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生物技术 生命科学

香蕉束顶病毒Rep蛋白特性分析及纯化体系的建立

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摘要:

以香蕉束顶病毒海口分离物DNA1(Accession NO. FJ463042)的Rep蛋白为研究对象,利用生物信息学软件对该蛋白的理化性质、结构组成、信号肽、磷酸化、二级结构、三级结构与功能结构域等作了较为详细的分析与预测,并配置了相关纯化试剂。将含重组质粒pET32b-Rep的E.coli BL21(DE3),接种到LB液体培养基,在20℃、0.1 mmol/L IPTG条件诱导4 h,产生大量的可溶性重组蛋白,经AKTA Explorer 100系统亲和层析柱后,用不同梯度的Washing buffer洗脱杂蛋白并对各阶段产物进行12% SDS-PAGE分析,确定了100 mmol/L咪唑的Washing buffer洗脱浓度,最终获得大量高纯度的重组蛋白。以His*Tag Monoclonal Antibody为一抗,Western blot鉴定结果表明纯化重组蛋白即为His-Rep融合蛋白。该纯化体系的建立为研究BBTV Rep蛋白的结晶奠定了基础,也为今后ABTV、FBNYV、MVDV、PNYDV等病毒的相关复制蛋白纯化提供了重要数据。

关键词: 香蕉束顶病毒;Rep蛋白;特性分析;纯化体系;建立

Characteristics Analysis of Banana Bunchy Top Virus Rep Protein and Establishment of its Purification System

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Abstract:

With Rep protein from banana bunchy top virus Haikou isolates DNA1 (Accession NO. FJ463042) as the research object, physical and chemical properties, composition, signal peptide, phosphorylation, secondary structure, tertiary structure and functional domains of the protein, etc. were analyzed and forecasted using bioinformatics software, and associated protein purification reagents were configured according to these features. The recombinant plasmid pET32b-Rep of E. coli BL21 (DE3) was inoculated into LB liquid medium and was induced at 20℃, 0.1 mmol/L IPTG for 4 h. A lot of soluble recombinant protein was generated and the supernatant was added to the AKTA Explorer 100 system by affinity chromatography. A different gradient of Washing buffer eluted impurity proteins and products of various stages via 12% SDS-PAGE electrophoresis to determine 100 mmol/L imidazole as washing buffer concentration, and a large number of high-purity recombinant proteins were obtained ultimately. His*Tag Monoclonal Antibody as the first antibody, Western blot showed that the purified recombinant protein was the His-Rep fusion protein. The establishment of this purification system could not only provide basis for studying the crystallization of BBTV Rep, but also provide important data for purification of ABTV, FBNYV, MVDV, PNYDV, etc.

Keywords: BBTV(Banana bunchy top virus) Rep protein characteristics analysis purification system establishment

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