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The opportunistic human fungal pathogen *Candida albicans* promotes the growth and proliferation of commensal *Escherichia coli* through an iron-responsive pathway

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ABSTRACT

Candida albicans is a commensal fungal species that commonly colonizes a heterogeneous mixture of human body where it intimately interacts with other microbes in the host environment such as the gastrointestinal (GI) tract. Most studies in fungal-bacterial interactions are about synergistic or antagonistic effects of bacterial functions on fungal physiological activities including pathogenicity. Very few studies have been demonstrated about the role of fungi on bacteria. In this study, we investigated the interactions between *C. albicans* and the bacterium *Escherichia coli* and unexpectedly observed that *C. albicans* enhances growth and proliferation of *Escherichia coli* strain K12 by facilitating its cell division. Importantly, we found, based on our genetic screens, that both fungus- and bacterium-derived factors, including the iron-responsive transcription factors Sef1 and Sfu1 in *C. albicans* and the siderophere enterobactin transporters FepD and FepG in *E. coli*, actively contribute to this transkingdom interaction. Deletion of *SFU1* or *SEF1* caused a dramatic reduction in growth enhancement of *E. coli.* Compared to the wild type *E. coli*, the enhanced growth of both *fepD* and *fepG* null mutants were largely dampened. However, the *E. coli* mutant lacking entB, a key enzyme catalyzing the biosynthesis of siderophore enterobactin, showed similar growth enhancement as the wild type when co-inoculated with *C. albicans. C. albicans* promotes growth and proliferation of the commensal bacterium *E. coli* and an iron-responsive signaling pathway appears to be required. *C. albicans* may act to supply a siderophere-like molecule that captures the environmental iron to promote the growth of *E. coli*. Our studies gave insight into a novel interacting mechanism operative in interspecies communication that occurs when bacteria and fungi co-exist.

1. Introduction

Bacteria and fungi coexist and interact in many natural environments including various niches within the human body. The interactions are mediated by physical association and molecular communications and play important roles in both fungal and bacterial development and functioning (Frey-Klett et al., 2011). Such interplays were found to influence not only environmental processes such as growth or diseases of plants and animals, but also our regular life including food production and human disease control. For example, bacteria-fungi interactions significantly affect human health. It has been documented that mixed bacteria-fungi infections may cause diseases that differ from single species infections (Peleg et al., 2010). In case of *Cryptococcus neoformans*, an encapsulated yeast that causes cryptococcal meningitis and encephalitis in people who have weakened immune systems, particularly those who have advanced HIV/AIDS (Jarvis and Harrison, 2007), it has been reported that the bacterium *Klebsiella aerogenes* provides dopamine that can be utilized by *C. neoformans* for synthesis of melanin (Frases et al., 2006), a pigment known to protect the fungus against environmental stress and human immune defense (Casadevall et al., 2000). However, it remains largely unknown about the molecular basis underlying these important interkingdom interactions and their importance on human health.

Candida albicans is normally recognized as a dimorphic commensal

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Table 1
Strains Used in this Study.

Strain	Relevant Genotype	Full Genotype	Reference
SN250	SN250	*leu2Δ::C.m.LEU2/leu2Δ::C.d.HIS1, his1Δ/his1Δ, arg4Δ/arg4Δ, leu2Δ/leu2Δ ura3Δ/URA3, iro1Δ/IRO1*	Noble et al. (2010)
SN515	*sfu1ΔΔ*	*sfu1Δ::C.m.LEU2/sfu1Δ::C.d.HIS1, his1Δ/his1Δ, arg4Δ/arg4Δ, leu2Δ/leu2Δ, ura3Δ/URA3, iro1Δ/IRO1*	Noble et al. (2010)
SN900	*sfu1ΔΔsef1ΔΔ*	*sef1Δ::C.m.LEU2/sef1Δ::C.d.HIS1, sfu1Δ::FLP/sfu1Δ:FLP-SAT1-FLP,arg4Δ/arg4Δ, ura3Δ/URA3, iro1Δ/IRO1*	this study
SN742	$SFU1^{OE}$	*arg4Δ/arg4Δ, leu2Δ/leu2Δ::C.d.ARG4-TDH3p-SFU1, his1Δ/his1Δ, ura3Δ/URA3, iro1Δ/IRO1, sfu1Δ::C.d.HIS1/sfu1Δ::C.m.LEU2*	Noble et al. (2010)
SN330	*sef1ΔΔ*	*sef1Δ::C.m.LEU2/sef1Δ::C.d.HIS1, his1Δ/his1Δ, arg4Δ/arg4Δ, leu2Δ/leu2Δ, ura3Δ/URA3, iro1Δ/IRO1*	Noble et al. (2010)

fungus that colonizes the skin, mucosa and the reproductive tract in up to 70% healthy individuals (Perlroth et al., 2007). However, this fungus was able to cause disease manifestations such as life-threatening disseminated candidiasis and chronic mucocutaneous candidiasis in immunocompromised patients (Netea et al., 2008; Gow et al., 2011). Interactions between *C. albicans* and bacterial species have been studied recently and those interplays have been found to have clinical importance. For example, a restrospective human study identified a higher mortality rate for a mixed bacteria-*Candida spp*. bloodstream infection than for a single infection with *C. albican spp* (Dyess et al., 1985), suggesting that more complicated mechanisms may exist in a polymicrobial infection. The bacteria-fungi interactions during an infection can be synergistic, in which the two organisms act together against the human host. Co-colonization of the commensal bacterium *Streptococcus oralis* with *C. albicans* in the oral mucosa synergizes to compromise the integrity of the oral mucosal barrier by activating epithelial calpain 1 and thus significantly enhance fungal invasion (Xu et al., 2016). Another bacterium *Streptococcus gordonii* was also found to enhance fungal virulence by promoting hyphal growth and biofilm formation of *C. albicans* during the co-colonization in the oral cavity and the interaction was mediated through both physical and chemical signals (Bamford et al., 2009). A recent study by Liang et al. revealed that unlike its role in suppressing filamentation of *C. albicans* by production of lactic acid and other metabolites, lactic acid bacteria can take advantage of environmental conditions such as pH and temperature to differentially promote filamentation when *C. albicans* switch between two heritable cell types, white and opaque (Liang et al., 2016). Interestingly, studies based on murine models revealed that infection with *C. albicans* and *Escherichia coli* could yield opposite disease outcomes, depending on the administration orders. For instance, intravenous or intraperitoneal administration of a sublethal dose of *E. coli* before a lethal dose of *C. albicans* significantly decreased the host killing by *C. albicans* (Gale and Sandoval, 1957), whereas enhanced killing was observed if the *E. coli* was delivered simultaneous or after inoculation with *C. albican* (Burd et al., 1992; Akagawa et al., 1995; Klaerner et al., 1997). On the other hand, interspecies interactions between bacteria and fungi can also be antagonistic. The bacterium *Pseudomonas aeruginosa*, an opportunistic pathogen frequently co-isolated with *C. albicans* from burn victims and cystic fibrosis patients, produces a range of secondary metabolites suchs the anti fungal molecule phenazine pyocyanin to inhibit or even kill *C. albicans* (Gibson et al., 2009; Morales et al., 2010). In addition, *C. albicans* generated the quorum-sensing molecule farnesol to inhibit the swarming ability of *P. aeruginosa* (McAlester et al., 2008) and antagonize the production of anti-fungal pyocyanin while *P. aeruginosa* produced 3-oxo-C12-homoserine lactone, a quorum-sensing molecule that is structurally similar to farnesol and significantly reduces *C. albicans* virulence by inhibiting hyphal growth (Hogan et al., 2004). Intriguingly, most studies in bacteria-fungi interactions are focusing on the synergistic or antagonistic effects of bacterial functions on fungal physiological activities including pathogenicity. Very few studies have been demonstrated about the role of fungi on bacteria. A good example is mixed infection of *C. albicans* and *Staphylococcus aureus*. When *S. aureus* alone was inoculated intraperitoneally, it can be easily cleared. However, simultaneous administration with *C. albicans* was able to protect *S. aureus* in the peritoneal cavity and facilitate dissemination of the bacterium to the peripheral tissues, and enhance bacterial virulence (Carlson and Johnson, 1985).

In this study, we surprisingly found that *C. albicans* promotes growth and proliferation of the commensal bacterium *E. coli* and an iron-responsive signaling pathway appears to be required. Our studies illustrate a novel interacting mechanism operative in interspecies communication that occurs between *C. albicans* and *E. coli*.

2. Materials and methods

2.1. Strains and media

The *Candida albicans* strains used in this study are listed in Table 1.

Bothe yeast and bacterial cells were routinely grown in either the Luria-Burtani (LB) (1% tryptone, 0.5% yeast extract, 0.5% NaCl) or YEPD (1% yeast extract, 2% peptone, 2% dextrose).

2.2. Library screening

Cells from the *C. albicans* mutant library were patched on YPD agar plate in a 96-well format. The wild type *E. coli* was spotted along with each of the *C. albicans* mutant colony, separating with a distance of 1 cm. All inoculations were manipulated using a robotic replicator.

2.3. In vitro interaction assay

Overnight cultures of *C. albicans* or *E. coli K12* were harvested, washed three times with sterile water, and resuspended in PBS buffer (OD_{600} of *C. albicans* is ~20; OD_{600} of *E. coli* is ~4). 5ul cells of each strain were transferred to YPD medium where both strains were grown adjacent to one another with a distance of 1.5 cm. Plates were incubated at 30 °C and the growth was photographed at indicated time. As indicated, Siderophore complexes (FC; Sigma) were prepared by overnight incubation of ferrichrome with FeCl3 at a ratio of 1:1. All siderophore complexes were filter-sterilized (0.2 μM filter). For plate assays, Fe-siderophore complexes were added to a final concentration of 10uM and the plates allowed to dry at 30 °C overnight. Yeast or bacterial cells were spotted onto plates and grown for 7 days.

2.4. Growth assay

The *Candida* cells were freshly streaked by subculture on the YPD agar plate. A loopful of inoculum was introduced into the YPD broth and cells were grown for 16 h at 30 °C before use. Overnight culture of YPD only, and the *Candida* cells were spin down and supernatant was filtered by 0.2um membrane. Overnight culture of K12 was added to 30 ml of filtered sup to original OD to 0.03 and incubated at 30 °C and 200 rpm. Growth was measured at 600 nm using Thermo Multiskan spectrophotometer. Optical density was recorded for each concentration against time (h) at indicated time points.