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OBSERVATION
Host-Microbe Biology

Filamentation Is Associated with Reduced Pathogenicity of Multiple Non-*albicans Candida* Species

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ABSTRACT Candidiasis affects a wide variety of immunocompromised and medically compromised patients. *Candida albicans*, a major human fungal pathogen, accounts for about 50% of all cases, while the remainder are caused by the less pathogenic non-*albicans Candida* species (NACS). These species are believed to be less pathogenic, in part, because they do not filament as readily or robustly as *C. albicans*, although definitive evidence is lacking. To address this question, we used strains for two NACS, *Candida tropicalis* and *Candida parapsilosis*, which were genetically engineered to constitutively express the key transcriptional regulator *UME6* and drive strong filamentation both *in vitro* and during infection *in vivo*. Unexpectedly, both strains showed a dramatic reduction in organ fungal burden in response to *UME6* expression. Consistent with these findings, we observed that a *C. tropicalis* hyperfilamentous mutant was significantly reduced and a filamentation-defective mutant was slightly increased for organ fungal burden. Comprehensive immune profiling generally did not reveal any significant changes in the host response to *UME6* expression in the NACS that could explain the increased clearance of infection. Interestingly, whole-genome transcriptional profiling indicated that while genes important for filamentation were induced by *UME6* expression in *C. tropicalis* and *C. parapsilosis*, other genes involved in a variety of processes important for pathogenesis were strongly downregulated. These findings suggest that there are fundamental evolutionary differences in the relationship between morphology and pathogenicity among *Candida* species and that NACS do not necessarily possess the same virulence properties as *C. albicans*.

IMPORTANCE Many immunocompromised individuals, including HIV/AIDS and cancer patients, are susceptible to candidiasis. About half of all cases are caused by the major fungal pathogen *Candida albicans*, whereas the remainder are due to less pathogenic non-*albicans Candida* species (NACS). Generation of filamentous cells represents a major virulence property of *C. albicans*, and the NACS are believed to be less pathogenic, in part, because they do not filament as well as *C. albicans* does. To address this question, we determined the pathogenicity of two NACS strains that have been genetically engineered to promote filamentation during infection. Surprisingly, these strains showed a dramatic reduction in pathogenicity. The host immune response did not appear to be affected. However, unlike *C. albicans*, filamentation of the NACS was associated with downregulation of several genes important for pathogenicity processes. Our results suggest that there are fundamental evolutionary differences in the relationship between filamentation and pathogenesis in NACS compared to *C. albicans*.

KEYWORDS candidiasis, infectious disease, mycology, morphology, pathogenicity, *Candida* species, evolution, filamentation, gene expression

Candida species account for the large majority of human fungal infections. These species can cause both mucosal infections, such as oral and vaginal thrush, and more serious life-threatening systemic bloodstream infections (1–3). Individuals with a

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compromised immune system, including cancer patients on chemotherapy, organ transplant recipients, and HIV/AIDS patients, are highly susceptible to infection (2, 3). Approximately 50% of all *Candida* bloodstream infections in the United States can be attributed to *Candida albicans*, while the remainder are due to a variety of inherently less pathogenic non-*albicans Candida* species (NACS) (4). Infections by NACS are on the rise, and several of these species show increased resistance to commonly used antifungal therapies (5).

C. albicans possesses a variety of virulence traits, including the ability to undergo a reversible morphological transition from single budding yeast cells to filaments, which are best described as elongated cells attached end to end (6). Multiple independent lines of evidence have suggested a strong association between the *C. albicans* yeast-filament transition and virulence (7–11), including our previous demonstration that constitutive high-level expression of the filament-specific transcriptional regulator *UME6* promotes this morphological transition and enhances *C. albicans* virulence, as well as tissue invasion, in a mouse model of systemic candidiasis (12).

Considerably less research has focused on NACS. These species are thought to be less pathogenic than *C. albicans* for a variety of reasons, including a reduced ability to adhere to host cells, secrete degradative enzymes, and form biofilms (13, 14). They are also generally more sensitive to cell stresses encountered in the host environment and do not filament as readily or robustly as *C. albicans* (14). We have previously shown that certain *C. albicans* morphological regulatory functions are evolutionarily conserved in several NACS, including *Candida tropicalis* and *Candida parapsilosis* (15). As in *C. albicans*, orthologs of *UME6* are transcriptionally induced in these species during filamentation, although at a reduced level and, in the case of *C. parapsilosis*, with delayed timing. In addition, as is the case for *C. albicans*, constitutive high-level expression of *UME6* orthologs is sufficient to promote strong filamentation in *C. tropicalis* and *C. parapsilosis*; orthologs of several, but not all, *C. albicans* filament-specific genes were also induced in response to *UME6* expression in these species (15).

In order to determine the specific effect of filamentation on the pathogenicity of *C. parapsilosis* and *C. tropicalis* during infection *in vivo*, we used our previously constructed *tetO-CtUME6* and *tetO-CpUME6* strains (15). In the absence of doxycycline (Dox), a repressor of the tetracycline operator (*tetO*), one allele of *UME6* in these strains is expressed at constitutive high levels, generating a highly filamentous morphology, whereas in the presence of Dox this allele is shut off and cells grow as yeast. Both *C. tropicalis* and *C. parapsilosis tetO-UME6* strains were used to inoculate female BALB/c mice by tail vein injection. Half the mice in each group were supplied with drinking water containing Dox. At day 16 postinfection, all mice were sacrificed and kidneys were harvested for fungal burden analysis. Unexpectedly, as shown in Fig. 1A, there was a significant reduction in fungal burden in the −Dox versus +Dox group for both species. Indeed, fungal burdens in the −Dox groups were below the limit of detection, suggesting that the infections had cleared. In a similar experiment, mice sacrificed at 24 h postinfection showed equivalent kidney (Fig. 1B) and brain (data not shown) fungal burdens in both +Dox and −Dox groups for each species. In order to determine the time course for organ clearance and whether clearance occurred in multiple organs, we next performed a timed-sacrifice experiment using both *C. parapsilosis* and *C. tropicalis tetO-UME6* strains and examined fungal burdens in both kidneys and brains at different days postinfection (Fig. 1C). Interestingly, at the day 3 postinfection time point the +Dox and −Dox groups inoculated with the *C. parapsilosis tetO-UME6* strain showed roughly equivalent kidney fungal burdens, although fungal burden was partly reduced in the brains of −Dox versus +Dox animals. At day 6 and day 10, there was a dramatic reduction in both kidney and brain fungal burden in the −Dox versus +Dox groups. As in the previous experiment, fungal burdens for the −Dox groups at these later time points were below the limit of detection. A similar trend in results, though less pronounced, was observed for the *C. tropicalis tetO-UME6* strain (Fig. 1C). Interestingly, these results suggested that constitutive high-level *UME6* expression is sufficient to significantly reduce both *C. parapsilosis* and *C. tropicalis* fungal burden, eventually

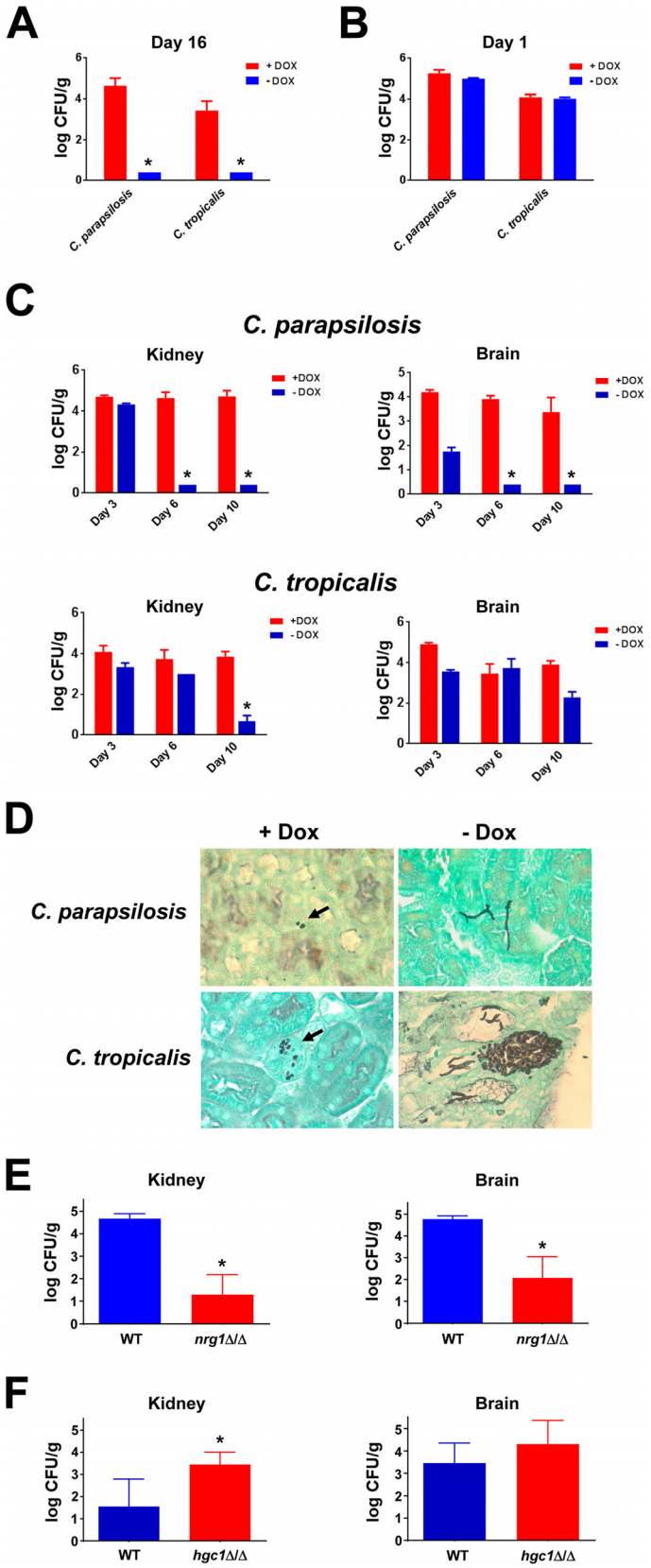

FIG 1 Filamentation is associated with reduced fungal burden of *C. tropicalis* and *C. parapsilosis* in a mouse model of systemic candidiasis. (A) *C. tropicalis* (4×10^4 CFU) and *C. parapsilosis* (4×10^6 CFU) *tetO-UME6* strains were used to inoculate female BALB/c mice (6 to 8 weeks old) by tail vein injection. Half

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leading to clearance of the infection from multiple organs. Histological analysis of infected kidneys was performed to confirm that *UME6* expression was able to promote filamentation during infection *in vivo*. As shown in Fig. 1D, both strains were observed to grow primarily in the yeast form in infected kidneys from the +Dox group. In contrast, in the absence of Dox the *C. tropicalis tetO-UME6* strain was found as a mixture of yeast and filaments and the *C. parapsilosis tetO-UME6* strain grew primarily as filaments (Fig. 1D). In order to determine whether organ clearance was more generally associated with morphology, mice were inoculated with either a hyperfilamentous *C. tropicalis nrg1*Δ/Δ mutant (15) or a filamentation-defective *C. tropicalis hgc1*Δ/Δ strain (16). Consistent with our previous results, the *nrg1*Δ/Δ mutant showed significantly reduced fungal burden compared to the wild-type (WT) strain in both the kidneys and brain (Fig. 1E). Also, in contrast to most filamentation-defective mutants of *C. albicans*, the *C. tropicalis hgc1*Δ/Δ mutant showed slightly increased organ fungal burdens compared to those of the WT control (Fig. 1F).

We next sought to determine whether organ clearance that is observed in response to *UME6* expression in *C. tropicalis* and *C. parapsilosis* occurs as the result of an altered host response. Kidney homogenates were prepared from mice placed on drinking water in the presence or absence of Dox, inoculated with *C. tropicalis* and *C. parapsilosis tetO-UME6* strains, and sacrificed at day 1 postinfection when fungal burdens are equivalent in +Dox and −Dox groups. These homogenates were then used to carry out comprehensive multianalyte profiling of over 50 different cytokines, chemokines, and other host markers of infection. In general, we did not detect significant differences in the levels of these analytes when comparing −Dox with +Dox groups, which could indicate an altered immune response (see Fig. S1 in the supplemental material). However, we did observe a significant reduction in both the *C. tropicalis* and *C. parapsilosis* −Dox versus +Dox ratio for myoglobin. Given that myoglobin levels are known to be correlated with tissue damage (17), these results are consistent with our previous findings and suggest that greater tissue damage occurs in response to *C. tropicalis* and *C. parapsilosis* yeast than to filamentous cells.

We were also interested in gaining a better understanding of the basis for organ clearance in response to *UME6* expression in *C. tropicalis* and *C. parapsilosis*. Thus, we performed whole-genome transcriptional profiling experiments. Both *tetO-CtUME6* and *tetO-CpUME6* strains were grown in the presence and absence of Dox to generate yeast and filaments (Fig. S2), respectively, and cells were harvested for RNA preparation and transcriptome sequencing (RNA-seq) analysis. As indicated in Fig. 2A as well as Tables S1 and S2, we observed that large sets of genes showed significantly increased and

FIG 1 Legend (Continued)
the mice were placed on drinking water with 2 mg/ml Dox ($n = 5$ mice/group). All mice were sacrificed at 16 days postinfection, kidneys were harvested, and fungal burdens were determined. For both species, the reduction in fungal burden in the −Dox versus +Dox groups was statistically significant (*, $P < 0.01$) as determined by a Mann-Whitney test. (B) The experiment in panel A was repeated using 2.1×10^5 CFU of *C. tropicalis* and 4.2×10^6 CFU of *C. parapsilosis tetO-UME6* strains, and all mice were sacrificed at 24 h postinfection for kidney fungal burden determination. (C) The experiment in panel A was repeated for the *C. parapsilosis* and *C. tropicalis tetO-UME6* strains using inoculum sizes of 4.5×10^6 CFU and 3.0×10^4 CFU, respectively. Mice were sacrificed at the indicated postinfection time points, and fungal burdens were determined for the indicated organs (*, $P < 0.05$, using a Mann-Whitney test). Please note that the fungal burden value for kidneys infected with the *C. tropicalis tetO-UME6* strain in the absence of Dox at day 6 represents the lower limit of detection. (D) Examples showing the effect of *UME6* expression on *C. tropicalis* and *C. parapsilosis* morphology during infection *in vivo*. Kidneys from mice infected with *tetO-UME6* strains from the indicated species were harvested, fixed, embedded in paraffin, stained with Grocott-Gomori methenamine silver (GMS), and visualized by light microscopy (fungal cells shown in black). Black arrows indicate yeast cells. (E) The *C. tropicalis* WT (3.7×10^5 CFU) and *nrg1*Δ/Δ (3.5×10^5 CFU) strains were used to inoculate female BALB/c mice (6 to 8 weeks old) by tail vein injection ($n = 5$). All mice were sacrificed at 6 days postinfection, kidneys were harvested, and fungal burdens were determined. The reduction in fungal burdens in mice infected with *nrg1*Δ/Δ versus WT strains was statistically significant (*, $P < 0.01$) as determined by a Mann-Whitney test. (F) The experiment in panel E was repeated using 2.9×10^4 and 3.1×10^4 CFU of *C. tropicalis* WT and *hgc1*Δ/Δ strains, respectively. The increase in kidney fungal burden in mice infected with *hgc1*Δ/Δ versus WT strains was statistically significant (*, $P < 0.05$) as determined by a Mann-Whitney test.

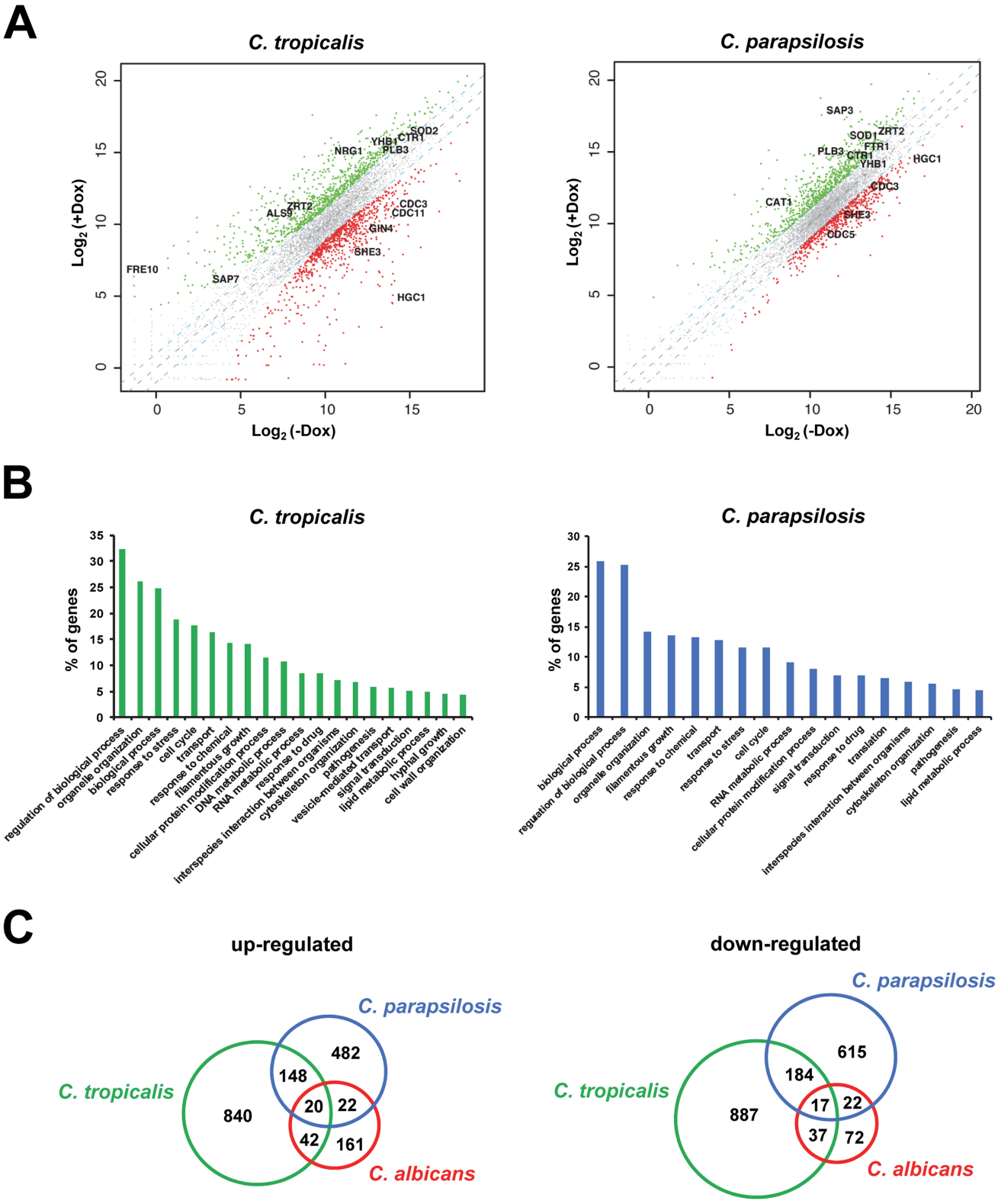

FIG 2 Transcriptional profile of *C. tropicalis* and *C. parapsilosis* in response to *UME6* expression. (A) Scatter plots showing gene expression changes for *C. tropicalis* and *C. parapsilosis tetO-UME6* strains grown in the absence versus presence of doxycycline (Dox). Axes represent $\log_2$(averaged normalized read counts) for +Dox and −Dox. Transcripts that are differentially expressed ≥2-fold (dashed lines) are indicated in red (upregulated) and green (downregulated). Transcripts of interest are labeled in black. (B) GO slim mapper (www.candidagenome.org) analysis showing percent representation of process gene classes in the sets of genes induced ≥2-fold in response to *UME6* expression in *C. tropicalis* and *C. parapsilosis*. Gene classes showing less than 4% representation are not shown. (C) Venn diagrams showing overlap of gene orthologs that are upregulated or downregulated in response to *UME6* expression in *C. tropicalis*, *C. parapsilosis*, and *C. albicans*. *C. albicans* genes showing differential expression in response to *UME6* induction have been described previously (18).

decreased expression in response to *UME6* induction in both strains. In contrast, as expected, very few genes showed significant expression changes in the absence versus presence of Dox for both *C. tropicalis* and *C. parapsilosis* WT control strains. A gene ontology (GO) analysis identified several common gene classes that were induced in response to *UME6* expression in both *C. tropicalis* and *C. parapsilosis* (Fig. 2B and Data Sets S1 and S3). In addition to filamentous growth, as expected, these gene classes included organelle organization, stress response, cell cycle, and transport. In *C. parapsilosis*, genes involved in amino acid metabolism were overrepresented compared to the genome as a whole, whereas genes associated with mitosis and the cell cycle were overrepresented in *C. tropicalis*. Among downregulated genes, those involved in carbohydrate metabolism were overrepresented in both *C. tropicalis* and *C. parapsilosis*, whereas genes associated with lipid catabolic processes were strongly overrepresented only in *C. parapsilosis* (Data Sets S2 and S4).

Orthologs of several genes known to be induced in response to *C. albicans UME6* expression (18) were also induced in both *C. tropicalis* and *C. parapsilosis*. As an independent confirmation of our RNA-seq results, we have previously demonstrated by Northern analysis that a few of these genes (e.g., *UME6*, *HGC1*, and *PHR1*) are induced in both NACS (15). In addition to *HGC1*, which encodes a cyclin-like protein important for septin phosphorylation (19), a number of other genes that play direct roles in the filamentation process, including the *CDC3* septin and *SHE3* mRNA-binding protein, were induced in all three *Candida* species (Fig. 2A and Data Sets S1 and S3); the *CDC11* septin, previously shown to be induced by *UME6* in *C. albicans* (18), was also induced in *C. tropicalis*. Interestingly, however, orthologs of several *C. albicans* genes involved in processes important for pathogenesis were significantly downregulated in response to *UME6* expression in both *C. tropicalis* and *C. parapsilosis* but not *C. albicans* (Fig. 2A and Data Sets S2 and S4) (18). These genes included *SOD2*, a superoxide dismutase important for combating oxidative stress, and *YHB1*, important for adapting to nitrosative stress, as well as a variety of genes important for iron, zinc, and copper transport/regulation (e.g., *CTR1*, *ZRT1*, *ZRT2*, and *FRE9*). Multiple members of the secreted aspartyl protease (*SAP*) gene family as well as the *PLB3* phospholipase, which is likely important for host cell degradation, were also downregulated in response to *UME6* expression in both *C. tropicalis* and *C. parapsilosis*. As in *C. albicans* (18), *CtNRG1*, previously shown to function as a repressor of filamentation in *C. tropicalis* (15), was downregulated in response to *CtUME6* expression, which is again consistent with the notion that mechanisms important for induction of filamentation by *UME6* are conserved among *Candida* species. A three-way comparison of gene orthologs regulated by *UME6* in *C. tropicalis*, *C. parapsilosis*, and *C. albicans* (Fig. 2C) revealed that orthologs of about one-third of *C. albicans* genes are upregulated in *C. tropicalis* and/or *C. parapsilosis* whereas orthologs of over one-half of *C. albicans* genes downregulated by *UME6* were also downregulated in *C. tropicalis* and *C. parapsilosis*. In the future, it will be useful to determine whether similar gene expression patterns are observed in response to expression of other activators of filamentation in NACS.

In this study, we have used a variety of approaches to more specifically define the relationship between morphology and pathogenicity in two NACS, *C. tropicalis* and *C. parapsilosis*. Surprisingly, unlike the situation in *C. albicans*, we find that under our experimental conditions the transition from yeast cells to filaments is associated with reduced pathogenicity as well as reduced expression of certain genes involved in pathogenicity-related processes. At this point, however, it is unclear whether the clearance effect is due to filamentation *per se* or some other process associated with filamentation (e.g., lowered expression of pathogenicity genes). With respect to *C. parapsilosis*, our results are consistent with those of a previous study demonstrating that the ability of this species to invade the oral epithelium does not correlate with formation of pseudohyphae (20). Also in support of our findings, a different study has found that a hyperfilamentous *C. parapsilosis* mutant is significantly attenuated for pathogenicity in both a *Galleria* model and a mouse model of candidiasis (21). While previous reports have suggested a correlation between *C. tropicalis* filamentation ability

and the ability to invade/damage epithelial cells, it is important to note that these experiments were carried out using *in vitro* systems rather than animal models (22, 23). A previous study showing this correlation during infection used immunosuppressed animals and a significantly larger inoculum size (24). We have also observed that increasing inoculum size for the *tetO-CtUME6* strain reduces the organ clearance effect (our unpublished results). An independent previous study has shown, by histological analysis, that *C. tropicalis* cells injected at smaller inoculum sizes equivalent to those used in our experiments typically grow in the yeast form, whereas greater inoculum sizes of *C. tropicalis* are correlated with an increased proportion of filamentous cells in kidneys (25). Interestingly, our results suggest that forcing filamentation of *C. tropicalis* at smaller inoculum sizes, at which cells would otherwise grow in the yeast form during infection *in vivo*, leads to organ clearance. Importantly, these findings suggest not only that filamentation can confer an evolutionary disadvantage for *C. tropicalis* and *C. parapsilosis* during infection but also that there are fundamental evolutionary differences in the relationship between morphology and pathogenicity among *Candida* species. More specifically, in NACS other processes that are independent of the morphological transition are likely to play a more prominent role in pathogenesis. On a broader level, our findings are significant because they suggest that not everything we learn about pathogenicity and virulence-related processes in *C. albicans* can be directly applied to NACS, but rather the virulence traits of these species need to be studied in their own right.

For a detailed description of experimental procedures used in this study, please see Text S1. All research using animals was approved by the Institutional Animal Care and Use Committee at The University of Texas at San Antonio.

Data availability. Raw RNA sequencing data for this study have been deposited at the NCBI Gene Expression Omnibus (GEO) database (accession number GSE134321).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/mSphere.00656-19.

TEXT S1, DOCX file, 0.04 MB.
FIG S1, PDF file, 0.01 MB.
FIG S2, PDF file, 0.6 MB.
TABLE S1, DOCX file, 0.02 MB.
TABLE S2, DOCX file, 0.02 MB.
TABLE S3, DOCX file, 0.01 MB.
DATA SET S1, XLSX file, 0.3 MB.
DATA SET S2, XLSX file, 0.2 MB.
DATA SET S3, XLSX file, 0.1 MB.
DATA SET S4, XLSX file, 0.2 MB.

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