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硕士学位论文

大肠杆菌表达重组Beta-1, 3-葡聚糖基因

Expression of Beta-1,3-Glucanase gene in Escherichia coli

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摘 要

在本实验中，来自酿酒酵母HS1185的胞外－1， 3－葡聚糖基因被插入TA克隆载体pMD-18中，并被转入大肠杆菌JM109中。重组质粒命名为pMDT-18-GLU。通过Xho I和Nco I双酶切质粒pMDT-18-GLU获得的－1， 3－葡聚糖基因片段插入pET22b(+)的Xho I和Nco I酶切位点。此重组质粒命名为pET22b/GLU。质粒pET22b-GLU被转入大肠杆菌BL21(DE3)，通过菌落PCR筛选出阳性克隆。

提取出来已经插入Beta－1， 3－葡聚糖基因的pET22b-GLU质粒和pET22b(+)通过PCR扩增和限制性内切酶验证分析。PCR 质粒pET22b-GLU扩增出1337 bp的条带，与酿酒酵母中Beta－1， 3－葡聚糖基因大小一致。在酶切验证中，重组质粒pET22b/GLU被Xho I和Nco I双酶切，得到了5431 bp和1337 bp的两条带，分别为pET22b(+)和－1， 3－葡聚糖基因。最后通过测序来进一步验证正确的基因序列被插入质粒中。测序结果显示，插入的基因序列与与酿酒酵母中－1， 3－葡聚糖基因序列有98%的序列同源性。

培养携带pET22b-GLU质粒的重组大肠杆菌BL21(DE3)，并通过IPTG诱导蛋白质表达。通过DNS测定残糖结果显示并没有酶活。但是酿酒酵母中－1， 3－葡聚糖基因已经成功插入pET22b(+)质粒，并转入大肠杆菌BL21(DE3)中。

关键词：Beta－1, 3－葡聚糖基因；酿酒酵母；重组质粒

Abstract

In this study the exo--1,3-glucanase gene (GenBank Accession No. X59259; EC No. 3.2.1.58) from Saccharomyces cerevisiae strain HS1185 was ligated with TA cloning vector pMDT-18 and transformed into Escherichia coli strain JM109. The constructed plasmid was named pMDT-18-GLU. Plasmid pMDT-18-GLU was digested with Xho I and Nco I restriction enzymes and the obtained fragment of -1,3-glucanase gene was ligated with plasmid pET22b(+) at Xho I and Nco I restriction sites. The constructed plasmid was named pET22b-GLU. Plasmid pET22b-GLU was transformed into Escherichia coli strain BL21 (DE3) and the positive colonies were identified by performing colony PCR.

The extracted plasmid pET22b-GLU that contained the correct insert of the -1,3-glucanase gene and plasmid pET22b(+) was confirmed using PCR amplification and restriction enzyme analysis. The performed PCR gave amplified fragments of about 1337 bp-the size which was in accordance with the original size of -1,3-glucanase gene from Saccharomyces cerevisiae. For the restriction enzyme analysis, the recombinant plasmid pET22b-GLU was double digested with enzymes Xho I and Nco I and gave two bands of sizes 5431 bp and 1337 bp for plasmid pET22b(+) and -1,3-glucanase gene, respectively. Further confirmation of the correct insert was done by carrying out DNA sequencing of the cloned gene. The nucleotide sequence results showed high identity of 98% to the original beta-1,3-glucanase gene from Saccharomyces cerevisiae.

The recombinant Escherichia coli strain BL21 (DE3) carrying plasmid pET22b-GLU was cultivated and induced with IPTG for protein expression. The results showed that there was no activity using the DNS assay for reducing sugar. However, based on the results obtained, -1,3-glucanase gene from

Saccharomyces cerevisiae strain HS185 was successfully cloned into plasmid pET22b(+) and transformed in Escherichia coli strain BL21(DE3).

Keywords: Beta-1,3-glucanaserecombinant plasmidSaccharomyces cerevisiae

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