

# Production and characterization of milk-clotting enzyme from isolated *Bacillus subtilis* DYU6

王曼瑩、吳建一

E-mail: 345441@mail.dyu.edu.tw

## ABSTRACT

Rennet (milk-clotting enzyme, MCE) is mainly in the animals, plants and microorganisms. Due to the high price and shortage of animal rennet supply there have so many studies attempt to replace calf rennet with other milk clotting proteases. The aim of this work was to investigate the characterization of the milk-clotting enzyme from isolated *Bacillus subtilis* DYU6. The effect of different culture variable on the production of MCE by *B. subtilis* DYU6 in flask and 5-L jar fermentor. In the flask, the optimal carbon sources was starch (20 g/ L) and the medium addition of NaCl (50mM) the results shown that the maximum enzyme activity was 1,000 SU, the maximum proteolytic activity was 0.16 U/ mL. In a 5-L jar fermentor, we achieved the maximum enzyme activity was 600 SU when the pH, aeration rate and aeration rate were controlled at 6, 100 rpm and 0.5 vvm, respectively. A partial purified enzyme was obtained by fractional precipitation with  $(\text{NH}_4)_2\text{SO}_4$ . The fractions obtained by precipitation, 50-70% possessed the milk-clotting activity (MCA) (1,333 SU/ mg). The 50-70%  $(\text{NH}_4)_2\text{SO}_4$  fraction was further purified by column chromatography (superdex<sup>TM</sup> grade) with MCA (4,176 SU/mg). Additionally, to study the effects of some of exogenous factors that can affect the processing properties of milk (coagulation temperature,  $\text{CaCl}_2$  concentration, NaCl concentration, pH). The MCA increased with the decreasing of milk pH from 7.5 to 5.5. Some metal ions (such as  $\text{Al}^{3+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Fe}^{2+}$ ) had a very clear function to accelerate milk coagulation whereas  $\text{Ni}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Na}^+$  decelerated the activity slightly. Both crude and partially purified extract were active at the range pH 5 – 11, however, optimum activity was displayed at pH 7. The enzyme was highly stable, retaining 70 % of its original activity when the liquid and solid enzyme storage at 4 °C for 240 and 140 days. Moreover, a model involved with *B. subtilis* DYU6 growth on the Logistic model were good agreement with the experimental observation.

Keywords : *Bacillus subtilis* DYU6、milk-clotting enzyme、purified

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