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生物技术 生命科学

乳杆菌乳糖酶的基因异源表达及酶学性质分析

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摘要:

采用简并引物PCR和TAIL-PCR方法,从乳杆菌Lactobacillus sp. B164中克隆得到一个乳糖酶基因bg42-164。基因全长2 031 bp,编码676个氨基酸和一个终止密码子,预测分子量76 kDa,无信号肽序列。将bg42-164连接pET-30a(+)载体并转入大肠杆菌BL21(DE3),经IPTG诱导表达后可检测到乳糖酶活力。SDS-PAGE分析乳糖酶BG42-164的蛋白分子量为76 kDa,使用组氨酸标签亲和层析方法进行蛋白纯化,并对纯化的乳糖酶BG42-164进行了酶学性质分析。该酶最适反应温度为50℃,经50℃处理30 min后,剩余酶活力保留80%以上,pH 6.0时该酶的水解活力最高。牛奶水解试验表明,乳糖酶BG42-164对乳糖的水解效果良好,5 mL牛奶中添加250 U酶蛋白,在50℃条件下2 h乳糖水解率为100%。该酶温度范围宽,乳糖水解效果好,为其在低乳糖奶生产中应用奠定了理论基础。

关键词: 乳杆菌;乳糖酶;β-半乳糖苷酶;基因表达

Expression of Lactase Gene from Lactobacillus sp. and its Enzymology Characteristics Analysis

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Abstract:

A lactase gene bg42-164 was cloned from a Lactobacillus sp. B164 strain using degenerate PCR and TAIL-PCR technology. The 2 031 bp bg42-164 gene encodes 676 amino acid residues and one stop codon, and its predicted molecular weight was 76 kDa without signal peptide. The bg42-164 gene was inserted into pET-30a(+) vector and then transformed into Escherichia coli BL21(DE3) to detect the lactase activity after induced by IPTG. Apparent molecular weight of the recombinant enzyme was about 76 kDa by SDS-PAGE. The enzymology character of this enzyme was analyzed after purified by histidine-tag affinity chromatography. The optimum temperature of BG42-164 was 50℃, approximately 80% of the activity remained after incubation at 50℃ for 30 min. The optimum pH of lactase activity was 6.0. With additional 250 U lactase BG42-164 per 5 mL milk, the hydrolysis rate of lactose was 100% at 50℃ for 2 h. The results illustrate considerable thermo-stability and hydrolysis ability of the BG42-164, which would provide a theoretical basis for further preparation of lactose-free milk.

Keywords: Lactobacillus lactase β-galactosidase gene expression

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