Abstract

*Candida albicans* is a yeast commonly found in the gastrointestinal and genitourinary tracts of healthy individuals. Although part of the normal human microbiome, *C. albicans* can be pathogenic in those with compromised immune systems or those taking broad-spectrum antibiotics. *C. albicans* is the cause of diaper rash, thrush, vaginal yeast infections, and is a leading cause of systemic infections in hospitals. The focus of *C. albicans* research has almost entirely been focused on a single strain background, SC5314, found in clade I. This research will contribute to a larger project to delete several markers (URA3, LEU2, ARG4, HIS3) that are commonly used for *C. albicans* genetics in order to create mutant strains. These genetic tools can then be used to compare and better understand the pathogenesis and antifungal drug resistance across the four major clades that, along with clade I containing SC5314, cause most *C. albicans* infections in the United States.

Introduction

• The five major clades of *C. albicans* evolved clonally, which means the strains in each clade have unique genetic backgrounds [1]. Differing genetic backgrounds result in differences in pathogenicity and antifungal drug sensitivity between strains in different clades [2].
• The goal was to generate “marked” auxotrophic strains from each clade that will provide researchers with the resources necessary to pursue further *C. albicans* studies in a diverse array of genetic backgrounds.
• Mutations will be generated in *C. albicans* strains from each clade/family by utilizing the SAT1 flipper cassette [3]. The SAT1 flipper cassette contains a nourseothricin resistance marker that when incorporated into the genome, grants resistance to the anti-fungal drug nourseothricin, allowing for the generation of mutants to occur [3].
• The focus of my research is to create the URA3 mutations using the SAT1 flipper cassette (Figure 1) and to transform the mutations into *C. albicans* strains by homologous recombination.

Methods

• One region upstream and one region downstream of *URA3* were amplified by Polymerase Chain Reaction (PCR) to be cloned upstream and downstream of the SAT1 flipper cassette.
• Ultra Competent *Escherichia coli* were used for bacterial transformations. Transformations were plated on YPD-Amp plates and grown at 37 °C overnight (Figures 3, 6, and 7) [4].
• Plasmids were isolated using the GeneJET Plasmid Miniprep Kit and procedure [4].
• Gel electrophoresis was run at 120 volts for 45 minutes with 0.8% agarose gels (Figures 2, 4, 5, and 8) [4].
• Restriction enzyme digests contained either lambda-HF and xhol (if upstream digest), or sacII-HF and sac II restriction enzyme endonucleases (if downstream digest). Digests were incubated at 37 °C for one hour and 30 minutes before being checked by gel electrophoresis (Figures 5, 8, and 9) [4].
• DNA bands were cut from agarose gels of digests and purified using the Gel Extraction Kit and procedure [4].
• Two reactions were set up for each ligation: one a base plasmid (pSFS2) + insert and the other containing just base plasmid (pSFS2 only) as a negative control (Figures 6 and 7).

Results and Discussion

• Successful PCR amplification of the region downstream of *URA3* was confirmed by the presence of bands at ~500 bp.
• The URA3 up and downstream PCR products were cloned and transformed into *E. coli* cells and colonies resulted (Figure 3).
• Colonies containing the plasmid were grown overnight. The plasmid and PCR products were isolated with the GeneJET Plasmid Miniprep Kit and procedure [4].
• The results of the agarose gels ran confirmed that both the URA3 up and downstream (Figure 4) inserts were transformed successfully into *E. coli*. The smaller URA3 up and downstream bands were cut from the gels and purified.
• The region downstream of the SAT1 flipper cassette was then digested for ligation of the URA3 downstream insert. A successful transformation confirmed successful digestion (Figure 5). The larger bands (pSFS2) were cut from the gel and purified.
• Ligation of the URA3 downstream insert into the pSFS2 plasmid downstream of the SAT1 flipper cassette was performed as well as a negative control ligation. The small number of colonies on the negative control plate suggest successful ligation (Figure 6).
• The digestion and ligation was repeated with the URA3 upstream insert (Figure 7).
• Three digests of each URA3 up and downstream ligated plasmids were performed including: URA3 upstream, URA3 downstream, and the URA3 upstream + SAT1 flipper cassette + URA3 downstream marker gene (Figure 8).
• The results in Figure 8 do not show successful assembly of the marker gene. The same two bands appear in the undigested plasmid in lane 6, as in the URA3 down and URA3 upstream + SAT1 flipper cassette + URA3 downstream digests. This suggests that either the downstream and full marker gene digests were not successful, or that the ligations into pSFS2 were not successful and should be repeated.
• When PCR amplification of the ligations had been successfully ligated upstream and downstream of the SAT1 flipper cassette (Figure 1), the amplified marker gene will be transformed into *C. albicans* through homologous recombination.
• The SAT1 flipper cassette will then be allowed to flip out, and the process will be repeated to knock out the second copy of the gene as well.
• This will be performed for the four divergent family strains and for the SC5314 strain as a control.

References


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Construction of Auxotrophic Strains of *Candida albicans* Clinical Strains

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