

Study of fermentation products of bacillus subtilis natto and their emulsifying and milk-clotting activities

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ABSTRACT

Natto, a fermented soybean by natto bacteria, has been concerned because of its usefulness and high nutrient components. In addition, many biopolymers, such as γ -polyglutamic acid (γ -PGA), levan (a fructan), and enzymes were found in natto. Recently, many applications of γ -PGA and fructan were used in food industry. It was found by accident, that liquid extract of natto displayed emulsifying activity and milk-clotting activity in the process of making bakery products. This research investigates what in the fermentation products of *Bacillus subtilis* natto have contributed to these activities. Both liquid and solid fermentation of natto have shown emulsifying activity, but the solid fermentation of natto showed even higher emulsifying activity. In contrast the levan and γ -PGA did not display any emulsifying activities. Unfortunately, the emulsifying activity of natto preparation was not significant in comparison with commercial emulsifiers. Although *Bacillus subtilis* Takahashi produces milk-clotting enzyme on both solid and liquid natto preparation, γ -PGA and levan fermentation don't produce milk-clotting enzyme at all. The milk-clotting activity was affected by the variation of operational and nutritional parameters. Remarkable milk-clotting activity was produced when *Bacillus subtilis* (natto) Takahashi was cultivated in the medium containing sucrose (50g/L), NaCl (10g/L), MgSO₄·7H₂O (0.5g/L), NaH₂PO₄·2H₂O (3g/L) and NaHPO₄·12H₂O (3g/L) pH 6 at 37°C for 1 day shaking at 175rpm. The highest milk-clotting activity obtained was 685.7 SU/ml or 12,000 SU/g. The ratio of milk-clotting activity to proteolytic activity obtained was 2981 in liquid experiment and 52,174 in solid experiment. The milk-clotting activity was quite stable between pH 5 and 6. When heating at 70°C for 5 min, the fermentation broth lost its milk-clotting activity almost entirely. However, the milk-clotting activities of the fermentation broth were quite stable at RT and 40°C. The ratio of milk-clotting activity to proteolytic activity enzyme of *Bacillus subtilis* (natto) Takahashi was not as higher as other commercial proteases (calf rennet and *Mucor miehei* rennet). However, with the useful properties of *Bacillus subtilis* (natto) Takahashi and its fermentation products, milk-clotting enzyme of *Bacillus subtilis* (natto) Takahashi may be concerned and used in cheese-making.

Keywords : *Bacillus subtilis* natto ; emulsifying activity ; milk-clotting activity

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