Production of Recombinant Bovine Lactoferricin Trimer Peptide Using Pichia pastoris

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ABSTRACT

This study is using the generation of the recombinant yeast, Pichia pastoris,containing the bovine lactoferricin gene fragment of monomer, dimer and trimer and the feasibility of production. First, we selected 20-amino acid antibacterial corepeptide from bovine lactoferricin. The coding sequences of desired peptides are bysynthesizing oligonucleotides sequences, and cloned the target gene into pGAPZ α Cyeast-expression vector. After confirmed by DNA sequencing, the recombinant vector BLFcin／ pGAPZ α C monomer, dimer and trimer were transformed into Pichia pastoris GS115 by electroporation and the transformants were identified by expressing the biological antibacterial peptides. We could get high copy number strains selected by high multiple antibiotic, zeocin and culture B-LFcin／ pGAPZ α C Trimer 29 in the growing medium by flasks. Collecting supernatant and analyzing by Tricine SDS-PAGE and Western blot. Data showed that a 10.8 kD predicted band was obtained in different culture periods. Therefore, we cultured recombinant B-LFcin／ pGAPZ α C Trimer 29 by fermentor and supernatant could be collected. Fast protein liquid chromatography was utilized to purify the lactofefficin from cellular extracts of BLFcin／ pGAPZ α C. The crude extracted protein was purify by Hitrap Heparin column and then was concentrated. The target protein showed that a 10.8 kD predicted band by Tricine SDS-PAGE. The condition of purification for lactofefficin needs further improvement.

Keywords : LFcin、Pichia pastoris

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