Production of Recombinant Casein Phosphopeptide Trimer Using Pichia pastoris and its Calcium Uptake Assay in Caco2 Cell

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ABSTRACT

Casein phosphopeptides (CPPs) are the protein fragment of casein (CN), which can bind with calcium to prevent precipitation and has potential to improve calcium absorption at the intestinal level. The properties of binding with calcium depend on the “ acidic motif ” which consists of three phosphoserines and two glutamic acids. Generally, CPPs are produced in vitro by tryptic and chymotryptic fragmentation of α S1-casein, α S2-casein and β -casein. Furthermore, most CPPs derived from casein hydrolysate are a mixture of various phosphopeptides in length, and this mixture can also limit pharmacological application. In this study, a gene encoding trimer of β -CN(1-25), a kind of CPPs, was constructed into the pGAPZ α C express vector, in Pichia pastoris GS115. Thus, we used a large-scale production of β -CN(1-25) trimer by fermentation in stirred-tank bioreactors. The recombinant product was purified by nickel column. Subsequently, activity assay of recombinant protein indicated that the recombinant trimer of β -CN(1-25) could improve Ca2+ uptake in Caco-2 cell line.

Keywords : Casein phosphopeptide、Pichia pastoris、Calcium absorption

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