Developing a Gene Deletion Strategy for *Candida krusei*

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Abstract:

Candida krusei is a pathogenic fungus that is a frequent cause of candidiasis in humans. In the United States, approximately 1.6% of fungal bloodstream infections and 4% of cancers, such as breast cancer and leukemia, are directly related to *C. krusei*. Although it is an important pathogenic species, *C. krusei* is not well studied because of the lack of genetic tools for its research. Here, we show a strategy for the targeted deletion of *CCC2* and *HOS2* from the genome of *C. krusei*. Copper-transporting P-type ATPase, encoded by *CCC2*, is required for iron uptake in *C. krusei*. In order begin the deletion process of *CCC2*, DNA purification was performed. After purifying the *C. krusei* genome, this gene was amplified via PCR using *CCC2* detection primers. *CCC2* was replaced by a *NAT1* cassette, which offers cell resistance to *nourseothricin,* an antibiotic to which many organisms are sensitive. The *NAT1* cassette was obtained through PCR amplification using *CCC2* disruption primers. In amplifying the *NAT1* cassette, the disruption primers attached 60 nucleotides of flanking sequence on each side of the cassette. This flanking sequence is homologous to the region upstream and downstream of *CCC2* in the *C. krusei* genome. The targeted deletion of *HOS2*, which is a gene required for NAD dependent/independent histone deacetylase activity, was performed following a similar protocol for the deletion of *CCC2*. Alteration to this procedure included the use of *HOS2* detection primers and *HOS2* disruption primers. The genomes of the *C. krusei* transformants were purified, and amplified via PCR, using the gene-specific detection primers. The transformant DNA was then visualized through gel electrophoresis. The results obtained from this experiment lay the ground work for future research on *C. krusei* by addressing a strategy for targeted gene deletion in this species.

C. krusei: A Fungal Pathogen Necessary for the Fermentation of Chocolate

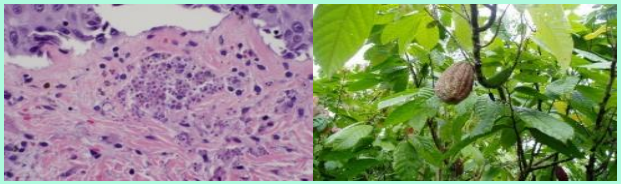

Figure 1. (*Left) Papillary dermis tissue infected by C. krusei (dermatalogy.cdlib.org). (Right) This pathogenic fungal species is also necessary for the fermentation of chocolate from the Cacao bean (botany.wisc.edu).*

Amplification of *NAT1 or HygB* using *Gene-Specific* DR Primers

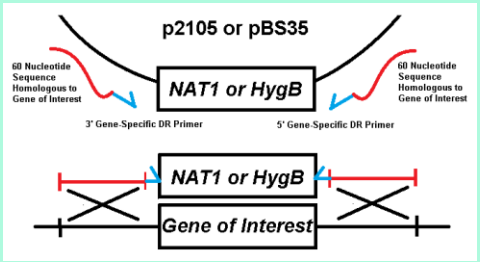

Figure 2. *Amplification of NAT1 or HygB using gene-specific disruption primers. A 60 nucleotide flank, which was homologous to the region upstream and downstream of the gene of interest, was added to NAT1 and HygB during amplification.*

Nourseothricin and Hygromycin Impede the Growth of Wild-Type *C. krusei*

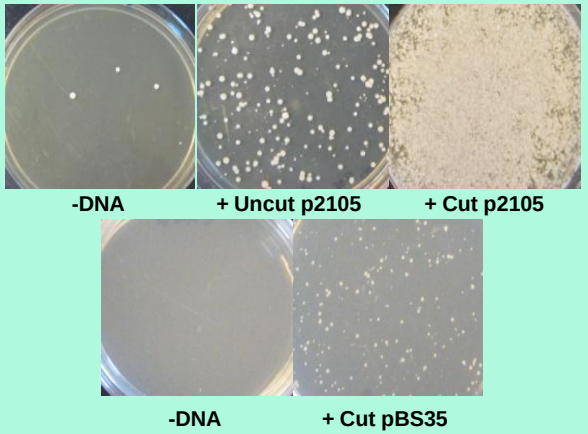

Figure 3. *(Top) Chemical transformation of C. krusei with cut and uncut p2105, a plasmid containing the NAT1 cassette. The cells transformed with cut p2105 exibited the most growth, relative to the untransformed cells and the cells transformed with uncut plasmid. (Bottom) Chemical transformation of C. krusei with cut pBS35, a plasmid containing HygB. These results indicate that cut plasmids integrate most efficiently in C. krusei.*

Chemical Transformation of *CCC2* Allele in *C. krusei* with the *NAT1* Cassette

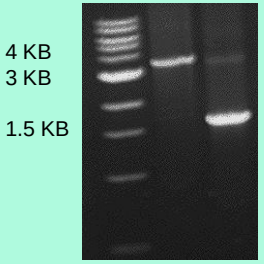

Figure 4. *Heterozygous deletion of CCC2 in C. krusei was achieved through chemical transformation using the NAT1 cassette. The marker used was a 1KB ladder, as shown in L1. L2 shows C. krusei WT. L3 shows C. krusei as a diploid organism, indicated by the band at approximately 3.3 KB, which represents one intact CCC2 allele. The band at approximately 1.6 KB represents the NAT1 cassette, which replaced one CCC2 allele.*

References:
Hager, Jonathan L. et al. (2010). "Candida krusei Fungemia in an Immunocompromised Patient." *Baylor College of Medicine*. <http://dermatology.cdlib.org/1604/case_reports/5_1000080/hsu.html>
Volk, Thomas J. (2007). "*Candida krusei, Geotrichum*, and *Acaulospora scrobiculata*, A Trio of Fungi Needed for Making Chocolate for Valentine's Day." *University of Wisconsin- La Crosse*. <http://botit.botany.wisc.edu/toms_fungi/feb2006.html>