BIOTECHNOLOGY & BIOENGINEERING

牛奶中分离的乳酸菌CGMCC1306中谷氨酸脱羧酶的分离纯化及酶学性质研究

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收稿日期 修回日期 网络版发布日期 接受日期

揚要 A Lactobacillus brevis CGMCC 1306 isolated from fresh milk without pasteurization was found to have higher glutamate decarboxylase (GAD) activity. An effective isolation and purification procedure of GAD from a cell-free extract of Lactobacillus brevis was developed, and the procedure included four steps: 30%—90% saturation (NH4)2S04 fractional precipitation, O sepharose FF anion-exchange chromatography, sephacryl S-200 gel filtration, and resource O anion-exchange chromatography, Using this protocol, the profiled GAD was electrophoretic homogeneity via SDS-PAGE. The purification fold and activity recovery of GAD were 43.78 and 16.95%, respectively. The molecular weight of the purified GAD was estimated to be approximately 62 kDa via SDS-PAGE. The optimum pH and temperature of the purified GAD was returned to the purified GAD were 4.4 and 37°C, respectively. The purified GAD had a half-life of 50min at 45°C and the Km value of the enzyme from Lineweaver-Burk plot was found to be 8.22. 5′-pyridoxal phosphate (PLP) had little effect on the regulation of its activity.

关键词 <u>Lactobacillus brevis</u> <u>glutamate decarboxylase</u> <u>purification</u> <u>anion-exchange chromatography</u> <u>characterization</u>

分类号 DOI:

Purification and characterization of glutamate decarboxylase of Lactobacillus brevis CGMCC 1306 isolated from fresh milk

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Received Revised Online Accepted

Abstract A Lactobacillus brevis CGMCC 1306 isolated from fresh milk without pasteurization was found to have higher glutamate decarboxylase (GAD) activity. An effective isolation and purification procedure of GAD from a cell-free extract of Lactobacillus brevis was developed, and the procedure included four steps: 30%—90% saturation (NH4)2SO4 fractional precipitation, Q sepharose FF anion-exchange chromatography, Using this protocol, the purified GAD was demonstrated to possess electrophoretic homogeneity via SDS-PAGE. The purification fold and activity recovery of GAD were 4.78 and 16.95%, respectively. The molecular weight of the purified GAD was estimated to be approximately 62 kDa via SDS-PAGE. The optimum pH and temperature of the purified GAD were 4.4 and 37°C, respectively. The purified GAD had a half-life of 50min at 45°C and the Km value of the enzyme from Lineweaver-Burk plot was found to be 8.22. 5′-pyridoxal phosphate (PLP) had little effect on the regulation of its activity.

Key words Lactobacillus brevis; glutamate decarboxylase; purification; anion-exchange chromatography; characterization

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