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Cellular Components Mediating Coadherence of *Candida albicans* and *Fusobacterium nucleatum*

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Abstract

Candida albicans is an opportunistic fungal pathogen found as part of the normal oral flora. It can be coisolated with *Fusobacterium nucleatum*, an opportunistic bacterial pathogen, from oral disease sites, such as those involved in refractory periodontitis and pulp necrosis. The physical coadherence between these 2 clinically important microbes has been well documented and suggested to play a role in facilitating their oral colonization and colocalization and contributing to polymicrobial pathogenesis. Previous studies indicated that the physical interaction between *C. albicans* and *F. nucleatum* was mediated by the carbohydrate components on the surface of *C. albicans* and the protein components on the *Fusobaterium* cell surface. However, the identities of the components involved still remain elusive. This study was aimed at identifying the genetic determinants involved in coaggregation between the 2 species. By screening a *C. albicans* SN152 mutant library and a panel of *F. nucleatum* 23726 outer membrane protein mutants, we identified *FLO9*, which encodes a putative adhesin-like cell wall mannoprotein of *C. albicans* and *radD*, an arginine-inhibitable adhesin-encoding gene in *F. nucleatum* that is involved in interspecies coadherence. Consistent with these findings, we demonstrated that the strong coaggregation between wild-type *F. nucleatum* 23726 and *C. albicans* SN152 in an in vitro assay could be greatly inhibited by arginine and mannose. Our study also suggested a complex multifaceted mechanism underlying physical interaction between *C. albicans* and *F. nucleatum* and for the first time revealed the identity of major genetic components involved in mediating the coaggregation. These observations provide useful knowledge for developing new targeted treatments for disrupting interactions between these 2 clinically relevant pathogens.

Keywords: coaggregation, interspecies interaction, oral microbiota, opportunistic pathogen, adhesin, mannoprotein

Introduction

Candida albicans is a commensal fungal species commonly colonizing human mucosal surfaces, which can be isolated from the oral cavity of as many as 60% of healthy adult subjects (Odds et al. 1988; Glick and Siegel 1999). It coexists with diverse oral microbial species and has long been adapted to the human host (Morales and Hogan 2010). In healthy individuals, *C. albicans* is usually a minor component of their oral microbial flora. However, under conditions of immune dysfunction or local predisposing factors such as poor oral hygiene or ill-fitted dentures, colonizing *C. albicans* strains can become opportunistic pathogens. In these patients, *C. albicans* becomes more predominant and invasive, causing recurrent mucosal infection, such as denture stomatitis or life-threatening disseminated infections (Boerlin et al. 1995; Coleman et al. 1997; Perlroth et al. 2007; Azie et al. 2012).

The colonization of *C. albicans* in the human oral cavity can be achieved through its adherence to a variety of salivary pellicle components, including proline-rich proteins and statherin (Calderone and Braun 1991). Moreover, it is able to directly bind to the oral epithelial cells through adhesion/ligand interactions (Salgado et al. 2011). In addition to oral tissue surfaces, increasing evidence suggests that *C. albicans* could establish themselves by incorporation into existing biofilms through adherence to a variety of oral bacterial species including streptococci, actinomyces, and fusobacteria (Shirtliff et al. 2009).

Fusobacterium nucleatum is another opportunistic oral pathogen that has been implicated in periodontal disease (Signat et al. 2011). Best known for its ability to adhere to a diverse range of oral bacteria, it is considered a "bridging" organism that links early commensal colonizers and late colonizing species, many of which are periodontal pathogens; therefore, it plays an important role in the succession of genera in oral polymicrobial communities (Kolenbrander 2000; Rickard et al. 2003). Other than interacting with bacterial species, fusobacteria can establish direct physical association with

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A supplemental appendix to this article is published electronically only at http://jdr.sagepub.com/supplemental.

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eukaryotic microbes such as fungi. The coadherence between *F. nucleatum* and *C. albicans* has been well documented and has been implicated in facilitating the colonization of *C. albicans* within the oral cavity (Grimaudo and Nesbitt 1997; Jabra-Rizk et al. 1999). Likewise, the coisolation of *C. albicans* with *F. nucleatum* from a variety of oral disease sites—including refractory periodontitis, such as therapy-resistant apical periodontitis (Waltimo et al. 1997) and pulp necrosis (Lana et al. 2001)—further suggested their interaction as well as involvement in polymicrobial pathogenesis.

Previous studies suggested that the coadherence between *C. albicans* and *F. nucleatum* was mediated by the carbohydrate components on the surface of *C. albicans* and the protein components on the *Fusobacterium* cell surface (Grimaudo and Nesbitt 1997; Jabra-Rizk et al. 1999). However, the identities of the components involved remain elusive. In the current study, by screening a *C. albicans* mutant library and a panel of *F. nucleatum* outer membrane protein mutants, we aimed to identify the genetic components involved in coadherence between these 2 clinically relevant opportunistic pathogens.

Materials and Methods

Strains and Growth Conditions

Wild-type strain *F. nucleatum* 23726 and its mutant derivatives lacking one of the large fusobacterial outer membrane proteins—including Fn0254, Fn0387, Fn1449 (Fap2), Fn1526 (RadD), Fn1554, Fn1893, Fn2047, or Fn2058 (Aim1; Kaplan et al. 2009; Kaplan et al. 2010; Gur et al. 2015)—were cultivated in Columbia broth (Difco, Detroit, MI, USA) at 37 °C anaerobically (nitrogen 90%, carbon dioxide 5%, hydrogen 5%).

A *C. albicans* SN152 mutant library was purchased from the University of California, San Francisco (Noble et al. 2010). The library contains 674 homozygous deletion mutants covering roughly 11% of the *C. albicans* genome. *C. albicans* wild-type and mutants were precultured in yeast peptone dextrose (YPD) medium (yeast extract, 10 g/L; peptone, 20 g/L; glucose, 20 g/L) at 30 °C in an atmosphere of 5% CO_2 in air. Hyphal growth of *C. albicans* was induced by inoculating overnight cultures in fresh YPD medium supplemented with 20% fetal bovine serum and aerobically incubated at 37 °C until the OD600 reached 0.2.

Coaggregation Assay

Coaggregation assays were preformed in modified coaggregation buffer (CAB) as previously described (He et al. 2012). Since hyphae-displaying *C. albicans* SN152 cells tend to precipitate even in the absence of coaggregating bacterial partner species, direct visual microscopic observation of coaggregation between *C. albicans* and *F. nucleatum* cells was performed. Briefly, exponential bacterial cells and hypha-displaying candida cells were collected and resuspended in CAB to an OD_{600} of 1 and 2, respectively. Equal volumes (250 μL) of bacterial and candida cells were mixed in reaction tubes. The tubes were vigorously vortexed for 30 s and allowed to stand at room temperature for 30 min. Coaggregating samples were taken and observed using light microscopy; images were taken from 10 random fields of view. For each coaggregation sample, 10 random candida hypha-displaying cells were chosen, and the attached fusobacterial cells were counted for each cell. Based on the average number (n) of attached bacterial cells per 20 μm of hyphal body length, coaggregation was graded on a 0 to 4 scale: score 4, $n \geq 20$; score 3, $10 \leq n < 20$; score 2, $5 \leq n < 10$; score 1, $1 \leq n < 5$; score 0, when no attached bacterial cells could be observed.

Fluorescence-based Coaggregation Assay

To achieve a more quantitative and sensitive readout, a fluorescence-based coaggregation assay was developed (see Appendix for detailed experimental procedure). Briefly, Syto 9 green fluorescent nucleic acid dye–stained fusobacterial cells were used to set up coaggregation assays with *Candida*. Coaggregation mixtures were then washed to remove unattached fusobacterial cells. The coaggregation between *Candida* and *Fusobacterium* was quantified by measuring the intensity of the florescence signal retained on the *Candida* cell surface.

Screening a C. albicans *SN152 Library for Mutants Defective in Coaggregating with* F. nucleatum *23726*

Two microliters of each mutant from a *C. albicans* SN152 mutant library stored in 96-well plates was transferred to plates containing 200 μL of fresh YPD medium in each well. Plates were incubated at 30 °C in an atmosphere of 5% CO_2 in air. After overnight growth, 60 mutant strains showing growth defects (with cell density <0.1 at OD_{600}) were excluded while the rest of the mutants were spun down and resuspended in fresh YPD medium supplemented with 20% fetal bovine serum. The plates were next further incubated aerobically at 37 °C for 4 h to induce hyphal growth. *C. albicans* cells were resuspended in CAB to a final OD_{600} of 0.5 to 1. Meanwhile, exponential-phase *F. nucleatum* cells were resuspended in CAB to a final OD_{600} of 1. One hundred microliters of bacterial cells were then added into each well of 96-well plates containing *C. albicans* mutants and mixed by pipetting. Plates were incubated at room temperature for 30 min before samples were taken and aggregation was observed under a light microscope. Mutants defective in coaggregating with wild-type *F. nucleatum* were confirmed by fluorescence-based coaggregation assays.

Inhibition of Coaggregation between F. nucleatum *and* C. albicans

By using the coaggregation assay described above, the following sugars or amino acids at a final concentration of 100 mM (Grimaudo and Nesbitt 1997) were tested for their ability to inhibit coaggregation between *C. albicans* and *F. nucleatum*:

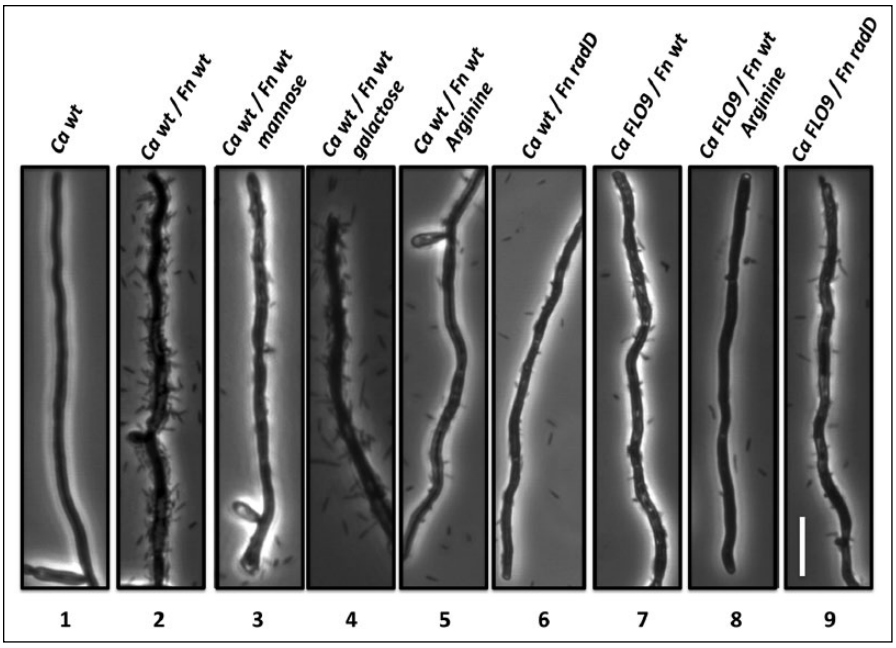

Figure 1. Microscopic observation of coaggregation between *Candida albicans* (*Ca*) and *Fusobacterium nucleatum* (*Fn*) under different treatments. For each interacting pair, the pictures of 10 random *Ca* cells were taken and 1 representative image shown. The scale bar is 10 µm. wt, wild type.

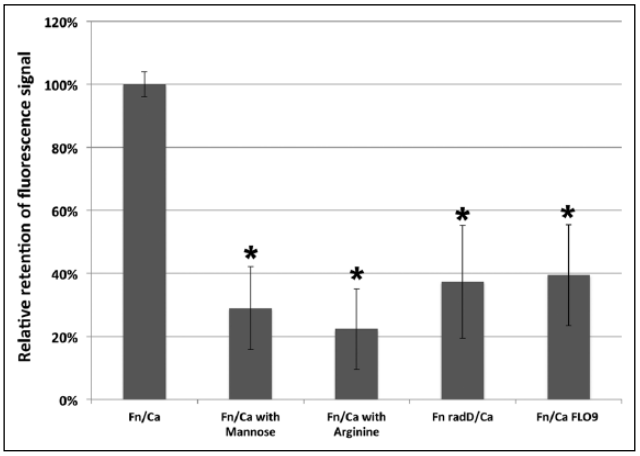

Figure 2. Coaggregation between *Candida albicans* (*Ca*) and *Fusobacterium nucleatum* (*Fn*). Fluorescence-based coaggregation assay (as described in Materials and Methods) was performed for *Fn* and *Ca*, both wild type and mutants. The result was represented as the percentage of fluorescence signal retained compared with the control group containing wild-type *Ca* and *Fn* as a coaggregating pair. Average values ± SD are shown. Asterisk indicates the significant difference between the testing group and the control group (wild-type *Fn*/*Ca; P* < 0.05, *t* test). Note: The absolute number of relative fluorescence units represented by 100% was 2,500, corresponding to about 2×10^6 colony-forming units per milliliter of *Fn* based on the experimental procedure described in the Materials and Methods.

D-galactose, D-lactose, D-mannose, L-leucine, N-acetyl-glucosamine, and L-arginine.

Recruitment Assay

Recruitment assays were designed and performed to test the ability of coaggregation-defective *C. albicans* and *F. nucleatum* mutants identified in this study to bind to monospecies biofilms of their wild-type interacting partner. Detailed procedures are provided in the Appendix. Briefly, planktonic cells of *C. albicans* (wild type and mutants) or *F. nucleatum* (wild type and mutants) were added to the monospecies biofilm of their wild-type interacting partner, and their ability to bind to the biofilm cells was determined by cell counting.

Results

C. albicans *SN152 Mutant FLO9 Exhibits Reduced Ability in Coaggregating with* F. nucleatum

The coaggregating ability between *C. albicans* SN152 and *F. nucleatum* 23726 was tested using cell suspensions in CAB. Coaggregation was monitored and recorded by light microscopy and fluorescence-based assays. Wild-type *C. albicans* SN152 hyphal cells and *F. nucleatum* 23726 displayed a strong coaggregation phenotype (Fig. 1, panel 2), while no aggregation was detected between the yeast form of *C. albicans* and *F. nucleatum* under the same coaggregating conditions tested (data not shown). To identify the genetic components of *C. albicans* involved in its coaggregation with *F. nucleatum*, a homozygous *C. albicans* SN152 gene deletion library (Noble et al. 2010) was screened for mutants that had normal growth but were defective in coadherence with *F. nucleatum*. The library contains 674 homozygous deletion mutants covering roughly 11% of the *C. albicans* genome. The screen led to the discovery of 1 mutant, *C. albicans* SN152 FLO9, which displayed the most drastically reduced ability in coaggregating with *F. nucleatum*. As revealed by microscopic observation, the surface of the FLO9 mutant was only sparsely decorated with *F. nucleatum* (Fig. 1, panel 7), as compared with wild-type *C. albicans*, whose hyphal surface was extensively covered by the coaggregating bacterial partner. Fluorescence-based coaggregation assay showed that, when compared with the wild type, the fluorescence signal retained decreased >60%, suggesting a severe reduction in the number of attached *F. nucleatum* cells (Fig. 2). *FLO9* encodes a putative adhesin-like cell wall mannoprotein (Noble et al. 2010), and our data suggested its involvement in mediating physical interaction with *F. nucleatum*.

F. nucleatum *RadD Mutant Is Defective in Coaggregating with* C. albicans *SN152*

A previous study indicated the involvement of the protein components on the *Fusobaterium* cell surface in its coaggregation

Table. Effect of Treatments on Coaggregation between *Candida albicans* and *Fusobacterium nucleatum*.

Coaggregating Pair	No Treatment	D-lactose	D-galactose	D-mannose	L-leucine	L-arginine	N-acetyl-glucosamine
Ca wt / *Fn* wt	4	4	4	2	4	2	4
Ca FLO9 / *Fn* wt	2	2	2	2	2	2	2
Ca wt / *Fn* RadD	2	2	2	2	2	2	2
Ca FLO9 / *Fn* RadD	2	2	2	2	2	2	2

For each coaggregation sample, 10 random hyphae-displaying *C. albicans* (*Ca*) cells were chosen; the attached *F. nucleatum* (*Fn*) cells were counted. Based on the average number (*n*) of attached bacterial cells per 20 µm of hyphal body length, coaggregation was graded on a 0 to 4 scale: score 4, $n \geq 20$; score 3, $10 \leq n < 20$; score 2, $5 \leq n < 10$; score 1, $1 \leq n < 5$; score 0, no attached bacterial cells could be found.

with *C. albicans* (Grimaudo and Nesbitt 1997). In this study, a panel of *F. nucleatum* outer membrane protein mutants generated in our laboratory (Kaplan et al. 2009; Kaplan et al. 2010) was tested for their coaggregating ability with *C. albicans* SN152. The results showed that among the 8 mutants tested, RadD was the only mutant defective in binding to the hypha-displaying *C. albicans* cells (Fig. 1, panel 6), with a >60% reduction in fluorescence signal recovered from the *C. albicans*–bound fusobacterial cells as compared with the wild type (Fig. 2). *RadD* encodes an arginine-inhibitable adhesin that has been shown to be required for interspecies coadherence between fusobacteria and oral streptococci (Edwards et al. 2007; Kaplan et al. 2009). Our data suggested that it also plays a role in mediating interaction with *C. albicans.*

We further tested the coaggregation phenotype between *F. nucleatum* RadD and *C. albicans* FLO9. Microscopic observation and fluorescence-based assay did not reveal any significant difference in coaggregation between the interacting pairs with either one or both mutant partners. In addition, there was visible residual coaggregation between fusobacterial and *Candida* cells when RadD and FLO9 mutants were used as interacting pair (Fig. 1, panel 9).

Mannose- and Arginine-inhibitable Coaggregation between C. albicans *and* F. nucleatum

Mutant screening revealed that the mannoprotein FLO9 from *C. albicans* and arginine-inhibitable RadD from *F. nucleatum* are involved in the interspecies coaggregation. These findings suggested that the observed strong coadherence phenotype between *C. albicans* and *F. nucleatum* might be mannose/arginine inhibitable. To confirm this, mannose and arginine, among other carbohydrates and amino acids, were tested for their ability in inhibiting the coadherence. The results showed that the presence of 0.1M mannose or arginine resulted in a drastic reduction in the coaggregation between the *Candida* and *Fusobacterium* pair, while other carbohydrates or amino acids tested did not have a significant effect on the coaggregation (Table). This was revealed by microscopic observation as well as the more quantitative fluorescence-based coaggregation assay showing about 70% reduction in the signal retention vs. the control (Fig. 1, panels 3, 5; Fig. 2), indicating the reduced number of *F. nucleatum* cells attached to *Candida* hyphal cells. Moreover, the addition of other monosaccharides (e.g., D-galactose) and amino acids (including L-leucine) had no apparent inhibitory effects on the coaggregation (Fig. 1, panel 4; Table).

C. albicans *SN152 FLO9 and* F. nucleatum *RadD Displayed Reduced Ability to Adhere to the Surface Attached Biofilm Cells of Their Respective Interacting Partners*

Our data showed that *C. albicans* and *F. nucleatum* were able to coaggregate under planktonic conditions, while mutation of *FLO9* in *C. albicans* and *radD* in *F. nucleatum* rendered the mutants defective in interspecies binding. To further investigate the observed FLO9- and RadD-dependent coadherence property between *C. albicans* and *F. nucleatum* under biofilm conditions, *C. albicans* SN152 FLO9 and *F. nucleatum* RadD mutants were tested for their ability to adhere to monobiofilms of wild-type *F. nucleatum* and *C. albicans*, respectively. The results showed that, compared with its wild-type counterparts, *F. nucleatum* RadD and *C. albicans* SN152 FLO9 displayed significantly reduced capabilities (with about 50% and 70% reductions, respectively) to adhere to monospecies biofilms of their respective wild-type interacting partners (Fig. 3).

Discussion

Coadhesion between different microbial species is a common feature of the oral microbiome. It plays a crucial role in colonization of the oral cavity, contributes to the formation of highly structured multispecies communities, and ultimately affects polymicrobial pathogenesis (Kolenbrander 1988, 1989; Kolenbrander et al. 1999; Kolenbrander et al. 2006; Peters et al. 2012). Such physical interspecies association not only exists among bacterial species but has also been observed for 2 clinically relevant opportunistic pathogens of different kingdoms, *C. albicans* and *F. nucleatum* (Grimaudo and Nesbitt 1997; Jabra-Rizk et al. 1999). Knowledge of the major genetic determinants involved in coadherence between these 2 microbes may help shed light on *Candida* succession and possible polymicrobial disease processes involving these 2 species.

Microscopic observations and fluorescence-based quantitative coaggregation assays clearly demonstrated the strong coadherence phenotype between the bacterial species *F. nucleatum* 23726 and the eukaryotic microbe *C. albicans* SN152 in the

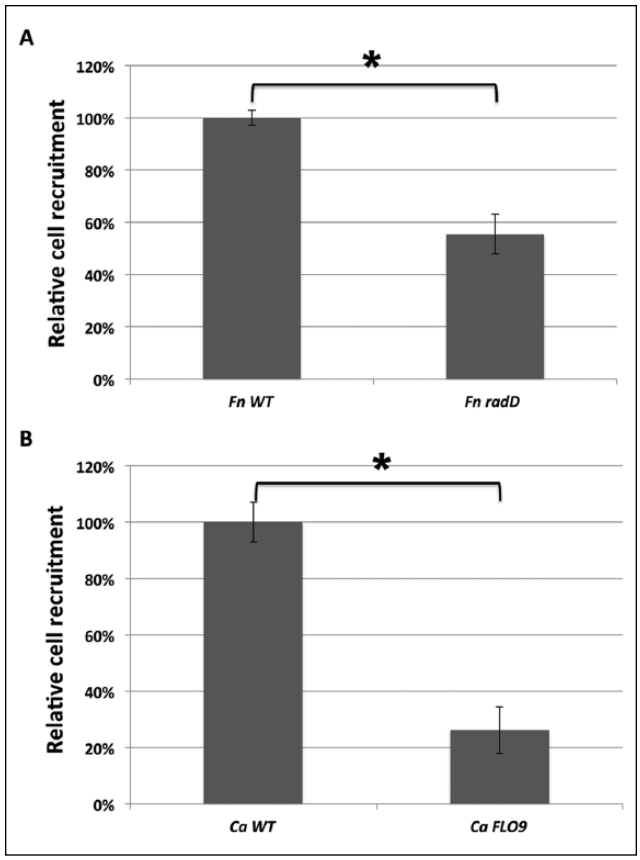

Figure 3. Binding of *Candida albicans* (*Ca*) and *Fusobacterium nucleatum* (*Fn*) to monospecies biofilm of their interacting partner species. Recruitment assay (as described in Materials and Methods) was performed to test the ability of *Fn* RadD and *Ca* FLO9 mutant to adhere to monospecies biofilm of wild type (WT) *Ca* (**A**) and *Fn* (**B**), respectively. The result was represented as the percentage of mutant cells adhered to biofilm of its coaggregating partner compared with the WT control. Average values ± SD are shown. Asterisk indicates significant difference between the 2 sample sets ($P < 0.05$, *t* test). Note: The absolute number of cells represented by 100% was about 4×10^7 colony-forming units per milliliter for *Fn* (A) and 2×10^6 colony-forming units per milliliter for *Ca* (B) based on the experimental procedure described in Materials and Methods.

hyphal form (Fig. 1, panel 2; Fig. 2). Screening of a *C. albicans* SN152 mutant library resulted in the identification of the *FLO9* gene, which is critical in establishing physical interaction with *F. nucleatum. FLO9* encodes a putative adhesin-like cell wall mannoprotein in *C. albicans* (Boisrame et al. 2011). Mutation in this gene does not affect its colony morphology or vegetative growth, nor does it affect competitive fitness in a mouse intravenous infection model (Noble et al. 2010). However, this mutant displayed a drastically reduced ability in coadherence with *F. nucleatum* as revealed by coaggregation assays, suggesting its role in mediating the observed duo-species interaction (Fig. 1, panel 7; Fig. 2). The identification of the mannoprotein FLO9 as the surface component mediating the coadherence of *C. albicans* with *F. nucleatum* was further confirmed by our observation that the coaggregation phenotype was mannose inhibitable (Fig. 1, panel 3). This is consistent with previous reports showing that the binding of *F. nucleatum* strains to *Candida* species, including *C. albicans* and *C. dubliniensis*, could be inhibited by addition of mannose but not other carbohydrates tested (Grimaudo and Nesbitt 1997; Jabra-Rizk et al. 1999).

C. albicans has a multilayered cell wall composed of an outer layer of proteins glycosylated with N- or O-linked mannosyl residues and an inner skeletal layer of beta-glucans and chitin (Gow and Hube 2012). The mannoproteins have been shown to play important roles in diverse cellular functions, such as acting as specific immunodeterminants (Gow and Hube 2012), binding to host-secreted macromolecules (Tronchin et al. 1988), as well as functioning as adhesins to mediate the direct *Candida*-bacteria and *Candida*–host cell recognitions (Salgado et al. 2011; Dutton et al. 2014). In *C. albicans, mnt1* and *mnt2* encode partially redundant α-1, 2-mannosyltransferases that catalyze the addition of the second and third mannose residues in an O-linked mannose pentamer, while *och1* encodes a α-1, 6-mannosyltransferase that is involved in N-glycan outer chain branch addition. In a recent study, Dutton et al. (2014) demonstrated that O-mannosylation via Mnt, specifically Mnt1, is critical for hyphal adhesion functions required for coadherence between *C. albicans* and *Streptococcus gordonii*. In our study, a *mnt1* mutant displayed a normal coaggregation phenotype with *F. nucleatum*, while the *och1* mutant showed drastically reduced coaggregation with *F. nucleatum* (Appendix Fig. 1) even though this mutant was not initially identified via library screening due to its growth defects. Our data suggested that N-linked glycosylation could be more important for FLO9 in determining its binding ability with its counterpart in *F. nucleatum*. Furthermore, FLO9 was predicted to be hyphally regulated and expressed during hyphal growth (http://www.candidagenome.org). This is in agreement with our data showing that the coadherence can be observed only between *F. nucleatum* and *C. albicans* hyphal, but not yeast cells.

Our study further revealed that RadD, an outer member protein in *F. nucleatum* 23726, is also involved in mediating the coadherence between fusobacterial and candidal cells. RadD is an arginine-binding protein (Edwards et al. 2007) and has been shown to be an adhesin required for the coadherence of *F. nucleatum* with gram-positive oral bacterial species, such as streptococci (Kaplan et al. 2009). More important, our data showed that no additive effects in the reduction of coaggregation can be observed when *F. nucleatum* RadD and *C. albicans* FLO9 were tested as a pair compared with interacting pairs containing either mutant (Fig. 1, panels 6 to 9). These data strongly suggested that RadD is acting as the counterpart component for FLO9 in *C. albicans* and that these 2 components could directly interact with each other and mediate coadherence. Consistent with this finding, exogenous arginine was able to achieve a similar inhibitory effect on the coaggregation between *F. nucleatum* and *C. albicans* compared with mannose (Fig. 1, panels 3 and 5; Table). Previous studies also suggested that other than a mannan-containing receptor on the *C. albicans*, the coadherence between *F. nucleatum* and *C. albicans* involves a heat-liable component on *F. nucleatum* (Grimaudo and

Nesbitt 1997). Meanwhile, sequence analysis of RadD revealed a domain close to the C-terminus (1513-1707aa) with homology to a sequence encoding a bacteriophage tail fiber. Previous studies showed that the adsorption of bacteriophage can be achieved through the binding of tail fibers to the polymannose O-antigens on the *Escherichia coli* cell surface (Heller and Braun 1982). These observations implied that RadD potentially has binding sites for both mannose and arginine. It could act as a receptor and bind to the specific mannan residue on the FLO9 mannoprotein on the surface of *C. albicans* to mediate physical interaction. Meanwhile, the binding of arginine might induce a conformational change in the binding pocket for mannose and result in the disruption of the coadherence between the 2 species. Further studies are needed to determine the respective binding sites in RadD for mannose and arginine.

It is worthwhile to point out that, in a study by Jabra-Rizk et al. (1999), the coaggregation between *C. albicans* and *F. nucleatum* was mannose inhibitable, while arginine had no such effects. In that study, *F. nucleatum* ATCC 49256 was tested—a *F. nucleatum* subsp. *vincentii* strain instead of a *F. nucleatum* subsp. *nucleatum* strain. Furthermore, the *C. albicans* strains used by Jabra-Rizk et al. were all clinical isolates, and they displayed different phenotypes when coaggregating with *F. nucleatum* ATCC 49256. Although FLO9 is conserved among many *Candida* species and *C. albicans* subspecies, RadD homologues have been found in different *Fusobacterium* species and subspecies, including *F. nucleatum* subsp. *vincentii.* It remains to be determined if these homologues could display differential adhesion/ligand specificities. Our recent study showed that different *F. nucleatum* subspecies display significantly differential coaggregation abilities with the same *Streptococcus mutans* strain (unpublished data), suggesting the involvement of different adhesins or the differential binding specificity of homologous adhesins in different subspecies when interacting with their partner species. Thus, it is likely that the observed difference in arginine inhibitability of the coaggregation could be due to the different strains used. The observed strain specificity in interspecies coadhesion could have an impact on host susceptibility to disease and/or the effectiveness of coadherence preventive measures.

In addition, residual coaggregation was observed even when *F. nucleatum* RadD and *C. albicans* FLO9 were used as interacting pairs (Fig. 1, panel 9), which suggested the presence of other receptor/ligand pairs playing minor roles in mediating the observed interspecies coadherence. This is consistent with the multimodal nature of adhesion of *C. albicans* in its coadherence with other oral species, such as *S. gordonii* (Holmes et al. 1996; Silverman et al. 2010) as well as host cells (Calderone and Braun 1991).

We also demonstrated that compared with their wild-type counterparts, both *F. nucleatum* RadD and *C. albicans* FLO9 were defective in coadhering to the monospecies biofilms of wild-type *C. albicans* and *F. nucleatum*, respectively (Fig. 3; Appendix Fig. 2). Oral colonization is a crucial step in bacterial/fungal infections, which can be achieved via directly binding to host tissue surfaces or coadhering to the microbes that have already been established within surface-attached biofilm. Increasing evidence suggests that the coadherence between *Candida* and bacterial species could promote the formation of mix-species biofilms and affect polymicrobial pathogenicity (Adam et al. 2002; Fox et al. 2014; Xu et al. 2014). Thus, intergenic interaction between *C. albicans* and *F. nucleatum*, 2 clinically relevant oral species, not only contributes to their succession in the oral cavity but could also ultimately affect their pathogenicity during polymicrobial infections and so warrants further investigation. In addition, further characterization of the interacting cellular components could help develop new targeted treatments to interfere with the coadherence, a process that could play an important role in polymicrobial pathogenesis involving these 2 microbes.

Author Contributions

T. Wu, contributed to design, data acquisition, analysis, and interpretation, drafted the manuscript; L. Cen, contributed to data acquisition and interpretation, critically revised the manuscript; C. Kaplan, contributed to data acquisition, critically revised the manuscript; X. Zhou, contributed to data analysis and interpretation, critically revised the manuscript; R. Lux, contributed to design, data analysis, and interpretation, critically revised the manuscript; W. Shi, X. He, contributed to conception, design, data analysis, and interpretation, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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References

Adam B, Baillie GS, Douglas LJ. 2002. Mixed species biofilms of *Candida albicans* and *Staphylococcus* epidermidis. J Med Microbiol. 51(4):344–349.

Azie N, Neofytos D, Pfaller M, Meier-Kriesche HU, Quan SP, Horn D. 2012. The PATH (Prospective Antifugal Therapy) Alliance® registry and invasive fungal infections: update 2012. Diagn Microbiol Infect Dis. 73(4):293–300.

Boerlin P, Boerlin-Petzold F, Durussel C, Addo M, Pagani JL, Bille J. 1995. Cluster of oral atypical *Candida albicans* isolates in a group of human immunodeficiency virus–positive drug users. J Clin Microbiol. 33(5):1129–1135.

Boisrame A, Cornu A, Da Costa G, Richard ML. 2011. Unexpected role for a serine/threonine-rich domain in the *Candida albicans* Iff protein family. Eukaryot Cell. 10(10):1317–1330.

Calderone RA, Braun PC. 1991. Adherence and receptor relationships of *Candida albicans*. Microbiol Rev. 55(1):1–20.

Coleman DC, Sullivan DJ, Bennett DE, Moran GP, Barry HJ, Shanley DB. 1997. Candidiasis: the emergence of a novel species, *Candida dubliniensis*. AIDS. 11(5):557–567.

Dutton LC, Nobbs AH, Jepson K, Jepson MA, Vickerman MM, Aqeel Alawfi S, Munro CA, Lamont RJ, Jenkinson HF. 2014. O-mannosylation in *Candida albicans* enables development of interkingdom biofilm. MBio. 5(2):e00911.

Edwards AM, Grossman TJ, Rudney JD. 2007. Association of a high-molecular weight arginine-binding protein of *Fusobacterium nucleatum* ATCC 10953 with adhesion to secretory immunoglobulin A and coaggregation with *Streptococcus cristatus*. Oral Microbiol Immunol. 22(4):217–224.

Fox EP, Cowley ES, Nobile CJ, Hartooni N, Newman DK, Johnson AD. 2014. Anaerobic bacteria grow within *Candida albicans* biofilms and induce biofilm formation in suspension cultures. Curr Biol. 24(20):2411–2416.

Glick M, Siegel MA. 1999. Viral and fungal infections of the oral cavity in immunocompetent patients. Infect Dis Clin North Am. 13(4):817–831.

Gow NA, Hube B. 2012. Importance of the *Candida albicans* cell wall during commensalism and infection. Curr Opin Microbiol. 15 (4):406–412.

Grimaudo NJ, Nesbitt WE. 1997. Coaggregation of *Candida albicans* with oral *Fusobacterium* species. Oral Microbiol Immunol. 12(3):168–173.

Gur C, Ibrahim Y, Isaacson B, Yamin R, Abed J, Gamliel M, Enk J, Bar-On Y, Stanietsky-Kaynan N, Coppenhagen-Glazer S, et al. 2015. Binding of the Fap2 protein of *Fusobacterium nucleatum* to human inhibitory receptor TIGIT protects tumors from immune cell attack. Immunity. 42(2):344–355.

He X, Hu W, Kaplan CW, Guo L, Shi W, Lux R. 2012. Adherence to streptococci facilitates *Fusobacterium nucleatum* integration into an oral microbial community. Microb Ecol. 63(3):532–542.

Heller K, Braun V. 1982. Polymannose O-antigens of *Escherichia coli*, the binding sites for the reversible adsorption of bacteriophage T5+ via the L-shaped tail fibers. J Virol. 41(1):222–227.

Holmes AR, McNab R, Jenkinson HF. 1996. *Candida albicans* binding to the oral bacterium *Streptococcus gordonii* involves multiple adhesin-receptor interactions. Infect Immun. 64(11):4680–4685.

Jabra-Rizk MA, Falkler WA, Merz WG, Kelley JI, Baqui AA, Meiller TF. 1999. Coaggregation of *Candida dubliniensis* with *Fusobacterium nucleatum*. J Clin Microbiol. 37(5):1464–1468.

Kaplan CW, Lux R, Haake SK, Shi W. 2009. The *Fusobacterium nucleatum* outer membrane protein RadD is an arginine-inhibitable adhesin required for inter-species adherence and the structured architecture of multispecies biofilm. Mol Microbiol. 71(1):35–47.

Kaplan CW, Ma X, Paranjpe A, Jewett A, Lux R, Kinder-Haake S, Shi W. 2010. *Fusobacterium nucleatum* outer membrane proteins Fap2 and RadD induce cell death in human lymphocytes. Infect Immun. 78(11):4773–4778.

Kolenbrander PE. 1988. Intergeneric coaggregation among human oral bacteria and ecology of dental plaque. Annu Rev Microbiol. 42:627–656.

Kolenbrander PE. 1989. Surface recognition among oral bacteria: multigeneric coaggregations and their mediators. Crit Rev Microbiol. 17(2):137–159.

Kolenbrander PE. 2000. Oral microbial communities: biofilms, interactions, and genetic systems. Annu Rev Microbiol. 54:413–437.

Kolenbrander PE, Andersen RN, Kazmerzak K, Wu R, Palmer RJ Jr. 1999. Spatial organization of oral bacteria in biofilms. Methods Enzymol. 310:322–332.

Kolenbrander PE, Palmer RJ Jr, Rickard AH, Jakubovics NS, Chalmers NI, Diaz PI. 2006. Bacterial interactions and successions during plaque development. Periodontol. 42:47–79.

Lana MA, Ribeiro-Sobrinho AP, Stehling R, Garcia GD, Silva BK, Hamdan JS, Nicoli JR, Carvalho MA, Farias Lde M. 2001. Microorganisms isolated from root canals presenting necrotic pulp and their drug susceptibility in vitro. Oral Microbiol Immunol. 16(2):100–105.

Morales DK, Hogan DA. 2010. *Candida albicans* interactions with bacteria in the context of human health and disease. PLoS Pathog. 6(4):e1000886.

Noble SM, French S, Kohn LA, Chen V, Johnson AD. 2010. Systematic screens of a *Candida albicans* homozygous deletion library decouple morphogenetic switching and pathogenicity. Nat Genet. 42(7):590–598.

Odds FC, Webster CE, Mayuranathan P, Simmons PD. 1988. Candida concentrations in the vagina and their association with signs and symptoms of vaginal candidosis. J Med Vet Mycol. 26(5):277–283.

Perlroth JB, Choi B, Spellberg B. 2007. Nosocomial fungal infections: epidemiology, diagnosis, and treatment. Med Mycol. 45(4):321–346.

Peters BM, Jabra-Rizk MA, O'May GA, Costerton JW, Shirtliff ME. 2012. Polymicrobial interactions: impact on pathogenesis and human disease. Clin Microbiol Rev. 25(1):193–213.

Rickard AH, Gilbert P, High HJ, Kolenbrander PE, Handley PS. 2003. Bacterial coaggregation: an integral process in the development of multi-species biofilms. Trends Microbiol. 11(2):94–100.

Salgado PS, Yan R, Taylor JD, Burchell L, Jones R, Hoyer LL, Matthews SJ, Simpson PJ, Cota E. 2011. Structureal basis for the broad specificity to host-cell ligands by the pathogenic fungus *Candida albicans*. Proc Natl Acad Sci U S A. 108(38):15775–15779.

Shirtliff ME, Peters BM, Jabra-Rizk MA. 2009. Cross-kingdom interactions: *Candida albicans* and bacteria. FEMS Microbiol Lett. 299(1):1–8.

Signat BC, Roques C, Poulet P, Duffaut D. 2011. *Fusobacterium nucleatum* in periodontal health and disease. Curr Issues Mol Biol. 13(2):25–36.

Silverman RJ, Nobbs AH, Vickerman MM, Barbour ME, Jenkinson HF. 2010. Interaction of *Candida albicans* cell wall Als3 protein with *Streptococcus gordonii* SspB adhesin promotes development of mixed-species communities. Infect Immun. 78(11):4644–4652.

Tronchin GJ, Bouchara JP, Robert R, Senet JM. 1988. Adherence of *Candida albicans* germ tubes to plastic: ultrastructural and molecular studies of fibrillar adhesins. Infect Immun. 56(8):1987–1993.

Waltimo TM, Siren EK, Torkko HL, Olsen I, Haapasalo MP. 1997. Fungi in therapy-resistant apical periodontitis. Int Endod J. 30(2):96–101.

Xu H, Jenkinson HF, Dongari-Bagtzoglou A. 2014. Innocent until proven guilty: mechanisms and roles of *Streptococcus-Candida* interactions in oral health and disease. Mol Oral Microbiol. 29(3):99–116.