GENERATION OF DELETION AND OVER-EXPRESSION LIBRARY IN THE PATHOGENIC YEAST CANDIDA PARAPSILOSIS

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Over recent years there has been exponential growth in the number of yeast genome sequences. Despite the growth of sequence information, a large number of fungal genes remain uncharacterized. For studying gene function the two methods are to generate over-expression or knock-out mutations.
In our previous work several fungal transcriptional factors have been identified using RNA-Seq data, that were overexpressed during host-pathogen interactions. Based on these data and using the Gateway™ technology we were able to generate *C. parapsilosis* strains that over-express our genes of interest. For this, *C. parapsilosis* CLIB 214 leu- strain was used. Using the *caSAT1* flipper system we have integrated the *RP10* locus of *C. albicans* SC5314 to the *RP10* locus of *C. parapsilosis* CLIB 214 leu- strain. With this integration we were able to adopt the *TDH3p-CLP10* over-expression system established in *C. albicans*. A *TDH3p-CLP10-GFP* constuct was used to test wether this system is able to express the genes in *C. parapsilosis*. For entry vectors the pDONR 221, while for destination vectors the *TDH3p-CLP10* containing vectors were applied. All of the mutants are barcoded using a 20bp tag. For gene knock out mutation, upstream and downstream gene specific flanking PCR products were generated and additionally, HIS1 and LEU2 markers were amplified from the vectors pSN52 and pSN40. After fusion PCR chemical transformations were carried out in *C. parapsilosis* CLIB leu-/his- auxotrophic strain. All of the transformants were barcoded using a 20bp tag. The null mutant strains are being tested in different conditions. We found mutants that show different phenotypes such as increased pseudohyphae formation or regressed growth in different temperatures. In the future, with these methods we are able to identify key regulators that may play a role in the virulence of *C. parapsilosis*. This research was realized in the frame of TÁMOP 4.2.4. A/2-11-1-2012-0001 „National Excellence Program – Elaborating and operating an inland student and researcher personal support systems convergence program". The project was subsidized by the European Union and co-financed by the European Social Fund.