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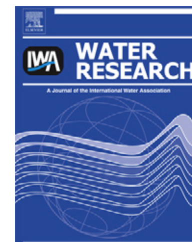

Hartmannella vermiformis can promote proliferation of *Candida* spp. in tap-water

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ABSTRACT

Candida yeasts colonize humans' oral cavities as commensals or opportunistic pathogens. They may be isolated from water circulating in dental unit waterlines mixed with saliva traces mainly because of dysfunction of anti-retraction valves. Free-living amoebae (FLA), like *Hartmannella vermiformis*, are frequently found in aquatic environments and they have also been already isolated from dental unit waterlines. They can be implicated as reservoir for pathogens or directly in infections. This work deals with the survival of three species of *Candida* (*Candida albicans*, *Candida glabrata* and *Candida parapsilosis*), in co-cultivation with FLA in tap-water. One strain of each *Candida* species was studied. Microbiological and microscopic approaches were used; amoebae–yeasts co-cultivation assays were performed at different temperatures of incubation. Results have shown that *H. vermiformis* were able to internalize *Candida* yeasts and promote their proliferation in tap-water with or without saliva traces (2% v/v). Amoebae interact differently with *Candida* depending on the temperature used and the studied species of yeasts. In conclusion, this study emphasizes the survival of yeasts and/or FLA in tap-water. The ability of yeasts and amoebae to interact should be taken into account because it could lead to candidiasis infection for the frailest patients after a dental treatment.

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1. Introduction

Changes in the qualitative or quantitative composition of microbiota may enhance the pathogenicity of *Candida* yeasts, resulting in superficial or systemic infections, depending on the immune status of the patient; these yeasts are classified as opportunistic pathogens (Odds, 1988; Hube, 2004). Among them, *Candida albicans*, *Candida glabrata* and *Candida parapsilosis* are important human fungal commensals of mucosa or skin. The wearing of dentures (or dental implant) and inappropriate denture cleaning are thought to allow the development in the oral cavity of a polymicrobial biofilm, on their surface, acting as microbial reservoir able of causing infections; this biofilm contains large numbers of bacteria in addition to *Candida* spp. (Budtz-Jorgensen, 1990; Cannon et al., 1995; Webb et al., 1998; Szymanska, 2005; Kamemizu et al., 2008). Moreover, *Candida* yeasts are also implicated in the biofilm architecture on natural teeth and *C. albicans* has been

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recently shown to form corncob structures in supragingival plaque (Jewtuchowicz et al., 2007; Zijnge et al., 2010). Biofilms thus act as *Candida* reservoirs in the oral cavity.

C. parapsilosis, *C. albicans* and other organisms commonly found in the oral cavity have also been isolated in tap water and in biofilm obtained from dental unit waterlines (DUWL) (Witt and Hart, 1990; Walker et al., 2000; Szymanska, 2005; Castiglia et al., 2008). The risk of contamination of the lines with oral microorganisms suggests back siphonage and failure of anti-retraction devices which limit re-aspiration of fluid from the oral cavity (Bagga et al., 1984; Kumar et al., 2010). Actually, the most common cause of DUWL contamination is believed to be the formation of a biofilm and the subsequent detachment of sessile microorganisms from the lines surfaces. The average temperature of most DUWL is 23 °C (Pankhurst, 2003); this may encourage the proliferation of microorganisms. As a consequence, dental unit waterlines system often deliver water to patients with microbial levels exceeding those considered safe for drinking water (Walker et al., 2000; Barben et al., 2009; Chate, 2010).

However, organisms isolated from DUWL mainly originate from the incoming main water. Free-living amoebae (FLA) such as *Acanthamoeba* spp. or *Hartmannella* spp. are ubiquitous in nature and widely distributed (Inoue et al., 1998); these protists are also widely isolated in recreational water systems and DUWL (Michel and Just, 1984; Williams et al., 1993; Barbeau and Buhler, 2001; Trabelsi et al., 2010). The flow of DUWL, which is maximal at the center of the lumen and minimal at the periphery, and the intermittent use patterns of dental lines lead to the deposition of oral and environmental microorganisms onto the surface of the tubing allowing a multispecies biofilm development (Williams et al., 1996). So, the DUWL and surgery plumbing act as a persistent reservoir of infection and aerosols from the dental unit instruments may increase the infectious risk for immunocompromised (or suffering from severe diseases) patients and dental staff. Actually FLA present a rare but serious risk to human health as direct causative agents, but mainly may serve as vector for other microorganisms as is well-known for *Legionella* (Kuiper et al., 2004; Bouyer et al., 2007; Lorenzo-Morales et al., 2007). However, rare studies showed interactions between fungal and amoebal cells; Steenbergen et al. (2001) have demonstrated that *Acanthamoeba castellanii* can be used by pathogen yeast, *Cryptococcus neoformans*, as a replication niche. In contrast, *C. albicans* were killed by *A. castellanii* (Steenbergen et al., 2001). It has been demonstrated that several fungal determinants of virulence needed for mammalian pathogenicity are also important for surviving predation by amoeba (Steenbergen and Casadevall, 2003; Casadevall, 2007). So, these potential interactions between fungi and amoebae may be problematic; indeed, if fungi are able to survive in amoebae, it may enable them to survive in macrophages and evade human immune system.

The aim of this work was to investigate the possible interaction between *Candida* spp. and *Hartmannella vermiformis* which can both be isolated in biofilms associated with DUWL, taking into account the influence of saliva presence. The survival of yeasts in co-cultivation with amoebae was evaluated by microbiological and microscopic approaches, using different incubation temperatures chosen to be comparable with those in DUWL. The FLA may digest the yeast after phagocytosis or otherwise encourage their multiplication, thus allowing them to escape more easily to anti-microbial treatment. The main *Candida* species isolated in water were studied (*C. albicans*, *C. glabrata* and *C. parapsilosis*).

2. Material and methods

2.1. Culture of FLA

H. vermiformis (ATCC 50802) was cultured in 75 cm^2 culture flasks containing PYNFH medium (modified ATCC medium 1034; De Jonckheere, 1977) supplemented with 10% of fetal calf serum and antibiotics (Streptomycin 2 μg/mL, Penicillin G 500 U/mL and Gentamycin 4 μg/mL). Cultures were incubated at 20 °C for 5 days.

2.2. Culture of yeasts

C. albicans (ATCC 3153), *C. glabrata* (IHEM 9556) and *C. parapsilosis* (ATCC 22019) were cultured on Sabouraud dextrose agar plates at 20 °C for 48 h.

2.3. Saliva collection

Whole unstimulated saliva was collected on ice from 11 healthy adult volunteers who rinsed their mouths gently with water before sampling to decrease bacterial contamination (Barbot et al., 2011). Saliva was then pooled and centrifuged (15 min, 3000 g, 4 °C). The resulting supernatant was stored at −80 °C until used.

2.4. Preparation of amoebae supernatants

Amoebae were cultured in 75 cm^2 culture flasks containing 10 mL of distilled water at 20 °C. After 3 days of incubation, the culture medium was removed and centrifuged (10 min, 800 g). The resulting supernatant was stored at −80 °C until used.

2.5. Co-cultivations

Amoebae suspension was prepared by eliminating culture medium, scraping adherent cells and centrifuging them (7 min, 300 g). For experiments, amoebae were used at a final concentration of approximately 5.7 logs cells/mL (or 5×10^5 cells/mL) in 0.22 μm filtered tap-water. Likewise, yeasts suspension was prepared in filtered tap-water with the same final concentration. The multiplicity of infection (MOI) was 1. A preliminary study suggested that an MOI of 1 made the investigation of amoebae–yeasts interactions easier (data not shown).

Co-cultivations were performed in 24-well plates. Amoebae were first distributed into wells and incubated 2 h at 27 or 20 °C. Yeasts and/or saliva 2% (v/v) were then added, and co-cultivations were incubated over a time course of 360 h. In order to study the indirect influence of amoebae on *Candida* spp., 1 mL of yeasts suspension was added to 1 mL of amoebae supernatant and incubated at 20 °C for 360 h.

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