Anaerobiosis and serum promote mycelium formation by *Candida albicans* in colonies on TSBV agar

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Following long-term periodontal treatment with tetracycline a superinfection with *Candida* may arise. The reduced environment and the serum transudate of the periodontal pocket may promote such infection. The present in vitro study was performed to ascertain whether yeast–mycelium transformation in a fresh periodontal isolate was promoted under anaerobic conditions and in the presence of serum. *C. albicans*, isolated from a patient with tetracycline-treated refractory periodontitis, was cultured anaerobically or aerobically on TSBV or Sabouraud's dextrose agar at 29°C or 37°C for 72 h, with the pH of the medium being 5.6 or 7.2. TSBV medium was also tested with its horse serum or yeast extract removed. Mycelial growth was recorded visually and by stereo and scanning electron microscopy. Anaerobic culture at 29°C or 37°C on TSBV provided abundant mycelium at both pHs. After aerobic culture the mycelial phase was less pronounced and more abundant at pH 7.2 than at 5.6. TSBV without serum or yeast extract yielded more mycelium after anaerobic than after aerobic culture, although less than when both components were included. Sabouraud's medium provided sparse mycelium after anaerobic culture irrespective of the pH, and no mycelium after aerobic culture. □ *Atmosphere; dimorphism; nutrients; periodontology; pH; temperature; yeasts*

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Broad-spectred antibacterial substances such as tetracyclines are increasingly being used as supplements to conventional treatment of marginal periodontitis—generally considered a bacterial disease. After long-term treatment with such drugs it has been observed that a superinfection with *Candida* may arise in the periodontal pocket (1). Sophisticated techniques such as immunofluorescence, anaerobic culture, and electron microscopy have led to the concept that microorganisms may routinely be identified in gingival tissues of periodontal disease (2). It is well known that deep periodontal pockets have a reduced milieu promoting anaerobic infection and that they are bathed by gingival fluid, which is a serum transudate. Since the mycelial (M) phase of the dimorphic *Candida* is believed to play a pathogenic role in the initial process of tissue invasion (3), the present in vitro study was performed to examine whether yeast (Y)–mycelium (M) transformation in *C. albicans*, recognized as the most common and virulent *Candida* species of the oral cavity, is promoted under anaerobic conditions and in the presence of serum. Slots selective medium (TSBV agar), devised for recovery from dental plaque of the suspected periodontopathogenic bacterium *Actinobacillus actinomycetemcomitans*, has proved to be a suitable substrate for yeasts (1, 4). This medium contains tryptic soy agar, bacitracin, vancomycin, horse serum, and yeast extract. In this study TSBV was compared with the frequently used yeast medium Sabouraud's dextrose agar, which contains mycologic peptone and dextrose.

Materials and methods

Candida albicans, isolated recently from a patient with tetracycline-treated refractory periodontitis, was cultured on TSBV medium (4) and on Sabouraud's dextrose

Table 1. Mycelium formation in colonies of *Candida albicans* on agar media as influenced by atmosphere, temperature, pH, serum, and yeast extract after culture for 72 h. Mycelium was assessed visually and by stereo and scanning electron microscopy

Medium	Anaerobic culture		Aerobic culture	
	37°C	29°C	37°C	29°C
TSBV (pH 7.2)	***	***	**	**
TSBV (pH 5.6)	***	***	*	*
TSBV without horse serum	**	**	*	*
TSBV without yeast extract	**	**	*	*
SAB (pH 7.2)	*	*	–	–
SAB (pH 5.6)	*	*	–	–

TSBV = Slots medium (4); SAB = Sabouraud's dextrose agar.
*** = abundant mycelium in colonies; ** = moderate mycelium in colonies; * = little mycelium in colonies; – = no mycelium in colonies.

agar. The organism was identified by fermentation and assimilation tests and germ tube formation in serum. The TSBV medium contained per liter of distilled, deionized water, 40 g of tryptic soy agar, 1 g of yeast extract, 100 ml of horse serum, 75 mg of bacitracin, and 5 mg of vancomycin (pH 7.2). Sabouraud's dextrose agar contained per liter, 10 g of Mycological peptone (Oxoid L 40), 40 g of dextrose, and 15 g of agar no. 1 (Oxoid L 11) (pH 5.6). After seeding, the yeast was cultured in air, in 10% CO_2 in air (chamber), and in 10% H_2, 10% CO_2, and 80% N_2 (anaerobic chamber). To test the effect of a microaerophilic environment on Y–M transformation, the yeast was cultured in a GasPak jar supplied with a CampyPak disposable hydrogen + carbon dioxide generating envelope (BBL Microbiology Systems, Becton Dickinson and Co., Cockeysville, Md., USA), which, according to the manufacturer, produces an atmosphere of approximately 5–12% CO_2 and a residual atmosphere of approximately 5–15% O_2. Aerobic and anaerobic cultures on TSBV and Sabouraud's dextrose agar were produced with the pH of the medium being either 7.2 or 5.6, which were the formula pHs of the TSBV and Sabouraud dextrose agar, respectively. The TSBV medium was also tested with its horse serum or yeast extract removed (pH 7.2). Two growth temperatures were used: 37°C and 29°C. The latter was the temperature in the anaerobic glove box outside the incubator. This temperature was similar to the incubation temperature (30°C) recommended as superior for yeast blood cultures (5). Mycelial growth was recorded visually under a stereo microscope and by scanning electron microscopy after critical-point drying and sputter-coating of colonies on the agar with gold–palladium. All cultures were made at least in duplicate on different days.

Results

When *C. albicans* was cultured anaerobically at 29°C or 37°C for 72 h, the TSBV agar provided abundant M growth at both pHs (Table 1). After aerobic culture the M phase was less pronounced (Table 1, Figs. 1 and 2). Culture on TSBV medium in a microaerophilic atmosphere at 37°C yielded an M-phase abundance between that of anaerobic and aerobic culture (Fig. 2). This was similar to the culture obtained on TSBV in 10% CO_2 in air at 37°C. After aerobic culture, M formation on the TSBV medium was more abundant at pH 7.2 than at 5.6 (Table 1).

TSBV medium without serum or yeast extract yielded more mycelium during anaerobic than during aerobic culture, although less mycelium than when both these components were included in the medium (Table 1). Sabouraud's dextrose agar provided sparse mycelium during anaerobic culture

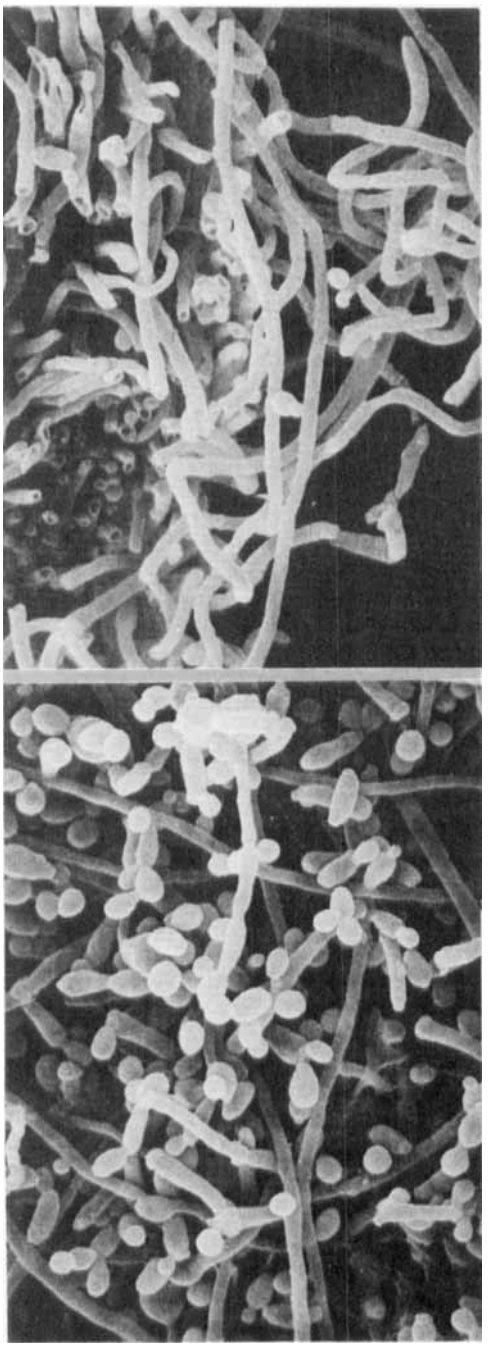

Fig. 1. Scanning electron microscopy photo of *Candida albicans* grown on TSBV (Slots) medium (pH 7.2) for 72 h at 37°C. Note abundant mycelium (top) formed during anaerobic culture (10% CO_2, 10% H_2, 80% N_2) and abundant blastospore formation during aerobic culture (bottom).

irrespective of the pH, and no mycelium during culture in 10% CO_2 in air, in a microaerophilic atmosphere, or in air. The anaerobically produced M-rich colonies of *C. albicans* on TSBV agar showed a greater tendency to penetrate the agar than did the aerobically provided M-poor colonies (Fig. 3). The anaerobic colonies on TSBV also contained a pronounced aerial mycelium, which gave them characteristics of molds.

Discussion

The present study used a fresh clinical isolate of *C. albicans* from refractory tetracycline-treated periodontitis, in which it was present in abundance. The study demonstrated that TSBV agar (4), originally designed for selective recovery of *A. actinomycetemcomitans* from dental plaque, is well suited for studying dimorphism of *Candida* in colonies. Sabouraud's dextrose agar, which is a medium frequently used for culture of yeasts, was quite unsuitable in this respect. Studies of factors promoting Y–M transformation in colonies should be encouraged, since the M phase of *Candida* is believed to play a pathogenic role in the initial process of tissue invasion (3) and because Y–M studies so far have mainly been performed in liquid culture (6, 7). Microorganisms of dental plaque are frequently located in microcolonies. Furthermore, it is increasingly being recognized that microorganisms may penetrate tissues in periodontal diseases (2).

C. albicans and the other pathogenic *Candida* species are generally considered aerobic yeasts that are able to grow anaerobically (3, 8–10). Partial anaerobiosis is believed to favor production of the filamentous form of *C. albicans* in vitro (3). This idea was supported by the present study, which found anaerobicity to be the most important factor promoting colonial Y–M transformation in a periodontal *C. albicans* isolate, followed by horse serum and yeast extract. Serum and 'suboptimal levels of yeast extract' are additional factors promoting production of hyphae from *C. albicans* in vitro (3).

There was no indication that 10% CO_2 had a major effect on Y–M transformation

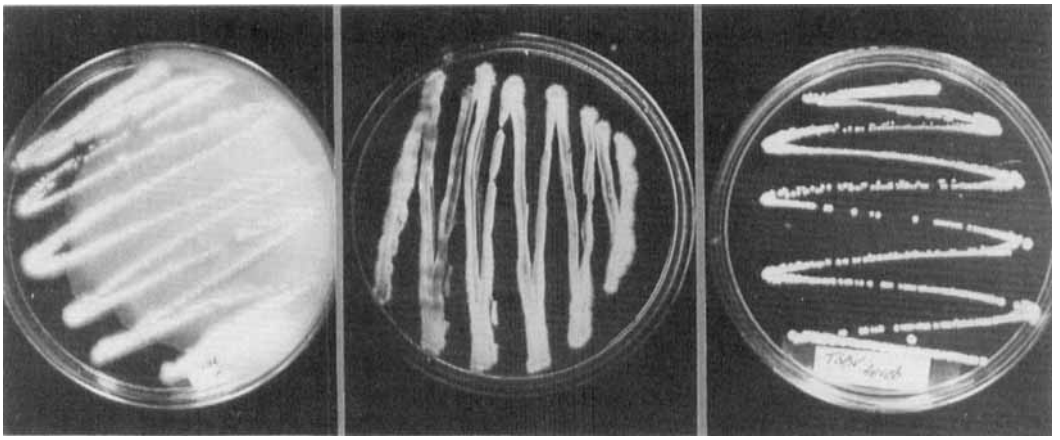

Fig. 2. Colonies of *Candida albicans* on TSBV agar (pH 7.2) after culture for 72 h at 37°C: anaerobic culture (left), microaerophilic culture (middle), and aerobic culture (right). Note that anaerobically grown cultures show abundant aerial mycelium, whereas aerobically grown cultures do not.

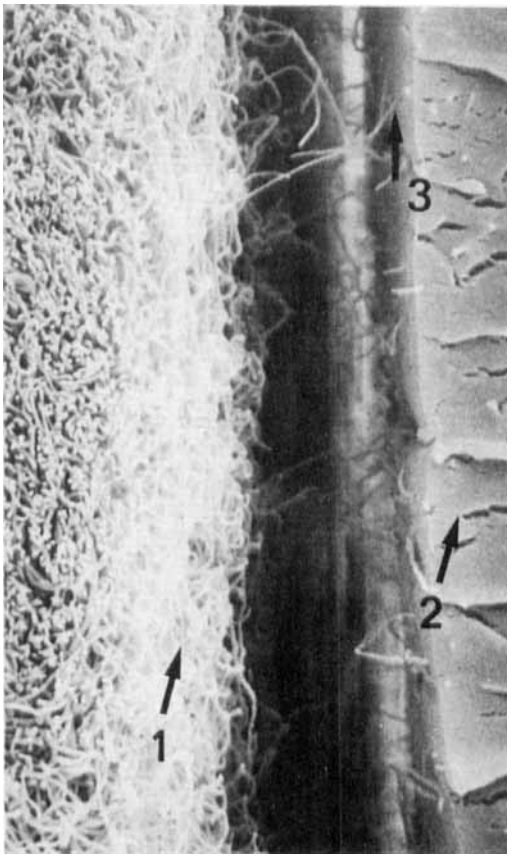

Fig. 3. Scanning electron microscopy photo of *Candida albicans* colony showing penetration of TSBV agar (pH 7.2) by hyphae (M phase) after anaerobic culture for 72 h at 37°C. 1 = surface colony; 2 = agar medium; and 3 = penetrating hypha.

in the present strain. However, hyphae production by *C. albicans* in vitro is promoted by an atmosphere with elevated CO_2 concentration (CO_2:O_2 ratio $\geq$ 2:1) (3, 6, 11).

C. albicans developed a pronounced aerial mycelium on TSBV agar under anaerobic conditions, thereby exhibiting characteristics of a mold. The formation of aerial mycelium supported previous findings in *C. albicans* (12), so far considered unsubstantiated (3). The great tendency for the present strain of *C. albicans* to form hyphae may give it a preference in the initial process of tissue invasion, since hyphae adhere better than yeast cells to human buccal and vaginal epithelial cells (13).

Care must be taken when extrapolating conclusions from in vitro findings to the situation in vivo. However, it should not be overlooked that the site from which the present yeast had been isolated was a deep periodontal pocket that was bathed by serum transudate and in which a highly reduced atmosphere existed. *Candida* are known to produce an abundance of mycelium in vivo when anaerobic conditions prevail, such as under maxillary dentures (14). Besides, *C. albicans* has been found to invade sulcular epithelium and gingival connective tissues (15–17), forming pseudomycelium (17). Our study suggested that anaerobicity and gingival fluid may be major contributory factors to this event. It should be emphasized, how-

ever, that more clinical isolates must be examined to test this hypothesis. Having invaded periodontal tissues, *C. albicans* has several virulence factors that may counteract host defense: inhibition of polymorphonuclear functions, killing of monocytes, degradation of alpha-2-macroglobulin, production of enzymes such as collagenase, phospholipase, aminopeptidase, acid and alkaline phosphatase, keratin protease, and Ig1, IgA1, and IgA2 immunoglobulin proteases (1). Thus, the yeast may maintain periodontal disease even though broad-spectred antibacterial drugs with no antifungal effect are being administered.

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