the frequency of individuals with yeasts isolated from the palate was higher for the thrush and the denture stomatitis groups than for the orally healthy group ($P = 0.007$ and 0.026, respectively). In clusters I, III, IV and V the frequencies of the yeast-positive individuals were too low to be analyzed statistically. The frequency of individuals with yeasts isolated from the gingival sulci/periodontal pockets was lower in the denture stomatitis group than in the thrush and orally healthy groups ($P = 0.001$ and 0.024, respectively).

Discussion

RAPD has evolved as the most popular method of DNA fingerprinting for infectious fungi and is a powerful and reliable method for identification of *Candida* species (12, 23, 34). Pujol et al. (23) demonstrated a strong congruence in clustering between RAPD, multilocus enzyme electrophoresis and DNA fingerprinting with probe Ca3 by using the average S_{AB}. This coefficient was therefore applied for analysis of genotypic relatedness among the yeast strains of our study.

In the present study, the primer GC10/1 (7) was chosen because in pilot experiments it produced more discriminative fingerprints than the alternative (M13) (35) (data not shown). We have previously used this primer for RAPD analysis of *Candida* species because it yielded distinct and reproducible profiles when compared to CX5 (5′-ACA-CTGCTTC-3′) and PST (5′-CAG-TTCTGCAG-3′) (15). The fingerprint patterns obtained with GC10/1 generated one to six intense bands that conformed to the results of Pujol et al. (23). The carefully standardized procedures of the RAPD analyses, which were reproducible, were performed by the same examiners in the same laboratory to minimize methodological errors.

Yeasts were isolated from 100% of the thrush patients, 90.9% of the denture stomatitis patients and 42.1% of the oral health subjects (3). Radiation, chemotherapy and denture wearing significantly increased oral yeast carriage on the palate, particularly of cluster II strains. Leung et al. (14) also found that yeast carriage in irradiated individuals was significantly higher than in controls. *C. albicans* was the most predominant species identified both in disease and health. The *Candida* species are regarded as causative organisms in denture stomatitis (4) and our study discovered that 20 of the 22 denture stomatitis patients had oral yeasts. Also, previous studies (2, 4, 8) reported a very low frequency of yeast-free denture stomatitis.

Two species were recovered from healthy oral cavities. Although identification based entirely on physiological evidence cannot always be assumed to be reproducible between laboratories (20, 30) it seems reasonable that phenotypic classification provides fewer yeast species in healthy oral cavities than in those with candidosis. In a

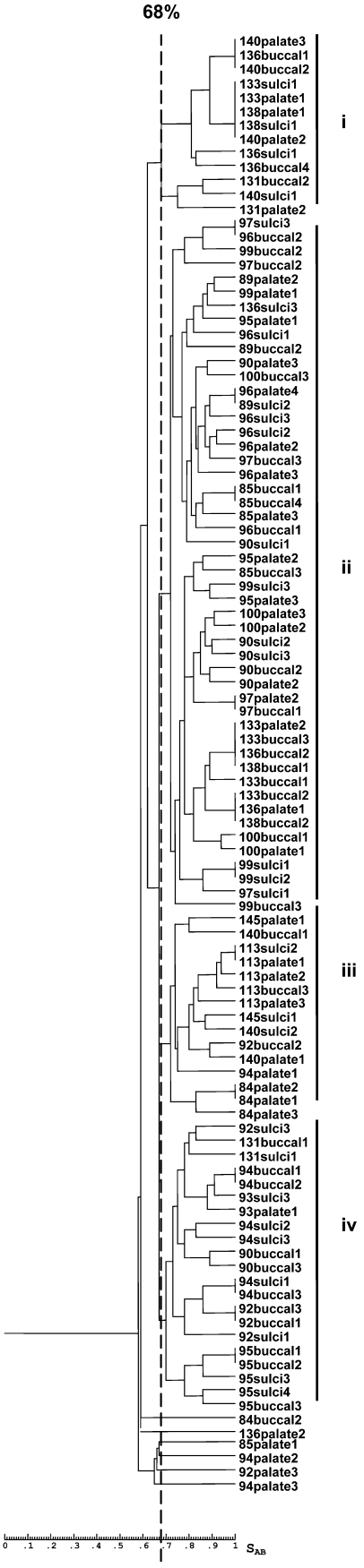

Fig. 3. Dendrogram of yeast fingerprints of thrush isolates. The isolates are designated as follows: patient number, oral site, and numbers of different colonies. i, ii and iii represent major genetic clusters. Sulci = gingival sulci/periodontal pockets.

recent study by Massonet et al. (18) ID32C compared favorably with ITS2-fragment length polymorphism analysis for identification of various yeast species.

C. glabrata was recovered only from diseased mouths. Redding et al. (24) reported three cases of *C. glabrata* oropharyngeal candidosis and it was regarded as an emerging pathogen in patients with head and neck cancer and HIV (24, 25).

In the dendrograms of yeasts from the thrush and denture stomatitis patients, multiple genotypes and genotypic clusters, high S_{AB} ranges and low average S_{AB} indicated that genetically heterogeneous yeasts are present in these commonest forms of oral candidosis. The yeasts from thrush were genetically more diverse than those of the orally healthy group, while the yeasts isolated from denture stomatitis patients were similar in genetic diversity to those of healthy subjects. Lupetti et al. (17) and Song et al. (32) found greater genetic diversity of oral yeasts in disease than in health, while Hellstein et al. (10) and Xu et al. (37) reported no significant difference in the genetic relatedness of yeast strains from HIV-positive patients, pregnant subjects and healthy subjects. Other studies (26, 27, 38) revealed less genotypic and genetic diversity among *C. albicans* isolates from clinical specimens, regardless of HIV/AIDS status, than in isolates derived from a non-clinical yeast population. The reasons for these discrepancies are not clear. Xu et al. (37) attributed different results of genetic diversity of yeasts to geographic heterogeneity, different methods of analysis and different host histories.

In Table 2 the high frequency of cluster II in different states suggested a majority of yeasts colonizing oral cavities from the same genetic group regardless of oral condition. This cluster, representing commensals in healthy oral cavities, possibly become pathogenic in thrush and denture stomatitis through phenotypic switching (17, 31). However, this has to be investigated further. These organisms shared a moderate genetic relatedness with the type strains *C. albicans* CBS 562 and *S. cerevisiae* CBS 1171. Correspondingly, Mathaba et al. (19) found that *C. albicans* strains from dental plates did not form a distinct genetic group. A number of studies (10, 17, 36, 37) reported common clones of *Candida* isolated from both candidosis patients and healthy subjects. It has been postulated that commensals in the oral cavity induce oral candidosis in compromised hosts (16, 17, 36).

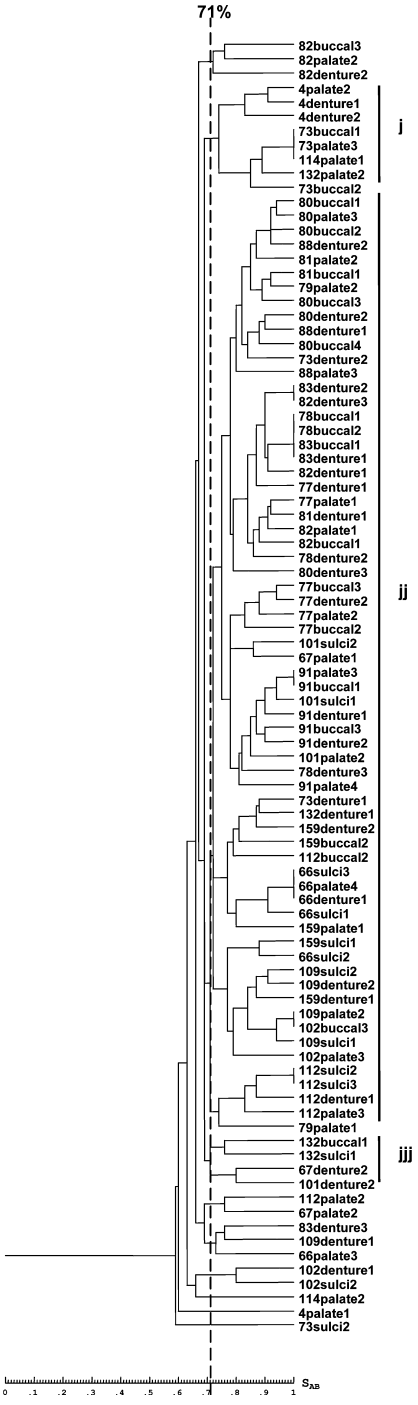

Fig. 4. Dendrogram of yeast fingerprints of denture stomatitis isolates. The isolates are designated as follows: patient number, oral site, and numbers of different colonies. j, jj and jjj represent major genetic clusters. Sulci = gingival sulci/periodontal pockets.

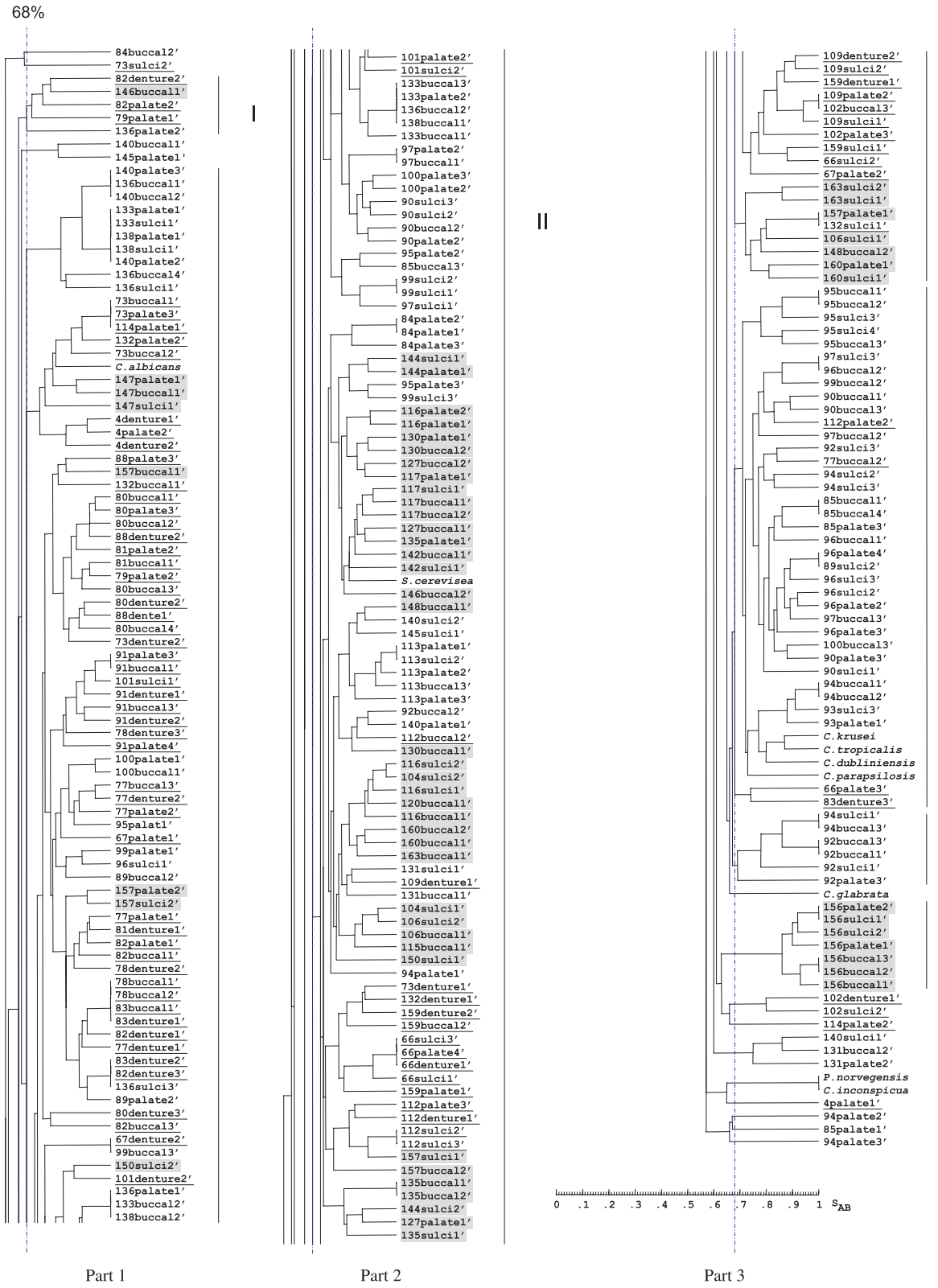

Fig. 5. Dendrogram of composite yeast fingerprints of isolates from thrush, denture stomatitis, oral health and yeast type species strains. The isolates are designated as follows: patient number, oral site, and numbers of different colonies. Underlined isolates are from denture stomatitis and shaded isolates from orally healthy subjects. I, II, III, IV and V represent major genetic clusters. Sulci = gingival sulci/periodontal pockets.

Clusters III and IV were found especially in thrush patients, suggesting that these clusters were unique for this condition. Previous studies show that certain clones are specific for infectious conditions (17, 22, 32). The emergence of unique genetic clones in thrush might be explained by the scenarios strain replacement or strain maintenance with minor genetic variation (16).

Table 2. Occurrence of yeast genotypic clusters in thrush, denture stomatitis and oral health[1]

Genotypic cluster	% of all individuals			% of yeast-positive individuals			% of total isolates		
	Thrush ($n = 19$)[2]	Denture stomatitis ($n = 22$)	Oral health ($n = 45$)	Thrush ($n = 19$)[3]	Denture stomatitis ($n = 20$)	Oral health ($n = 19$)	Thrush ($n = 105$)[4]	Denture stomatitis ($n = 91$)	Oral health ($n = 60$)
I	5.3	9.1	2.2	5.3	10	5.3	0.9	3.3	1.7
II	78.9	90.9	40	78.9	100	94.7	54.3	86.8	86.7
III	57.9	18.2	0	57.9	20	0	30.5	4.4	0
IV	10.5	0	0	10.5	0	0	5.7	0	0
V	0	0	2.2	0	0	5.3	0	0	11.7

[1]From Song et al. (33).
[2]n = number of all individuals.
[3]n = number of yeast-positive individuals.
[4]n = number of total yeast isolates.

Close similarity between groups of *Candida* strains has been reported in patients and it has been postulated that originally commensal strains are replaced by infection-inducing isolates (3, 5, 16, 27). There is also a possibility that *Candida* undergoes genetic transition during the progression of candidosis (16). However, the relatively low frequency of clusters III and IV indicated that such replacement or genetic transition occurs at a low frequency.

Although cluster V was recovered only from orally healthy subjects, it is thought to be an individual cluster rather than a common healthy cluster because all of its seven strains were from one subject.

The distribution of the isolates showed that the buccal mucosa was a favorable site for yeast colonization regardless of oral condition. Radiation, chemotherapy and denture wearing significantly increased yeast carriage on the palate, particularly of cluster II strains. For gingival sulci/periodontal pockets the yeast carriage in denture stomatitis was lower than in thrush. This may be related to the extensive loss of teeth in denture stomatitis. In general, yeasts with similar genotypes were recovered from different oral sites. Pizzo et al. (22) demonstrated genetic identity within most oral and subgingival *C. albicans* isolates collected from the same individual. Soll et al. (31) and Xu et al. (37) uncovered genetically similar *Candida* strains from different anatomic locations regardless of host condition. However, Pizzo et al. (22) and Lamster et al. (11) revealed subgingivally specific strains in HIV^+ individuals, which were possibly the result of a reduced Th1 lymphocyte response in the subgingival environment.

Our findings are based on yeasts with distinct colony morphology as described in the Yeast isolation, cultivation and identification section. Morphotyping has previously been used to differentiate between strains of *C. albicans* on the basis of the nature and extent of marginal fringing and the surface topography of the streak colony (21). It will be interesting to study further the genotypic features of yeasts with similar colonial morphology isolated from different oral conditions.

In conclusion, genetically heterogeneous yeasts were isolated from thrush and denture stomatitis. The yeasts of these commonest forms of oral candidosis were genetically more diverse than yeasts from orally healthy subjects. The common clone (II) comprising a majority of infecting yeasts and commensals suggested that commensals might become agents of subsequent oral candidosis. On the other hand, the unique genetic clones for thrush (III, IV) might have been established through strain replacement or strain maintenance with minor genetic variation. The buccal mucosa was a favorable site for yeast colonization regardless of oral condition, while radiation, chemotherapy and denture wearing significantly increased yeast carriage on the palate.

Acknowledgments

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