利用篩選之Bacillus subtilis DYU6菌株生產凝乳酵素及其特性之研究

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摘 要

凝乳酵素(milk-clotting enzyme, MCE)主要源自動物、植物以及微生物。由於動物性凝乳酵素供不應求，導致價格居高不下，所以，許多學者便開始研究其它替代性的蛋白?，以取代小牛凝乳酵素。本研究的目的是利用篩選之Bacillus subtilis DYU6菌株生產MCE，並探討其特性。利用搖瓶和5-L發酵槽，探討不同培養條件對B. subtilis DYU6菌株發酵生產MCE的影響。搖瓶部分，發現最佳碳源為澱粉(20 g/ L)，在培養基中添加50mM的NaCl，可得到酵素最佳活性為1,000 SU，最大的蛋白質水解活性為0.16 U/ mL。在5L發酵槽實驗方面，由實驗結果發現，當培養基pH調控在6、轉速為100 rpm、曝氣量為0.5 vvm時，可達到最高酵素活性為600 SU。使用(NH4)2SO4沉澱可獲得部分純化的沉澱物。當 (NH4)2SO4飽和度為50-70%時，由收集到的沉澱物，可測得凝乳酵素活性(milk-clotting activity, MCA)(1,333 SU/ mg)。將收集到的沉澱物再使用column chromatography (superdexTM grade)進一步地純化，由收集的沖提液中測得MCA為4,176 SU/ mg。研究外在因子(凝固的溫度、CaCl2濃度、NaCl濃度、pH)對於牛奶凝乳特性的影響。MCA隨著牛奶的pH遞減(從7.5-5.5)而增加。添加如：Al3+、Zn2+、Fe3+、Fe2+ 金屬離子可以明顯地促進凝乳，而添加Ni2+, Cu2+ 及Na+則會抑制活性。不論粗酵素或是純化酵素，其酵素活性範圍在pH 5-11，而擁有最佳活性的pH為7。酵素儲存穩定性則是在4°C最穩定，液態或粉末酵素分別在240天和140天內仍能保留70 %的初始活性。此外，對於B. subtilis DYU6菌體生長可利用Logistic model進行動力學解析，由結果顯示Logistic model對於菌體生長具有良好的模擬性。

關鍵詞 : 凝乳酵素、純化

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