

The Synthesis and Expression of Peptide CbnY

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Bacteriocins are small antimicrobial peptides produced by bacteria, and have great potential in the food industry as an alternative to antibiotics. The two-component bacteriocins, produced by various strains of lactic acid bacteria, display optimal activity when complexed together. A bacteriocin, CbnX, was recently isolated and reported, but initial structural studies and sequence homology suggests that it is part of a two-component bacteriocin. To test this hypothesis, the proposed second component CbnY, a 33 amino acid peptide predicted by genetics, was prepared by both solid phase peptide synthesis and overexpressed as a fusion protein. Solid phase peptide synthesis resulted in numerous truncated peptides that were difficult to separate by high-pressure liquid chromatography (HPLC). Therefore, CbnY was subsequently expressed as a SUMO-fusion protein and purified by affinity chromatography. A protease cleavage pilot study was performed on the isolated fusion protein, confirming its presence. Once cleaved, CbnY will be further purified by ion exchange and HPLC. It will then be tested for antimicrobial activity in conjunction with CbnX, and its structure will be determined by nuclear magnetic resonance spectroscopy.