吉林大学学报(医学版) 2012, 38(1) 70-74 DOI: ISSN: 1671—587X CN: 22—1342/R

基础研究

酿酒酵母无机焦磷酸酶基因在大肠杆菌中的表达及其意义

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摘要:

目的：利用大肠杆菌表达酿酒酵母无机焦磷酸酶(Y-IPPA)，获得具有活性的酿酒Y-IPPA。 方法:以 酿酒酵母基因组为模版，通过查询NCBI获得酿酒酵母无机焦磷酸酶序列，设计引物，用PCR方法获得酿酒Y-IPPA基因序列，插入克隆性载体pMD19-T Simple Vector中，并转化至大肠杆菌DH5α扩增，获得重组质粒，经酶切及测序验证正确后，将酶切的目的片段插入到原核表达载体p ET28(a)中，再转化大肠杆菌DH5α扩增，经酶切及测序验证正确后,转化大肠杆菌BL21(DE3)表达蛋白,通过IPTG诱导表达出Y-IPPA,以金属离子亲和层析蛋白纯化系统纯化蛋白，并测定其比活和酶学性质。结果：获得与pMD19-T Simple Vector一致的Y-IPPA碱基序列，构建了重组表达质粒pET28(a)-Y-IPPA,并转化大肠杆菌BL21(DE3),以IPTG诱导并进行SDS-PAGE电泳，在相对分子质量为32 000处可见Y-IPPA蛋白条带；镍柱亲和层析纯化目的蛋白，其中一组的蛋白浓度、活性和比活分别为0.312 $g.L^{-1}$、31.13 $units.mL^{-1}$和99.73 $uints.mg^{-1}$。结论:成 功制备Y-IPPA蛋白,为进一步研究其酶学性质奠定了基础。

关键词： 酿酒酵母；无机焦磷酸酶；大肠杆菌

Expression of Saccharomyces cerevisiae inorganic pyrophosphatase gene in Escherichia coli and its significance

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Abstract:

Objective To express Saccharomyces cerevisiae(S.cerevisiae) inorganic pyrophosphatase (Y-IPPA) inEscherichia coli (E.coli) and obtain the active yeast inorganic pyrophosphatase. Methods S. cerevisiae genome was used as a template,the yeast inorganic pyrophosphatase sequences were obtained by searching NCBI, the primers were designed,the yeast inorganic pyrophosphatase gene sequence was obtained by PCR.It was inserted in the T-simple 19 cloning vector and transformed into amplification of E.coli DH5α,the recombinant plasmid was se
quenced and verified correctly,the restriction fragments were inserted into prokaryotic expression vector pET28 (a),and then transformed into E.coli DH5α and then transformed into E.coli BL21 (DE3) after restriction analysis and sequencing correctly. Y-IPPA was expressed by IPTG induction.The protein purification system was used to purify the proteins and the protein level, activity and specific activity were determined. Results The T-simple 19 consistent with the Y-IPPA base sequence was obtained and the recombinant expression vector pET28 (a)-Y-IPPA was constructed,and transformed into E. coli BL21 (DE3) with IPTG induction and SDS-PAG Eelectrophoresis. The molecular weight of 32 000 was noted in Y-IPPA protein bands.Nickel affinity chromatography was performed to obtain the target protein.The protein level, activity and specific activity were 0.312 $g.L^{-1}$,31.13 $units.mL^{-1}$,and 99.73$uints.mg^{-1}$.Conclusion The Y-IPPA protein is obtained successfully,and it lays the foundation for further study on its enzymatic features.

Keywords: saccharomyces cerevisiae;inorganic pyrophosphatase;Escherichia coli

收稿日期 2011-06-29 **修回日期** **网络版发布日期** 2012-01-28

DOI:

基金项目:

江苏省科技厅科技支撑计划项目资助课题（BE2008121）

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