Heterologous expression of bacteriocins in *Saccharomyces cerevisiae*

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Abstract

The emergence of multidrug- and extreme drug-resistant bacteria is of great concern and there is a growing need for new or alternative strategies to combat antibiotic resistance. Antimicrobial peptides, such as bacteriocins, are excellent candidates; however, their high manufacturing costs limit their application as alternative antimicrobials. Recombinant gene expression offers the potential to produce these peptides at a larger scale and more cost-effectively. Bactericidal yeast strains were constructed to express two class IIa bacteriocins. The relevant genes were cloned into episomal expression vectors containing either the *S. cerevisiae* alpha mating factor (*MFα1*) or the *Trichoderma reesei* xylanase 2 (*XYNSEC*) secretion signals. The native and codon-optimized variants were also investigated to determine the effect of codon bias. The successful expression of the recombinant peptides was confirmed using agar overlay assays against the indicator organism, *Listeria monocytogenes*. Agar well diffusion assays indicated activity in the supernatant from the strains secreting the AMPs via the *MFα1* secretion signal, but not the *XYNSEC* secretion signal. Tricine-SDS-PAGE analysis confirmed the presence of the *MFα1*-secreted peptides in the yeast supernatant. Bacteriocin activity assays indicated that codon optimization of the one gene resulted in an 8-fold increase in activity of the recombinant peptide, while no significant difference in antimicrobial activity was observed between the codon-optimized and native variant of the other gene. Scanning electron microscopy confirmed the activity of the recombinant peptides; *L. monocytogenes* cells treated with the peptides appeared shriveled and intracellular components were leaking out of the cells. The development of an optimized yeast expression system for the production of antimicrobial peptides could lead to improved peptide bio-manufacturing. The bactericidal yeast strains could also be used as a biological control agent for the prevention of bacterial contamination in commercial fermentations.

Key words: yeast, bacteriocins, *Saccharomyces cerevisiae*, heterologous expression