

Turning Probiotics into Antibiotics: Engineering a Broad-Spectrum Antibacterial Probiotic via Inclusion of Antimicrobial Peptide-Encoding DNA, Year Two

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Probiotics were engineered to deliver antimicrobial peptides (AMPs) that show promise as an alternative to antibiotics. This work involved designing a broad-spectrum *Escherichia coli* Nissle 1917 AMP delivery system to target enteropathogenic bacterial infections by producing and secreting the AMPs microcin L and enterocin A, which show antimicrobial activity against gram-negative and gram-positive enteropathogens, respectively. This is the second year of a two-year study. Plasmid pMK-P+ was engineered by incorporating the strong promoter proTeOn+ into the pMK-RQ-Bb backbone. The next step was to include secretion machinery that would enable bacteria to secrete the produced AMP. The microcin L secretion machinery was expensive, so the operon from an available plasmid (pHK22) containing the highly homologous microcin V secretion machinery mutated to halt production of microcin V while maintaining production of the microcin V secretion machinery. The mutated microcin V operon was inserted into pMK-P+ to create the pMK-P+-V plasmid. Finally, gene blocks that encode for production of the AMPs microcin L and enterocin A were designed and inserted into the pMK-P+-V plasmid. Preliminary results indicate that both microcin L and enterocin A can be produced by *E. coli* and secreted via the microcin V secretion machinery. This work is an important step in engineering a broad-spectrum AMP delivery system that has activity against multiple species of enteropathogenic bacteria. Successful synthesis of such a broad-spectrum probiotic would be a tremendous breakthrough in developing an alternative to antibiotics.

Awards Won:

Second Award of \$2,000