Genetically engineering probiotics to combat human gut diseases: Expression of human interleukin-10 in *Saccharomyces boulardii*

Rachel Kwan, Dr. David Stuart*

Department of Biochemistry, University of Alberta

POSTER

In order to harness the probiotic *Saccharomyces boulardii* for therapeutic purposes, its capacity for genetic engineering was explored. This study proposes the engineering of *S. boulardii* to overexpress and secrete therapeutic peptides such as interleukin-10 (IL-10) and interleukin-8 (IL-8). The probiotic then serves as a transiently colonizing microbe, allowing direct delivery of therapeutic peptides to the intestine in high-concentrations. Consequently, the recombinant probiotic may be beneficial over current oral peptide deliveries or treatments for Inflammatory Bowel Diseases. This study tests the probiotic's ability to produce therapeutic peptides under control of regulatory elements from the closely related yeast *Saccharomyces cerevisiae* (e.g., *PYK1* promoter, *GAL1* promoter, and *MAT*α secretion signal). To demonstrate proper processing in yeast, the model *S. cerevisiae* was transformed with the IL-10 and IL-8 expressing plasmids. Western Blots showed the tagged IL-8 was expressed and secreted in high quantities, with one major product by 24 hours after induction. In the first construct, IL-10 was expressed, but not secreted. The ability for *S. cerevisiae* to express and/or secrete short, recombinant peptides suggests similar potential in the related *S. boulardii*. An engineered strain of *S. boulardii* was created by a knocking out both copies of the *URA3* gene using homologous recombination. The expression, secretion, and activity of IL-8 and IL-10 will be tested by mass spectrometry, enzyme-linked immunosorbent assay (ELISA), and a signalling bioassay from J224 macrophages. Given the demonstration in transferred vectors, a delivery system may be developed by swapping in other therapeutic drugs for expression in *S. boulardii*.