

Release of bioactive peptides - characterization of two cell-envelope associated proteinases

Bo Li, Torben Kliche, Diana Meske, Michael de Vrese, Wilhelm Bockelmann, Knut J. Heller

Department of Microbiology and Biotechnology, Max Rubner-Institut, Kiel, Germany

Abstract

Lactic acid bacteria (LAB) are extensively used as starter strains in the manufacture of various fermented products. They are increasingly marketed as health-promoting bacteria. Certain strains have been shown to produce bioactive peptides from milk protein, which are released by proteolytic enzymes. The proteolysis of milk protein by LAB is initiated by cell-envelope proteinases (CEP), which degrade the protein into oligopeptides [1].

In our study, we screened for proteolytically active lactobacilli and lactococci using casein labelled with FITC fluorescence dye [2] and soluble fluorescence was released through proteolysis of casein by the cell-bound proteases. Bioactive activity such like ACE-inhibitory effect was found in the hydrolytes of casein.

Highest proteolytic activity was found in strain 92202, followed by strain 92059. For species identification purposes, we performed 16S rDNA sequencing and used bioinformatical tools (ARB) for species-tree construction. Nucleotide sequence analyses together with physiological sugar fermentation tests suggested that strain 92059 is a *Lactobacillus delbrueckii* subsp. *bulgaricus* and 92202 is a *Lactobacillus delbrueckii* subsp. *lactis* strain, respectively. Presence of proteinase genes in both strains was confirmed by Southern blot assay. Proteinase genes from both strains were amplified via PCR with primers designed on the basis of published sequences of *Lactobacillus delbrueckii* and cloned into pSMART vector for sequencing. Expression of the genes are being optimized in *Lactococcus lactis* with the nisin controlled expression system (NICE), and purified enzymes will be used to degrade milk protein for release of bioactive peptides.

[1] Savijoki, Ingmer & Varmanen (2006) *Appl. Microbiol. Biotechnol.* 71: 394-406

[2] Twining (1984) *Anal. Biochem.* 143: 30-34

Abstract für:

11th International Symposium on Lactic Acid Bacteria: Health, sustainability, diversity, and application - A meeting on the fundamentals and application of the most important bacteria used in food and feed production.

Congress Centre "Hotel Zuiderduin", Egmond aan Zee, the Netherlands

August 31 to September 4, 2014.