

论文

荧光假单胞杆菌胞外蛋白酶的纯化及热稳定性

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摘要:

从原料乳中分离获得一株荧光假单胞菌Rm12, 该菌株分泌的胞外蛋白酶具有很强的耐热性, 其发酵上清液经过硫酸铵沉淀、阴离子层析、疏水层析和分子排阻层析纯化得到SDS-PAGE均一蛋白酶, 经鉴定确认该酶是一种新的金属蛋白酶, 命名为Ht12, 对Ht12的基本特性和热稳定性进行了研究. 结果表明, 此蛋白酶分子量为45000, 含有较高的脯氨酸和二硫键, N末端氨基酸残基序列为MSKVKDKAIVSAAQAS, Mn^{2+} 对蛋白酶活力有促进作用. 该蛋白酶具有较高的热稳定性, 于160 °C加热20 s, 保留活力3.8%.

关键词: 荧光假单胞杆菌 耐热蛋白酶 金属蛋白酶 原料乳

Purification and Heat-stable Properties of a Novel Protease from *Pseudomonads fluorescens* Rm12

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Abstract:

Heat-resistant proteases from psychrotrophic bacteria in raw milk may induce bitterness, gelation and hydrolyzation of sterilized milk. A novel extracellular heat-stable metalloprotease, named as Ht12, was found for the first time from *Pseudomonads fluorescens* Rm12, which was isolated from raw milk. Ht12 was purified to homogeneity from the culture supernatant via ammonium sulfate precipitation, ion-exchange chromatography, hydrophobic chromatography, and size exclusion chromatography and its properties of enzymology and heat-stable properties was studied. This protease in its native state was identified as a monomer of 45000 Da, containing Pro and disulfide bonds and the N-terminal sequence was MSKVKDKAIVSAAQAS. Mn^{2+} has positive effect on activity. The protease has a higher heat resistance. After treatment of 160 °C for 20 s, the residual activity was 3.8%.

Keywords: *Pseudomonads fluorescens* Heat-stable protease Metalloprotease Raw milk

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