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ORIGINAL ARTICLE

# Inhibition of *Candida albicans* biofilm development by unencapsulated *Enterococcus faecalis* cps2

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**Abstract** *Background/purpose:* In the oral environment, *Candida albicans* interacts with many bacteria, including *Enterococcus faecalis*. We investigated the susceptibility of *C. albicans* biofilm development to the presence of unencapsulated *E. faecalis* cps2 in comparison with reference strains (*E. faecalis* ATCC 29212) or their respective spent medium (collected at 6 hours).
*Material and methods:* Crystal violet stain was used to measure the total biofilm mass, whereas quantitative real-time polymerase chain reaction was used to analyze the change in expression of the mRNA of hypha morphology (ALS1 and ALS3) and biofilm maturation (EFB1).
*Results:* At the intermediate stage, *C. albicans* resisted the presence of each *E. faecalis* strain tested and their spent medium. However, at the maturation stage, the unencapsulated strain was stronger in reducing *C. albicans* biofilms than the reference strain ($P < 0.05$). At this maturation stage, the transcription levels of each gene tested decreased in the presence of either *E. faecalis* strains or their respective spent medium. The unencapsulated strain was more pronounced in reducing ALS1/ALS3 expression, whereas the respective spent medium had a similar capability to restrict the expression of EFB1.
*Conclusion:* This study showed, the unencapsulated strain is more effective in inhibiting *C. albicans* biofilm development compared with the reference strains. In contrast, the secreted molecules produced by each strain tested are necessary in controlling the growths of *C. albicans* biofilm.


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## Introduction

*Enterococcus faecalis* is a Gram-positive bacterium that has the ability to colonize a variety of sites in humans, including the oral niche.[1] Like *E. faecalis*, *Candida albicans* is a fungal species that exists in many niches in the human body, including the gastrointestinal tract and the oral cavity. Both organisms are often found as coisolates in samples collected from endodontic-related infections, especially those linked with chronic periodontitis, as well as from root-filled teeth with periapical lesions.[2] This indicates that conditions in root canal teeth favoring infection with either are similar.

A report by Pinheiro et al[3] showed that the majority of *E. faecalis* isolates taken from root canal teeth were of the CPS (capsular polysaccharide) type 1 (cps1) genotype. However, a report by our group[4] found that the dominant strain found in saliva and infected root canals is the *E. faecalis* cps2 genotype, a strain belonging to serotype C,[5] and its virulent traits are associated with the presence of the surface CPS.[6] This indicates that in infected root canal teeth, the *E. faecalis* cps2 strain may differ from those in other oral niches. Moreover, a previous report showed that *E. faecalis* QA29b (a nonstarter food isolate) is an incongruent cps2 strain. This strain carries the full-length *cps*2 locus, but its *cps* gene is not expressed because of an insertion sequence, IS6770, inserted in the cpsC—cpsK promoter region.[7] Thus, the presence of IS6770 suggests that at some points the nonencapsulated phenotype may be advantageous during some points in the *E. faecalis* life cycle and disadvantageous at others, suggesting a role in its adaptation. Because *C. albicans* and *E. faecalis* have shown antagonistic relationships in the *Caenorhabditis elegans* model,[8] where *E. faecalis* inhibits hyphal formation of *C. albicans*, we hypothesized that the absence of the cps expression could enable the bacterium to communicate with *C. albicans* through antagonistic interactions while the fungus grows as biofilm.

A number of studies have also described changes in gene expression levels during biofilm development of *C. albicans*.[9–11] Of these, *ALS1* and *ALS3*, which belong to the ALS (agglutinin-like sequence) gene family, encodes cell surface glycoproteins.[12,13] Another gene, *EFB1*, has been reported to be constitutively expressed under most growth conditions of *C. albicans*.[14] All genes have been demonstrated to be upregulated in *C. albicans* hyphae, which suggests that they may play a role in biofilm development by this organism.[13,15,16] Considering the above-mentioned information, we used an unencapsulated *E. faecalis* cps2 strain, based on the presence of IS6770, to investigate its effect on *C. albicans* growth and biofilm formation *in vitro*. For this reason, we used crystal violet (CV) assays to measure the biofilm mass and the quantitative polymerase chain reaction (qPCR) method to evaluate the altered mRNA expression of the *ALS1*, *ALS3*, and *EFB1* genes. Analysis of the *in vitro* interaction of *E. faecalis* and *C. albicans* may contribute to the understanding of the behavior of the unencapsulated *E. faecalis* cps2 strain in the human body environment.

## Materials and methods

The unencapsulated *E. faecalis* cps2 strain used in this study was a clinical isolate. This strain was isolated from one of the endodontic patients in our previous study.[4] PCR[5] and qPCR methods were used to determine CPS genotyping characterization and to determine the encapsulated or unencapsulated strain by detecting the presence of the insertion sequence (IS6770).[7] To visualize the presence or absence of the CPS, expressed by this strain, we used the staining method, stain-All (Sigma-Aldrich, St. Louis, MO, USA) (Figure 1).[17] *E. faecalis* strain ATCC 29212 was used as a control during testing. All *E. faecalis* strains were maintained in brain—heart infusion (BHI) broth (Oxoid, Basingstoke, United Kingdom) with 30% (v/v) glycerol at −80°C until testing.

*C. albicans* used in this study was *C. albicans* ATCC 10231 that was routinely propagated in yeast extract—peptone—dextrose (YPD; 1% yeast extract, 2% peptone, 2% glucose) agar plates or, when indicated, in yeast nitrogen base (YNB; Difco Laboratories, Detroit, MI, USA) medium (pH 7), supplemented with 50mM glucose.

To obtain spent medium samples, we used a method as described previously.[18] *E. faecalis* was grown in 20 mL BHI broth. Then, 10 mL of the medium was taken from overnight cultures at a middle exponential stage of growth (6 hours) and centrifuged at 5000 g, 10 minutes (Thermo Fisher Scientific, Waltham, MA, USA). The supernatant was filter sterilized through a 0.22-μm filter (Millipore, Billerica, MA, USA). Protein concentration in the spent medium was determined using the Bradford method. Spent medium was diluted in phosphate buffer saline (PBS; Sigma-Aldrich) to yield 10 μg/mL and 100 μg/mL concentrations and used immediately or stored for short periods at −20°C. The pH of the spent medium was adjusted to pH 7.

### Biofilm staining with CV and the colony-forming unit assay

For the biofilm assay, yeast cells of *C. albicans* were grown overnight at 35°C. After incubation for 16 hours at 37°C with

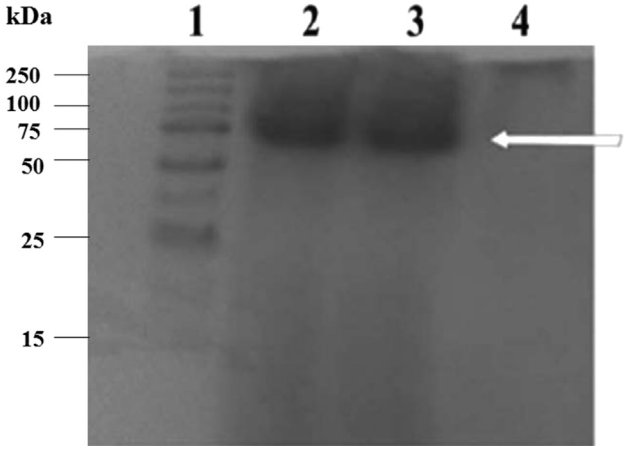


**Figure 1** Stain-all staining of heat extracts of different *Enterococcus faecalis* strains after electrophoresis in an acrylamide gel. Lanes: 1 = prestained protein markers (New England BioLabs); 2 = reference strain (ATCC 29212); 3 = encapsulated *E. faecalis* cps2 (strain that showed no positive result after qPCR); 4 = unencapsulated cps2 (strain that showed positive result after qPCR). The arrow indicates locations of CPS. CPS = capsular polysaccharide; qPCR = quantitative polymerase chain reaction.