Influence of SCP1 on Cryptococcus neoformans Virulence

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Background

Background: *SCP1*, encoded by *C. neoformans* CNAG_01580, was previously identified in a mutant screen looking for genes associated with pH adaptation. During the normal course of disease, *C. neoformans* survival is dependent on its ability to adjust to the pH of its surrounding environment that can vary over a broad pH range from acidic to alkaline. Indeed, *C. neoformans* must even be able to adapt from an acidic to alkaline environment and back again within its host. Our previous research involved deleting the *SCP1* gene in *C. neoformans* and evaluating the growth phenotypes related to the *scp1Δ* mutant. *SCP1* deletion resulted in growth inhibition at pH 8 and at 1.5M NaCl (1).

In this current study, we have successfully reconstituted the mutant strain using biolistic bombardment (2). We will now check for the restoration of growth phenotypes on pH 8, 1.5M NaCl, and $CoCl_2$. Understanding pH adaptation in *C. neoformans* allows us to identify genes that may be crucial for the adaptive survival of the fungal infection. Genetic manipulation provides an opportunity to identify novel targets for the development of antifungal therapies against the highest ranked pathogen on the Fungal Priority Pathogen List (WHO).

Introduction and Research Question

C. neoformans wild type strain causes a fungal pneumonia in the lungs and is often misdiagnosed as the more common bacterial pneumonia. However, unlike bacterial pneumonia, *C. neoformans* crosses the blood-brain barrier, enters the central nervous system, and causes meningoencephalitis; which is difficult to treat and has a mortality rate of 41-61%. Immunocompromised individuals, such as transplant or chemotherapy patients, along with the AIDS and HIV community, are the most susceptible to this infection. *C. neoformans* is also capable of adapting to the host's alkaline environment, allowing it to avoid removal (1). A number of genes have been identified that potentially influence pH adaptation, such as the *scp1Δ* gene.

Does the *SCP1* gene influence pH adaptation and virulence in *Cryptococcus neoformans?*

Methods

The *C. neoformans* gene *SCP1* (CNAG_01580) was previously deleted in strain KN99α using biolistic transformation. We successfully reconstituted the *scp1*Δ mutant strain using biolistic bombardment (i.e. PDS 1000/He Gene-Gun). The growth phenotypes of the wildtype, mutant, and reconstituted strains will now be evaluated to verify restoration of growth phenotypes on YPD pH 8, NaCl, and CoCl2.

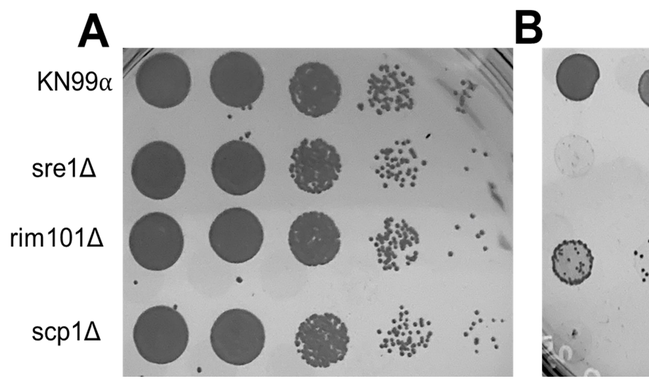

Figure 1: pH8 vs YPD Growth. SCP1 was plated on YPD medium (A) and YPD medium adjusted to pH8 (B). Strains that are unable to grow at pH8 recapitulate the phenotype observed in the original mutant screen (1).

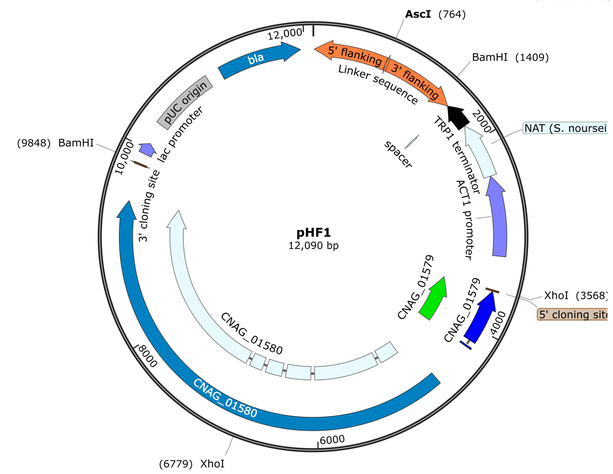

Figure 2: SCP1 reconstitution construct. The coding region of SCP1 including 1kb upstream and 0.5 kb downstream sequence was cloned into plasmid pSDMA25 at the HindIII site using the NEBuilder HiFi DNA Assembly Cloning kit to create plasmid pHF1.

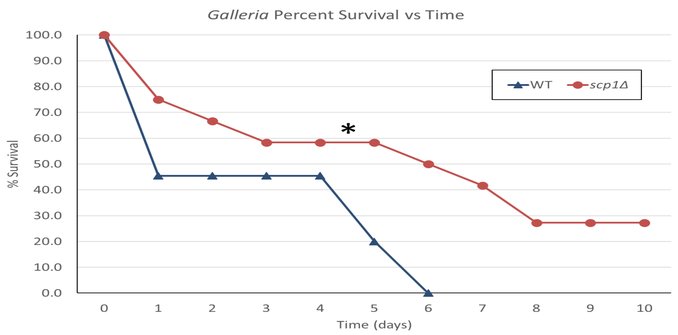

Figure 3: Virulence Assay in *Galleria* Hosts. The original *scp1Δ* mutant and the wild type strain were tested for virulence in a *Galleria* moth larvae model (3). The *scp1Δ* mutant suggests a lower virulence than the wild type strain, which demonstrates that the pH adaptation affects the virulence of the strain. This virulence assay will be repeated in a mouse model of cryptococcal disease (4).

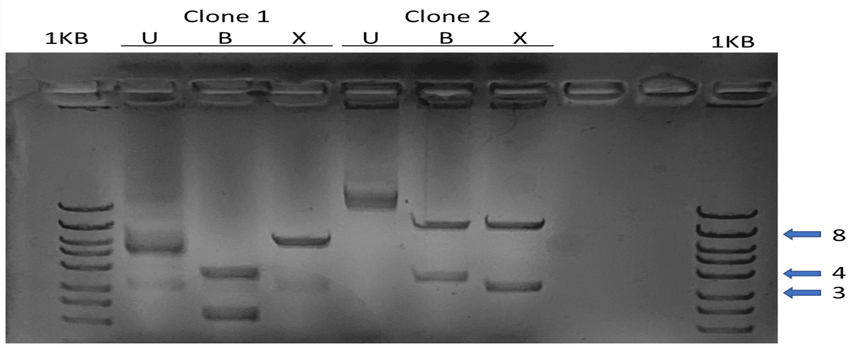

Figure 4: Verification of pHF1. The PCR products for two pHF1 clones were digested with BamH1 and XhoI to confirm cloning of the SCP1 insert. 1KB = DNA ladder; U = uncut plasmid; B = BamH1 digested plasmid; X = XhoI digested plasmid. Clone 2-B shows 3.6 and 8.4 kb bands from BamH1 digestion and Clone 2-X shows 3.2 and 8.8 kb bands from XhoI digestion as expected if the SCP1 insert is present.

Results

The reconstitution of the *scp1Δ* mutant via biolistic bombardment was successful. Previous attempts to transform the mutant using electroporation were unsuccessful due to the involvement of the *scp1Δ* mutant strain in the ergosterol biosynthetic pathway. The sterol ergosterol is involved in the cell membrane integrity of fungi. The process of electroporation was disruptive to the cell membrane (2), while biolistic transformation was not. This method of transformation yielded transformants that will be selected from to move forward. Once growth phenotypes are analyzed, the wild type, mutant, and reconstituted strains will be evaluated in virulence assays, including an invertebrate *Galleria* moth larvae model (3) and using a murine inhalation model (4). In both cases, animals will be monitored daily for signs of disease. Survival will be assessed using the Wilcoxon Log-rank test for both assays.

Future Work

1. Evaluate growth phenotypes on pH 8, 1.5 M NaCl, and 0.7 mM CoCl2 plates with reconstituted strains (1).
2. Assessment of virulence in a murine model of disease (4).
3. Verify the pH response pathway associated with SCP1 via qPCR.
4. Evaluate sensitivity to of the *scp1*Δ mutant to fluconazole.

References and Acknowledgments

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