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Diversity and frequency of yeasts from the dorsum of the tongue and necrotic root canals associated with primary apical periodontitis

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Abstract

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Aims To determine the frequency and diversity of yeasts from the dorsum of the tongue and necrotic root canals with teeth associated with primary apical periodontitis.

Methodology Detailed medical and dental histories of 168 patients were recorded. The samples were collected from the dorsum of tongue and from 168 teeth with root canals contained necrotic pulps. Yeasts were isolated on Sabouraud agar with 100 mg L^{-1} chloramphenicol, purified and characterized by standard methods. Identification was confirmed by EI1 PCR fingerprint technique. Yeast isolates of uncertain identity or with a different genetic fingerprint profile from the reference strains were identified by sequencing the D1/D2 variable domains of the large subunit rDNA.

Results Yeasts were isolated from 22.6% of teeth sampled and from 45.8% of tongue samples. *Candida albicans* was the most frequently isolated species at both investigated sites but other species were also found. *Saccharomyces cerevisiae* and *Kluyveromyces lactis* were recovered from the tongue.

Conclusions Although the detection of yeasts in the root canal does not imply an involvement in the disease process, the study suggests a frequency of *Candida* spp. in primary endodontic infections that deserves further clarification.

Keywords: lingual dorsum, periapical diseases, root canal, yeasts.

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Introduction

Yeast populations in the mouth are represented mainly by *Candida* species, with *Candida albicans* being the most prevalent species found in both healthy and immunocompromised individuals (Odds 1988). The frequency of oral *C. albicans* varies from 47% to 75%, while *C. krusei*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata* and *C. guilliermondii* represent less than 10% of the yeast species isolated from the mouth (Odds 1988, Cannon *et al.* 1995). Other yeast species, such as *Saccharomyces* spp. and *Geotrichum* spp., have also been found to be members of the indigenous oral microbiota (Odds 1988). The dorsum of the tongue is the primary habitat of the yeasts in the mouth; however, these microorganisms can be found at other oral sites such as the jugal membrane, palate mucosa, tooth surfaces and saliva (Borromeo *et al.* 1992). Yeasts can be detected in 7–18% of infected root canals, being commonly associated with persistent cases of apical periodontitis (Waltimo *et al.* 2004). *Candida albicans* is the most common yeast isolated from root canals in both primary (Egan *et al.* 2002) and secondary endodontic infections (Waltimo *et al.* 1997, Sundqvist *et al.* 1998, Peciuliene *et al.* 2001, Waltimo *et al.* 2003). Although studies have specifically sought to investigate the frequency of yeasts in root canal infections, the role

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of *Candida* species present in the root canal at the onset of periapical disease is unclear. Generally, *C. albicans* is isolated in association with facultative Gram-positive bacteria such as *Streptococcus gordonii*, *S. mutans*, *S. sanguis*, *S. mitis*, *S. pyogenes*, *Enterococcus faecalis*, *Actinomyces* sp. and Gram-negative bacteria such as *Fusobacterium nucleatum* (Waltimo *et al.* 2003). This fact may be a result of the isolation method used in the above studies. The majority of microbiological assays in endodontic microbiology studies use techniques that favour the isolation of bacteria, and the yeasts recovered in these surveys are seldom the result of contamination. When the main purpose of the study is to isolate yeasts from the mouth and teeth; in general, a much higher frequency of fungi is observed than in surveys looking exclusively for bacteria (Lana *et al.* 2001). In agreement with this statement, Egan *et al.* (2002) reported that the prevalence of yeasts in untreated root canals was 5.7% in contrast with other studies that did not use a selective agar for yeast cultivation, suggesting that the cultivation techniques may influence the recovery of yeasts from the endodontic niche. In this study, using standard techniques for yeast isolation, the frequency and diversity of these microorganisms on the dorsum of the tongue and in necrotic root canals associated with primary apical periodontitis were determined in dental patients. Special care was taken to minimize the possibility of cross contamination from saliva, other tooth or the patient tongue.

Materials and methods

Clinical material

Patients seeking treatment at the Endodontic Department of the Universidade Federal de Minas Gerais (UFMG) and Dental School of the Pontificia Universidade Católica (PUC-MG), Belo Horizonte, Brazil, were selected. A detailed medical and dental history was obtained from each patient. Patients who had received antibiotic and antifungal treatment during the previous 6 months, or who had a systemic disease, were excluded. The Ethical Committee in Research of the UFMG approved the protocol describing the specimen collection for this investigation, and all patients were informed of the method, objectives and risks of the procedures. Patients who met the inclusion criteria and agreed to take part in the experiment were asked to sign the written informed consent form.

Sampling procedure and yeast isolation

A total of 168 patients without pain, comprising 94 women and 74 men, from 20 to 65 years of age, were selected. One hundred and eighty-four teeth with intact pulp chambers and no cracks and therefore with no direct oral pulpal communication but presenting with pulpal necrosis and radiographic evidence of apical periodontitis were selected from the group and microbiological samples collected. Normally, a single tooth was sampled from each patient; however, in 16 patients, two teeth were sampled. For the statistic analysis of yeast frequency 16 teeth were randomly excluded, therefore, each patient contributed only one tooth. Pulpal necrosis was confirmed through heat, cold and electric pulp sensibility tests. All the teeth were subject to the initial standard endodontic procedure where a rubber dam was used for the complete isolation of the target tooth from the oral cavity, and the root canal was accessed using a sterilized dental drill without irrigation. Decontamination of the crown and of the surgical field was accomplished by the sequential application of 30% hydrogen peroxide, 5% iodine dye and 5% sodium thiosulfate solutions; all solutions were left in place for 1 min. The aseptic status of the crown was confirmed by rubbing sterilized swabs along the crown, and then using the swabs to streak modified Sabouraud agar (2% glucose, 1% peptone, 0.5% yeast extract, 2% agar and 100 mg L^{-1} chloramphenicol). After intracoronal access, the canals were irrigated with sterile saline to remove debris. Samples were collected from root canals flooded with sterile absorbent paper cones that remained inside the root canal for 1 min (Egan *et al.* 2002). The samples were collected aseptically in triplicate. In each case, a single root canal was sampled in order to restrict the microbial evaluation to a single ecological environment. In multirooted teeth with periapical lesions, only the widest root canal was selected. The paper points were transferred immediately aseptically to tubes containing 4 mL of modified Sabouraud broth with 100 mg L^{-1} chloramphenicol. After sampling, the teeth were restored temporarily using a standard cement and a base of zinc oxide. The tubes were immediately transported to the laboratory, and left at room temperature for 48 h. After incubation, aliquots of 0.1 mL were transferred to modified Sabouraud agar with 100 mg L^{-1} chloramphenicol, and the plates stand for 5 days at room temperature. Five colonies from each different morphotype were purified and stored in a freezer at −86 °C for later identification (Kurtzmann & Fell 1998). Sterile

swabs were used to collect samples from the dorsum of the tongue from each patient during the treatment. The swab was inserted inside the mouth, and the collection tip was rubbed along the tongue. The material was streaked directly on modified Sabouraud agar with 100 mg L^{-1} chloramphenicol and incubated for 5 days at room temperature. Furthermore, five colonies of each different yeast morphotype were purified and described as above.

Yeast identification

Yeasts were characterized by the standard methods of Yarrow (1998). The taxonomic keys of Kurtzmann & Fell (1998) were used for species identification. The PCR fingerprint technique was used to confirm identification of all the yeasts. DNA templates were prepared as described by Barros Lopes *et al.* (1998). The primer EI1 (5′CTGGCTTGGTGTATGT3′) targets intron-splicing sites in hypermutable regions of the yeast genome (Barros Lopes *et al.* 1998). PCR assays were performed as described by Barros Lopes *et al.* (1998), with minor modifications. To prepare DNA templates, pure yeast cultures were grown 48 h in YPD agar plates (1% yeast extract, 2% peptone, 2% glucose and 2% agar). An aliquot was re-suspended in a solution of 0.1% Triton X-100 (Sigma-Aldrich, São Paulo, SP, Brazil) in DNase-free water and the suspension was boiled for 10 min. A cell suspension of 2 μL (5×10^4 cells) was used for each PCR amplification with the primer EI1. PCR were performed in 25 μL with 25 pmol of primer EI1, 2 μL of DNA template, 0.2 mmol L^{-1} of each deoxynucleoside triphosphate, 1.5 mmol L^{-1} $MgCl_2$, and 1.5 U of Taq polymerase (Invitrogen, São Paulo, SP, Brazil). The reactions were performed in 33 cycles as follows: denaturation at 92 °C for 1 min, annealing at 45 °C for 2 min and extension at 72 °C for 1.5 min. An initial denaturation at 92 °C for 2 min and a 5-min final extension at 74 °C were used. Amplified PCR products were separated in 2% agarose gel in an electrophoresis chamber and visualized under UV light after stained with ethidium bromide solution. Authentic or type strains of the following yeast species were used as a standard for fingerprinting profile comparisons: *Candida albicans* ATCC 18804, *C. parapsilosis* ATCC 22019, *C. krusei* ATCC 2159, *C. tropicalis* UFMG-A10, *C. glabrata* NCYC 388, *C. lusitaniae* CBS 6936 and *C. dubliniensis* CBS 7987. Yeast isolates of uncertain identity or with a different genetic fingerprint profile were identified by sequencing the D1/D2 variable domains of the large subunit rDNA. D1/D2 divergent domains were amplified by PCR as described by Lachance *et al.* (1999) using the primers NL-1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL-4 (5′-GGTCCGTGTTTCAAGACGG-3′). The amplified DNA was concentrated and cleaned on WizardSV columns (Promega Corporation, Madison, WI, USA), and then sequenced in triplicate using a MegaBACE 1000 automated sequencing system (Amersham Biosciences Inc., Piscataway, NJ, USA). The sequences were edited with the program DNAMAN, version 4.1 (Lynnon Bio-Soft, Vaudreuil, QC, Canada). Existing sequences for other yeasts were retrieved from the GenBank and yeast isolates with D1/D2 sequences with more than 99% of homology were considered as been the same species (Kurtzmann & Robnett 1998).

Statistical analysis

Results were statistically analysed by means of McNemar test. Significance levels were always established at 5% ($P < 0.05$).

Results

A total of 115 yeast colonies were obtained; 77 originated from the tongue and 38 from root canals (Table 1). Yeasts were isolated from 22.6% of sampled teeth. The yeasts were identified as *C. albicans, C. parapsilosis, C. tropicalis*, *Candida* sp., *Kluyveromyces lactis* and *Saccharomyces cerevisiae*. The most frequently identified species at both sites was *C. albicans*, representing 90.9% of the total yeast isolates from the tongue and 89.5% from the necrotic root canal.

Table 1 Frequency of yeasts isolated from two oral sites (dorsum of tongue and root canals) in 168 healthy patients with apical periodontitis

Yeast species	Oral site		Frequency of species[c]
	Dorsum of tongue[a]	Root canals[b]	
Isolation frequency (% [number of isolates])			
Candida albicans	90.9 (70)	89.5 (34)	90.4 (104)
C. parapsilosis	5.2 (4)	7.9 (3)	6.1 (7)
C. tropicalis	1.3 (1)	0 (0)	0.9 (1)
Candida sp.	0 (0)	2.6 (1)	0.9 (1)
Kluyveromyces lactis	1.3 (1)	0 (0)	0.9 (1)
Saccharomyces cerevisiae	1.3 (1)	0 (0)	0.9 (1)

[a]Frequency of the 77 yeast isolates on the tongue.
[b]Frequency of the 38 yeast isolates in root canals.
[c]Frequency of the 115 yeast isolates in two different oral sites.

Candida parapsilosis represented 5.2% of the yeast isolates from the tongue and 7.9% from the root canals. One yeast isolate from the tongue was identified as *C. tropicalis* and one isolate from the root canal was identified as *Candida* sp. From the tongue of one patient, *S. cerevisiae* was isolated and from other *K. lactis* was obtained (Table 1). Ninety patients did not have yeast recovered from tongue and from those patients only one had yeast isolated from the root canal, the *Candida* sp. isolate referred to previously. Considering patients in whom yeasts were recovered from the tongue, 37 (48.1%) had yeasts isolated from the root canals.

There was a significant difference in the yeast frequency between the investigated sites ($P < 0.05$). Unfortunately, the molecular technique was unable to correlate the presence of a particular yeast strain in the root canal with its concomitant presence on the tongue.

Discussion

Several studies on primary root canal infections have focused on strict anaerobic bacteria due to their predominance in samples taken from untreated teeth with necrotic pulps (Sundqvist 1976, 1992, Le Goff *et al.* 1997, Dahlén *et al.* 2000). However, the present study suggests that yeast species from the genus *Candida* may also be involved in these infections. The frequency of yeasts in this study was 22.6%. Most studies using non-selective agar (Tronstad *et al.* 1987, Sundqvist *et al.* 1998, Molander *et al.* 1998) have reported a lower frequency of yeasts in root canal compared to the present study. Using the same conditions for *Candida* cultivation, Jackson & Halder (1963) and Najzar-Fleger *et al.* (1992) reported a higher frequency of yeasts than that obtained in the present study (26% and 55%, respectively).

Candida albicans was the most frequent species in both sites; tongue and root canal. This result confirms the findings of other studies that reported *C. albicans* as the most commonly found species in the root canal (Waltimo *et al.* 1997, Sundqvist *et al.* 1998, Cheung & Ho 2001, Peciuliene *et al.* 2001, Egan *et al.* 2002). It is known that *C. albicans* has special features that allow it to survive in and colonize the root canal system such as the ability to produce and secrete aspartyl proteases. These enzymes are the key to colonization, allowing the use of different protein substrates, including dental collagen, as energy sources (Hagihara *et al.* 1988). Studies involving animals have shown a direct relationship between the ability to produce these proteins and the aggressiveness of the strain (Togni *et al.* 1994). Some strains can also undergo phenotypic changes under conditions where nutrients are limiting, and these modifications can represent an adaptive advantage for the colonization of root canals. Pleomorphic patterns of growth and tigmotropism are factors possibly related to the higher frequency of *C. albicans* in endodontic infections (Kunamoto & Vinces 2005).

Candida parapsilosis was the second species most prevalent at the both oral sites. The presence of *C. parapsilosis* in endodontic infections was also reported by Lana *et al.* (2001). This yeast was isolated in canals after seven days of dressing and intra-canal medication. This may not only be an indication of contamination during the treatment, but may also indicate resistance towards the calcium hydroxide pastes used as an intracanal antimicrobial agent. The increasing numbers of patients with persistent endodontic disease associated with an increased microbial resistance towards antifungal treatment may result in difficulties in the future for endodontic treatment. The isolation of *C. parapsilosis* within dental root canals may be related to its presence as a saprophytic organism in the oral cavity (Odds 1988).

Candida tropicalis was found only on the tongue. This yeast is also a commensal found throughout the gastrointestinal tract. Its incidence in the oral cavity is usually related to the endogenous introduction of strains from the perianal region (Collin *et al.* 1999). Most studies in healthy carriers have shown that this species is the second most prevalent yeast isolated from samples obtained from oral sites, providing 7% of the total yeast population (Odds 1988, Cannon *et al.* 1995).

The strain identified as *Candida* sp. probably represents a new yeast species. This yeast showed only three substitutions in the D1/D2 domains of the rDNA sequence when compared with the undescribed *Candida* sp. CBS 7880 (Genbank access number AY559041), and probably are the same species. *Candida* sp. CBS 7880 was isolated from liquid sweetener (http://www.cbs.knaw.nl). The isolation of this yeast from the root canal shows that food strains may invade the root canal.

The ingestion of dairy products with *S. cerevisae* and *K. lactis* may allow occasional colonization of oral sites by these species. Although this study shows a low frequency of isolation of *S. cerevisiae*, its presence inside the oral cavity could represent a risk of subsequent infection in immunocompromised patients. Various cases of fungemia caused by this species in severely

debilitated, traumatized or immune-deficient patients have been reported in recent years, suggesting that this species could be an opportunistic pathogen (Graf & Gavazzi 2007). Debelian *et al.* (1997) reported the isolation of *S. cerevisiae* from an infected root canal and from the blood of a patient undergoing root canal treatment of a tooth with asymptomatic apical periodontitis. It was supposed that the root canal was the source of the blood isolate as result of unintentional transfer into the bloodstream during root canal treatment. In the present study, however, *S. cerevisiae* was not isolated from root canals with necrotic pulps.

The presence of yeasts in the oral cavity may be a primary source of inoculum for the root canal. The experimental design did not address this hypothesis, but in only one patient was yeast recovery from the root canal unaccompanied by recovery of the microorganism from the tongue. In the present study, 45.8% of the patients carried yeasts only in the mouth, a percentage similar to that observed in other surveys. Budtz-Jörgensen *et al.* (2000) reported the isolation of yeasts from 34% of healthy patients; however, Kleinegger *et al.* (2001) isolated yeasts from the oral cavity in 60% of healthy individuals. In a report by Egan *et al.* (2002), the prevalence of yeasts in root canals was greatly increased if the same yeast was found in the patient's saliva. Their conclusions were drawn from the analysis of six teeth from five patients and all teeth had some history of communication between the root canal and the oral cavity. Taken in isolation, their findings may suggest that the presence of yeasts in the root canal were due to a direct overgrowth of yeasts from the oral cavity through cracks or other tooth defects.

The presence of *C. albicans* as the sole microorganism in root canals with necrotic pulps was reported by Waltimo *et al.* (2004) in secondary endodontic infections. In the present study, only intact teeth were sampled yet a high frequency of teeth had viable yeast cells present in their canal systems. As the material used in this survey consisted of teeth with intact pulpal chambers, the isolation of yeasts in root canals is intriguing and may be suggestive of the capability of such microorganisms to invade the pulpal environment actively or be carried to the region passively via lateral root canals, resorption lacunae or periodontal sulci. The means by which *Candida* species invaded such an environment was not considered in this study, but its recovery in such high percentages indicates that yeast are able to penetrate root canals, probably through dentinal tubules as other studies have already indicated (Waltimo *et al.* 1997, 2003).

In spite of *C. dubliniensis* has been isolated in other studies (Waltimo *et al.* 1997, Lana *et al.* 2001, Egan *et al.* 2002), in the present study, it was picked up more than one colony with the same morphology to make sure that the morpho-species is a single species and according to the phenotypic characters of isolates combined with molecular-based criteria used, this species was not isolated. The medical history indicated that none of patients sampled was immunocompromised and none had received antifungal and antibiotic treatment during the previous six months. No correlation was found between patient characteristics (age, gender or ethnical group) and the presence of specific yeast species. Even though, the presence of yeasts in the root canal cannot currently be implicated as a primary factor driving apical periodontitis, the presence of such microorganisms at a high frequency cannot be ignored.

Conclusions

1. The frequency of *Candida* species in root canals associated with primary endodontic infections was 22.6%.
2. The frequency of yeasts on the tongue was 45.8%.
3. *Candida albicans* was the most prevalent specie at both sites.

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References

Barros Lopes CP, Soden A, Martens AL, Henschke PA, Langridge P (1998) Differentiation and species identification of yeasts using PCR. *International Journal of Systematic Bacteriology* **48**, 279–86.

Borromeo GL, Mccullough MJ, Reade PC (1992) Quantification and morphotyping of *Candida albicans* from healthy mouths and from mouths affected by erythematous candidosis. *Journal of Medical Veterinary Mycology* **30**, 477–80.

Budtz-Jörgensen E, Mojon E, Rentsch A, Deslauriers N (2000) Effects of oral healthy program on the occurrence of oral candidosis in a long term care facility. *Community Dental and Oral Epidemiology* **28**, 141–9.

Cannon RD, Holmes AR, Mason AB, Monk BC (1995) Oral *Candida*: clearance, colonization or candidiasis. *Journal of Dental Research* **74**, 1152–61.

Cheung GSP, Ho MWM (2001) Microbial flora of root canal-treated teeth associated with asymptomatic periapical radiolucent lesions. *Oral Microbiology and Immunology* **16**, 332–7.

Collin B, Clancy CJ, Nguyen MH (1999) Antifungal resistance in non-*albicans Candida* species. *Drug Resistance Update* **2**, 9–14.

Dahlén G, Samuelsson W, Molander A, Reit C (2000) Identification and antimicrobial susceptibility of enterococci isolated from the root canal. *Oral Microbiology and Immunology* **15**, 309–12.

Debelian GJ, Olsen I, Tronstad L (1997) Observation of *Saccharomyces cerevisiae* in blood of patient undergoing root canal treatment. *International Endodontic Journal* **30**, 313–7.

Egan MW, Spratt DA, Ng Y-L, Lam JM, Moles DR, Gulabivala K (2002) Prevalence of yeasts in saliva and root canals of teeth associated with apical periodontitis. *International Endodontic Journal* **35**, 321–9.

Graf C, Gavazzi G (2007) *Saccharomyces cerevisae* fungemia in an immunocompromised patient not treated with *Saccharomyces boulardii* preparation. *Journal of Infection* **54**, 310–1.

Hagihara Y, Kaminishi H, Cho T, Tanaka M, Kaita H (1988) Degradation of human dentine collagen by an enzyme produced by the yeast *Candida albicans*. *Archives of Oral Biology* **33**, 617–9.

Jackson FL, Halder AR (1963) Incidence of yeast in root canals during therapy. *British Dental Journal* **115**, 459–60.

Kleinegger CL, Stoeckel DC, Kurago ZB (2001) A comparison of salivary calcoprotein levels in subject with or without oral candidiasis. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **92**, 340–9.

Kunamoto CA, Vinces MD (2005) Alternative *Candida albicans* lifestyles: growth on surfaces. *Annual Review of Microbiology* **59**, 113–33.

Kurtzmann CP, Fell JW (1998) *The Yeasts – A Taxonomic Study*, 4th edn. Amsterdam: Elsevier, 1055 pp.

Kurtzmann CP, Robnett CJ (1998) Three new insect-associated species of the yeasts genus *Candida*. *Canadian Journal of Microbiology* **44**, 965–73.

Lachance MA, Bowles JM, Starmer WT, Barker JSF (1999) *Kodamea kakaduensis* and *Candida tolerans*, two new ascomycetous yeast species from Australian *Hibiscus* flowers. *Canadian Journal of Microbiology* **45**, 172–7.

Lana MA, Ribeiro-Sobrinho AP, Stehling R, Garcia GD, Silva BKC, Hamdan JS (2001) Microorganisms isolated from root canals presenting necrotic pulp and their drug susceptibility in vitro. *Oral Microbiology and Immunology* **46**, 100–5.

Le Goff A, Bunetel L, Mouton C, Bonnauremallet M (1997) Evaluation of root canal bacteria and their antimicrobial susceptibility in teeth with necrotic pulp. *Oral Microbiology and Immunology* **12**, 318–22.

Molander A, Reitt C, Dahlén G, Kvist T (1998) Microbiological status of root-filled teeth with apical periodontitis. *International Endodontic Journal* **31**, 1–7.

Najzar-Fleger D, Flipovic D, Prpic G, Kobler D (1992) *Candida* in root canals in accordance with oral ecology. *International Endodontic Journal* **25**, 40. (Abstract 1528).

Odds FC (1988) *Candida and Candidosis*, 2nd edn. London: Baillière Tindall, 300 pp.

Peciuliene V, Reynaud AH, Balciuniene I, Haapasalo M (2001) Isolation of yeasts and enteric bacteria in root-filled teeth with chronic apical periodontitis. *International Endodontic Journal* **34**, 429–34.

Sundqvist G (1976) Bacteriological studies of necrotic dental pulps. PhD Thesis, University of Ümea, Ümea, Sweden.

Sundqvist G (1992) Ecology of the root canal flora. *Journal of Endodontics* **18**, 427–30.

Sundqvist G, Figdor D, Persson S, Sjögren U (1998) Microbiologic analysis of teeth failed endodontic treatment and the outcome of conservative re-treatment. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **85**, 86–93.

Togni G, Sanglard D, Monod M (1994) Acid proteinase secreted by *Candida tropicalis*: virulence in mice of a proteinase negative mutant. *Journal of Medical Veterinary Mycology* **32**, 257–65.

Tronstad L, Barnett F, Riso K, Slots J (1987) Extraradicular endodontic infections. *Endodontics & Dental Traumatology* **3**, 86–90.

Waltimo TMT, Sirén EK, Torkko HLK, Olsen I, Haapasalo MPP (1997) Fungi in therapy-resistant apical periodontitis. *International Endodontic Journal* **30**, 96–101.

Waltimo TMT, Sen BH, Meurman JH, Orstavik D, Haapasalo MPP (2003) Yeasts in apical periodontitis. *Critical Reviews in Oral Biology and Medicine* **14**, 128–37.

Waltimo TMT, Haapasalo M, Zehnder M, Meyer J (2004) Clinical aspects related to endodontic yeast infections. *Endodontic Topics* **9**, 66–78.

Yarrow D (1998) Methods for the isolation, maintenance, classification and identification of yeasts. In: Kurtzmann CP, Fell JW, eds. *The Yeasts, a Taxonomic Study*. Amsterdam: Elsevier, pp. 77–100.

