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Analyzing the Role of a Protein Downregulated After Induction of Filamentous Growth In

Candida albicans

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Abstract

Candida albicans is a commensal fungus, normally living with its human host, however, it has the ability to cause invasive infection. *Candida albicans* is the fourth most frequent nosocomial infection affecting a vulnerable immunocompromised population. *Candida albicans* exhibits different morphologies including yeast, pseudohyphae, and hyphae. The varying morphological potential of this organism is a virulence trait. Because of this, research has focused on what drives activation of hyphal formation as well as what impedes it. During a filamentation assay, a novel observation pertaining to a subgroup of proteins being downregulated early after germination, was made. In this study, we constructed an over-expression strain of one of these proteins and have used known hypha inducing media to determine if it will have an impact on filamentation. With the conditions tested thus far, no significant impacts on morphology have been observed.

1 *Candida albicans* is a commensal fungus, normally colonizing the gastrointestinal tract and other
2 mucosal membranes of its human host. Yet, *Candida* has virulence potential associated with different
3 environmental cues affecting signal transduction pathways, making *Candida* an opportunistic
4 pathogen as well. Changes in host immunity and complex environmental factors play a large role in
5 *C. albicans* transformation from commensal to pathogen. *Candida* is able to change its phenotypic
6 state between a single celled yeast to filamentous, hyphal or pseudohyphal, morphologies under
7 varying circumstances. There are superficial types of *Candida* infections. Such infections are
8 prevalent in AIDS patients whom frequently suffer from oropharyngeal infections known as oral
9 thrush (1–3). Or, it is also known that 75% of women will suffer some form of *Candida* related
10 vulvogenital infection at least once in their lifetime, impacting their quality of life (4). *Candida*
11 *albicans* is a top cause of nosocomial infections worldwide (5). In fact, there are about 400,000
12 bloodstream infections worldwide with a 46-75% mortality rate and 10,000 deaths in the United
13 States alone, all attributed to a more severe form of *Candida* bloodstream infection (Reviewed in (6)).
14 The high mortality rate of this organism makes understanding the associated virulence mechanisms
15 paramount.

16 Aside from the high mortality rate, the associated prevalence of *Candida* infections is important. A
17 reason for rise in fungal infections can be associated with the growing immunocompromised
18 populations in a hospital setting, a side effect of the medical advancements granting an increased
19 volume of patients with hospital stays. Those advancements include organ transplantation, stem cell
20 transplantation, cancer therapies such as chemotherapy, premature infant deliveries and care, and
21 implanted medical devices (7). These medical treatments compromise the immunity of patients by
22 some degree enabling *Candida* bloodstream infections. Treatment difficulties have arisen as drug
23 resistance has occurred in virulent strains of *candida* (8). Treatment difficulties are also exacerbated

24 by the lack of efficacy in detecting techniques from cultured based diagnostic tests typically used in
25 detecting fungal infections; it is reported that about 50% of invasive candidiasis cases were missed
26 by this way of detection (9).

27 *C. albicans* has developed strategies in order to survive in different niches of a human host and cause
28 disease. A highlight here would be the ability of this organism to undergo morphological switching
29 under varying circumstances (10). Although the importance of morphological switching as a
30 virulence trait was accepted, there wasn't a manner of testing whether or not this theory was 'true' in
31 experimental studies until a group constructed a strain that could be regulated between yeast and
32 filamentous growth form (11). This study was the first time ever where the theory of morphological
33 switching being necessary for virulence, was proven evident. Morphology becomes important during
34 biofilm formation, another virulence trait of *C. albicans*. Biofilms are formed when yeast cells adhere
35 to a surface, commonly medical implanted devices like heart valves or pacemakers, followed by
36 hyphal morphogenesis, and eventually the film forms in a population of filamentous and yeast cells
37 which produce polysaccharides, proteins and nucleic acids (12–14). When biofilms form, they have
38 the potential of impairing the function of the devices they form on, having the correct surface to
39 adhere to, as well as maintaining the ability to sustain infection. (15).

40 Environmental factors responsible for activation of *Candida albicans* hyphal growth include:
41 presence of serum, neutral pH, 5% CO₂, and low nitrogen (10, 14, 16, 17). These environmental cues
42 activate important pathways such as the mitogen activated protein kinase (MAPK), cyclic adenosine
43 monophosphate (cAMP), calcineurin, and HOG pathways (10, 18). However, activation of hyphal
44 growth must be accompanied by downregulation of filament repressors, such as Nrg1 (14, 19).

45 Previous work in the lab identified a group of proteins that were seen to be degraded early during
46 filamentation when induced in 37°C, a known activation factor of hyphal formation. This finding

47 presented the question if the presence of these proteins was necessary for morphogenesis from yeast
48 form to filamentous growth, as well as what their true function is. The focus of this study is one of
49 these proteins. However, this protein remains uncharacterized and is called C1_05590_C. The
50 primary way of analyzing this protein was by increasing its expression levels followed by testing
51 over-expression strains under the influence of known filamentation activating cues. This allowed for
52 the determination of whether or not this protein influences the transition from yeast to filamentous
53 growth. Furthermore, the importance of deciphering events of morphological switching involves the
54 better understanding of this phenomenon to drive clinical applications directed in reducing the high
55 mortality rates associated with *Candida* infections.

56

57 **RESULTS AND DISCUSSION**

58

59 Wild-type strain SC5314 was used as a control and was grown along *sa*-C1_05590_C mutants
60 from the same cell line. There were no significant differences between the two mutant strains and
61 the wild-type strain when grown in different liquid media. When grown in yeast conditions (28
62 °C) in liquid YPD, all cells remained in yeast form. When grown in filamentous inducing
63 conditions corresponding to YPD, GLcNAc, YNB, RPMI, and Spider media, filamentation was
64 clearly observed amongst all streaked colonies examined. This is reflective of the growth
65 conditions of the mutants not being affecting differently in any significant pathways involved in
66 the morphological switching from yeast to filamentous growth form than the wild type strain.

67

68 When strains were grown on solid media under filamentous growth conditions, again, filamentous
69 growth was observed in all strains. For example, a distinct crenulation on spider media is a typical

70 macroscopic outcome of SC5314 and this was maintained in the mutant strains macroscopic
71 analysis. Invasion into the media was also apparent amongst all strains examined as washing of
72 the cells demonstrated this, indicating again, no impact on filamentous growth form observed in
73 the mutant strains tested. Lee's medium contains a variety of amino acids necessary for induction
74 of filamentous growth from yeast form growth. Similarly, Spider medium is known to induce
75 filamentous growth based on carbon availability. Lastly, GlcNAc is known to stimulate activating
76 pathways of hyphae inducing outcomes, such as activating the cAMP signaling pathway.

77

78 **METHODS AND MATERIALS**

79

80 Entailed in this study was the construction of a *Candida albicans* vector in order to insert, clone,
81 and express proteins of interest to analyze their effect under conditions where filamentous growth
82 is known to be activated. These constructs were made up of CIpSAT-SA, the CIp component being
83 a modified version of the CIp10 vector, created specifically for *Candida* integration. The SAT
84 component was introduced to CIp10, encoding a nourseothricin resistance and replacing the URA
85 component of CIp10. Finally, the SA component of CIpSAT-SA is the shorter of the two Actin
86 promoters, which are constitutively on, ensuring the maintenance of elevated levels of
87 C1_05590_C in mutant strains.

88

89 However, the protein of interest was first designed into a pMiniT which does not integrate well
90 into *Candida* cells. Xho1 and Mlu1 enzymes allowed for a sub-cloning of C1_05590_C from a
91 pMiniT vector into a CIpSAT-SA construct. Once the final transformation was done, the remainder

92 of the experiment involved culture assays as the primary way of attempting to characterize the role
93 and function of this novel protein.

94

95 Strains and Growth Conditions

96

97 *C. albicans* strain SC5314 and mutant strains *sa-C1_05590_C* were grown on yeast-peptone-
98 dextrose (YPD) agar in 28 °C. The mutant strain was engineered as follows: 5'-Xho1-AUG-His6-
99 C1_05590_C-Myc-TAA-Mlu1-3' in a pMiniT plasmid. The candida plasmid CIp10 was
100 reengineered to replace the URA component with a SAT component encoding nourseothricin
101 resistance for selectivity, as well as incorporating the short act promoter (SA) allowing for
102 constitutive production of the protein of interest. The name of this new construct is CIpSAT-*sa*,
103 maintaining a Xho-1 and Mlu-1 cutsite. The enzymes Xho-1 and Mlu-1 were used to cut out the
104 designed protein from pMiniT and to linearize CIpSAT-*sa*. The insert and linearized CIpSAT-*sa*
105 were ligated, followed by a Stu-1 digestion to prepare the DNA for an electroporation
106 transformation into SC5314 cells (20, 21). To ensure the insert integrated into CIpSAT-*sa*, a
107 double digestion using Xho-1 and Mlu-1 was done and the DNA lengths of the insert-C1_05590_C
108 and CIpSAT-*sa* empty plasmid were compared using gel electrophoresis. After transformation by
109 electroporation was performed, the plated colonies were picked and patched onto another plate.
110 This allowed for colonyPCR followed by gel electrophoresis to confirm that the correct product
111 was growing on the patched plate. Once gel electrophoresis on the double digested DNA and
112 colonyPCR confirmed the correct transformant was present, a YPD plate was streaked with two
113 transformants and a WT strain.

114

115 Filamentation Assay

116

117 Two approaches were taken for filamentation assays. One approach was by done by streaking YPD
118 grown strains onto plates of YPD, YPD+Serum, Spider, and SLAD. All but SLAD were placed in
119 37 °C to induce filamentation. SLAD was placed in 28 °C. A second approach involved liquid
120 media. A 1mL aliquot was taken, washed twice with water, and resuspended in water to dilute
121 (1:20) into fresh media including YPD, Spider, Lee's, GLcNAc, RPMI, and YNB with shaking at
122 37 °C for three hours. Following, microscopy was used to visualize the cells for phenotypic
123 comparison.

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September 6, 2019

David Martin, PhD
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Dear Dr. David Martin,

I will soon have a Bachelor of Science in Biochemistry from Grand Valley State University and I am currently a Ronald E. McNair Scholar at this institution. My regard for the sciences has led me to investigate a component of the organism *Candida albicans* in the Thomas-Cleary Lab, an established laboratory in the biomedical sciences department. Under their direction I have used a microbiological approach in uncovering some research questions regarding an uncharacterized protein in the organism.

In my research I used a microbiological approach to examine the impact of the uncharacterized protein, C1_05590_C, on filamentation. Morphogenesis is an important virulence trait of *C. albicans*. This fungal microorganism lives as a commensal in most of the human population, however, is a known opportunistic pathogen. As a pathogen, *Candida* is a top nosocomial infection, impacting already vulnerable populations. In my recent unpublished article, *Analyzing the Role of a Protein Downregulated After Induction of Filamentous Growth in Candida Albicans*, I explain how I conducted this research. This paper entails two focuses: One questioning what this proteins impact is during morphological switching in *Candida*, and two, my attempt to uncover what this proteins function in the organism is. To do this, I overexpressed the protein by placing it in a vector with a constitutive promoter, allowing for constitutive production of it. This was followed by testing the constitutive mutant under conditions known to induce filamentation.

Infection and Immunity is a journal within the American Society of Microbiology and is fitting for my article because *Candida* is known to cause a deep-seated infection. My article focuses on a novel protein possibly associated with an important virulence trait of *Candida* infections. The impact of this protein remains enigmatic as it has not been observed to evade morphological switching with the media it has been analyzed with. Its function also remains a mystery as the mutant has not been observed to function differently than the wild-type strain. The research I have conducted will bring attention to this uncharacterized protein, for others to continue understanding the underlying mechanisms intrinsic to the virulence trait of phenotypic switching. I have contributed to a starting point, where someone else who is intrigued, can carry on. The only other known information about this novel gene is given by computational predictions.

Thank you,



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Target publication

[American Society for Microbiology - Infection and Immunity](#)