Introduction

The human oral cavity is a diverse microbial ecosystem comprised of bacteria, fungi, protozoa, and viruses [1]. Over 700 species of bacteria are known to reside within the human oral cavity, and more than 100 species are found in the mouth of a healthy individual at one time [2]. Normally, the different species maintain an ecological homeostasis; however, due to constant competition for limited space and nutrients, any disturbance of the biofilm by environmental factors or interspecies interactions can favor the growth of certain species, leading to diseases [3].

One such disease is oral candidiasis (oral thrush), which is caused by Candida albicans, the most prevalent yeast isolated from the human body and a natural part of the commensal microbiota [4]. Studies showed that C. albicans can be found in 50-60% of clinically healthy mouths and are often associated with bacterial species in polymicrobial biofilms [5,6]. Under certain conditions, resident C. albicans strains can become invasive, causing recurrent mucosal or life-threatening infections [7]. Nevertheless, despite a considerable portion of the population carrying the yeast, very few of these people suffer from oral Candidiasis, suggesting the presence of factors suppressing the pathogenic attributes of C. albicans [4].

Researchers have attempted to explore the bacteria-fungal relationship in the oral environment. Co-culture studies utilizing various oral commensal bacterial species showed that higher concentrations of certain bacteria, like Actinomyces israelii, Porphyromonas gingivalis, and Prevotella nigrescens, result in a reduction of Candida in biofilms [5]. The mechanism of this reduction could be explained as a result of nutritional competition as suggested by Cannon et al. [8]. In addition, oral Streptococcus salivarius was reported to suppress yeast growth by interfering with its ability to adhere to the oral mucosal surfaces [9]. However, this finding was later challenged by de Miranda et al., who could not reproduce the suppressive effect of S. salivarius upon C. albicans in vivo and in vitro [10].

**Research Article**

Suppression of *Candida albicans* Growth and Filamentation by Oral *Staphylococcus aureus*

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**Abstract**

**Background:** Candida albicans is the most prevalent yeast isolated from the human body and a natural part of the commensal microbiota; however, under the right conditions, C. albicans can cause candidiasis (oral thrush). The oral cavity is colonized by hundreds of bacterial species, and the relationship between these oral bacteria and C. albicans has been a topic of interest in understanding the development of candidiasis. Several studies have attempted to explain the bacteria-fungal relationship in the oral environment, but few have focused on single bacterial species inhibiting growth and morphogenesis of C. albicans. The aim of this study was to identify specific bacterial species, isolated from the healthy human oral cavity, that can suppress C. albicans.

**Materials and methods:** Bacterial and yeast species were isolated and purified from the saliva samples of 12 healthy individuals. PCR was performed by using 16SrDNA and 18S rDNA as templates to identify bacterial and yeast species for all isolates, respectively. Real time qPCR and microscopic examination were carried out to determine the inhibitory effect of *S. aureus* on *C. albicans*.

**Result:** This study demonstrated that numerous bacterial isolates from the oral cavity of healthy humans could inhibit the growth of *C. albicans* oral isolates, including the model strain CS5314. Among these bacteria, one *Staphylococcus aureus* strain displayed the strongest inhibitory effect on the growth of yeasts. By qPCR assay, all isolated *C. albicans* strains were inhibited on the growth by *S. aureus* in various degrees. Mixed culture experiments also demonstrated suppression of *C. albicans* pseudo hyphae or hyphae formation by *S. aureus* but not by other bacterial species tested.

**Conclusion:** It is well known that *C. albicans* and *S. aureus* exist in a cooperative relationship and form substantial polymicrobial biofilm, thus, this is a first report about one clinical isolated *S. aureus* strain suppressing the growth and pseudo hyphae/hyphae formation of *C. albicans*, indicating the complexity of interspecies interaction in human oral cavity.
The mitis group streptococci (principally represented by Streptococcus mitis, Streptococcus oralis and Streptococcus gordonii) are considered ‘initial colonizers’, comprising greater than 80% of the early biofilm population on a professionally cleaned or newly emerged tooth surface [2,11,12]. S. gordonii has been shown to co-aggregate with C. albicans and form biofilms, and the interactions between the two species may result in pathogenic synergy, allowing C. albicans to integrate into the oral biofilms [13,14]. In the presence of S. gordonii, C. albicans hyphal morphogenesis was also found to be enhanced [15]. Like S. gordonii, Staphylococcus aureus, a pathogen that can cause opportunistic infections, has been shown to be able to form substantial polymicrobial biofilm when C. albicans presented [16]. Furthermore, co-infection of S. aureus-C. albicans results in synergistic effect and increased morbidity and mortality in vivo [17,18].

In this study, we isolated bacteria and yeast from the healthy human oral cavity and identified specific bacterial species that can suppress C. albicans growth or hyphal formation. Interestingly, among all isolated and identified bacteria, one clinical S. aureus strain showed significant suppression of growth and inhibition of pseudohyphae or hyphae formation for all clinically isolated C. albicans strains. To our knowledge, this is the first report of the inhibitive effect of S. aureus on C. albicans.

Materials and Methods

Bacteria and yeast isolation. Saliva from 12 healthy individuals was collected upon IRB approval (#1471). Bacterial species were isolated on brain heart infusion (BHI) agar (1.5% agar) plates under both aerobic and anaerobic conditions. Purified colonies were grown in BHI medium and visualized microscopically (Olympus Microscope 60X/1.40 Oil Ph3). Species were identified by 16S rDNA (the primers in Table 1) [19], and stored in BHI containing 15% glycerol at -80°C. Yeast strains were isolated on BHI rDNA sequencing (the primers in Table 1) [19], and stored in BHI Microscope 60X/1.40 Oil Ph3). Species were identified by 16S rDNA and 18S rDNA containing 15% glycerol at -80°C. Yeast strains were isolated on BHI plates grown aerobically, and species were identified by 18S rDNA sequencing (Table 1) (20). Candida albicans strain ATCC CS5314 was purchased from ATCC and routinely grown in YEP (1% yeast extract, 2% peptone supplemented with 2% glucose).

Identification of bacterial and yeast strains. The chromosomal DNA was isolated for bacteria or yeast using Wizard Genomic DNA Purification Kit (Promega, USA) or E.Z.N.A. Yeast DNA Kit (OMEGA, USA) according to the manufacturer’s protocol. The abundance of C. albicans was analyzed by qPCR using C. albicans-specific primers (Table 1). The fold change was expressed as 2-ΔΔCt (co-culture)-Ct (single culture). All experiments were repeated at least 3 times.

Statistical Analysis. The Student’s t-test was used for statistical analyses and the significance was accepted ATP < 0.05,0.01 < p < 0.05; 0.005 < p < 0.01; 0.001 < p < 0.005; 0.0005 < p < 0.001; 0.0001 < p < 0.0005.

Results

Inhibition of C. albicans strain CS5314 growth by oral bacteria. To determine the effect of oral bacteria on yeast growth, model C. albicans strain CS5314 was used in mixed cultures with 8 isolated oral bacterial strains, respectively. After 18h incubation, biofilm formation was visible at the bottom of all wells. Microscopic evaluation revealed a vast array of yeast-bacteria interactions, including bacterial attachment to yeast cells/hyphae, bacterial clumping and/or isolation away from yeast, and bacterial indifference to yeast (Figure 1). The presence of yeast cells, pseudohyphae, and hyphae also varied among yeast mixed with different bacterial strains. In addition, some bacteria appeared to be more effective than others in inhibiting the growth of C. albicans (data not shown).

Bacterial strains identification

The four most effective and four least effective bacterial strains in inhibiting yeast growth were chosen. 16S rDNA sequence analysis revealed S. aureus (Figure 1B), S. salivarius (Figure 1C), and

Table 1: Primers used in this study.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’ to 3’)</th>
<th>Purpose</th>
</tr>
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<tbody>
<tr>
<td>Bac-16S-F</td>
<td>AGAAGTTGATCCCTTGGCTCAG</td>
<td>Sequence of bacterial 16S rDNA</td>
</tr>
<tr>
<td>Bac-16S-R</td>
<td>TACGTTAATCTGTTAGACCTT</td>
<td>Sequence of bacterial 16S rDNA</td>
</tr>
<tr>
<td>Yeast-18S-F</td>
<td>ATCTGTTGATACCTGGCAGT</td>
<td>Sequence of yeast 18S rDNA</td>
</tr>
<tr>
<td>Yeast-18S-R</td>
<td>GATCTTCTCGCCAGGTTACC</td>
<td>Sequence of yeast 18S rDNA</td>
</tr>
<tr>
<td>Ca-18S-qPCR-F</td>
<td>GACTCAACAGGGGAAAACCT</td>
<td>qPCR for C. albicans</td>
</tr>
<tr>
<td>Ca-18S-qPCR-R</td>
<td>ATTCTCGTGTGAAAGAGCA</td>
<td>qPCR for C. albicans</td>
</tr>
</tbody>
</table>

Enterobacter aerogenes (Figure 1D) were the species identified as being able to inhibit yeast growth the most; whereas the four strains that were least effective were Neisseria subflava, an unidentifiable Neisseria species, Neisseria meningitides and Neisseria flavescens (data not shown). Among all eight bacterial species, S. aureus showed the highest capability of impairing the growth of C. albicans C5314 strain.

Inhibition of growth of clinically isolated yeast strains by S. aureus. To study the inhibitive effect of clinically isolated S. aureus strain on oral yeasts, 7 yeast strains were isolated and purified from the saliva of different healthy human subjects. The 18s rDNA sequencing showed that they all belong to C. albicans. Mixed culture experiments were carried out in semi-CDM [21]. After an 18 h co-incubation at 37°C, all cultures were harvested, and total genomic DNA was isolated, and qPCR was utilized for measuring the abundance of C. albicans by using C. albicans-specific primers [22]. This primer pair did not generate any PCR product when using S. aureus or S. gordonii chromosomal DNA as template (data not shown). As shown in Figure 2, compared to the single culture control, the growth of C. albicans #9 and #20 were reduced by 6.33-fold and 8.50-fold in the presence of S. aureus, respectively (0.0001 < P < 0.0005), and the other 5 strains displayed 2-3 times reduction as well. This difference of S. aureus inhibitory effects might be due to various characteristics of the 7 clinically isolated strains of C. albicans, thus, further investigation is required to determine the exact mechanism.

Inhibition of pseudohyphae or hyphae formation of clinically isolated yeast strains by S. aureus. It has been demonstrated that S. gordonii can enhance C. albicans hyphal morphogenesis [15], due to the inhibitive effect of this S. aureus strain on the growth of C. albicans; we hypothesized that it might be able to affect hyphal formation of latter. To study this, mixed culture experiments were implemented and showed that S. aureus suppressed the formation of pseudohyphae or hyphae of all seven clinical C. albicans strains in spite of the discrepant capability of generating pseudohyphae or hyphae (data not shown). C. albicans #7, generating the most luxurious hyphae, was chosen to be representative in this study, and S. gordonii was used as control because of its ability to promote C. albicans hyphal formation [15,23]. As shown in Figure 3, single culture of C. albicans #7 strain formed robust hyphae (Figure 3A), but in the presence of S. aureus, the formation of hyphae was nearly suppressed (Figure 3B). In contrast, S. gordonii lightly increased the C. albicans hyphal formation (Figure 3C). To our knowledge, this is the first report of S. aureus strain inhibiting the hyphal formation of C. albicans in vitro.

Discussion

Growth suppression of C. albicans by oral flora has been well documented in literature. The aim of this study was to investigate the effect of clinical isolated oral bacterial species on the growth and hyphal formation of C. albicans isolated from the oral cavity. Our findings indicate that some bacterial species isolated from saliva could suppress yeast growth to some extent, and one isolated S. aureus strain showed the strongest inhibitive effect on the growth and pseudohyphae/hyphae formation of all clinically isolated C. albicans strains.
Candida infections rely on the organism’s ability to switch morphology between yeast cells and hyphae forms in order to adhere to surfaces, form biofilms, and penetrate tissues [7,24]. Hyphae are believed to be the invasive and pathogenic form of Candida species while yeast is the commensal nonpathogenic form [4]. Some molecules generated by Gram-negative bacteria were reported to inhibit the formation of hyphae in C. albicans, e.g. 3-oxo-C12 homoserine lactone or cis-2-dodecenoic acid (BDSF) produced by Pseudomonas aeruginosa or Burkholderia conorpecia, respectively [25,26]. Gram-positive bacterium Streptococcus mutans also produces Competence Stimulating Peptide (CSP), trans-2-decenoic acid and secondary metabolites to suppress filamentation in C. albicans [27-29]. However, there is no report about hyphal repression of C. albicans by S. aureus, since it is well known that these two microbes usually form mixed fungal-bacterial biofilms, and this structure confers them extreme resistance to antimicrobial compounds or host clearance forces [30, 31]. Furthermore, C. albicans hyphal protein agglutinin-like sequence 3 (Als3p) can regulate S. aureus binding to C. albicans hyphae, thus, C. albicans establishes the biofilm base and facilitates the development of S. aureus biofilm [32-34]. In this study, we found one S. aureus strain, isolated from the mouth of a healthy people, apparently suppressed the formation of C. albicans pseudohyphae and hyphae in semi-CDM, which was often utilized to mimic human oral condition [21]. In contrast, similar to the report [15], S. gordonii did not affect C. albicans hyphae formation. Thus, this result provides a great evidence to prove the complexity of interspecies interaction in human oral cavity. The inhibitory effect of various bacteria on the development of C. albicans hyphae should play a crucial role for its pathogenicity.

Although most studies depicted both C. albicans and S. aureus exist in a competitive relationship, quorum sensing molecule farnesol generated by C. albicans was found to interrupt S. aureus cell membrane integrity and then inhibit its biofilm formation and viability [35,36]. Thus far, the inhibitory effect of S. aureus on C. albicans has not been reported. In this study, S. aureus, S. salivarius, and E. aerogenes were determined to be effective in inhibiting yeast growth, and S. aureus was the most effective species. Using qPCR assay, 2 strains in all 7 strains of C. albicans were identified as being strongest inhibited on the growth by S. aureus, and the rest five C. albicans strains showed reduced growth to some extent in the presence of S. aureus (Figure 3). In a study exploring the antagonistic fungal-bacterial interaction between C. albicans and P. aeruginosa, Hogan and Koler determined that P. aeruginosa was unable to bind or kill the yeast form of C. albicans, but it could form a dense biofilm on C. albicans hyphae, which killed the fungus [37]. Surprisingly, in this study, S. aureus strain apparently suppressed the pseudohyphal/hyphae formation of all isolated C. albicans strains, but displayed the obviously inhibitory effects on their growth. Thus, further studies are required to figure out the mechanism(s) of repression of S. aureus on C. albicans.

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References


