

木糖苷酶的基因工程菌发酵,产生 $\alpha$ -阿拉伯糖苷酶/木糖苷酶,可以避免自然菌生长密度低和酶系复杂给 $\alpha$ -阿拉伯糖苷酶/木糖苷酶的纯化带来的麻烦。在实验过程中,利用在常温菌 *E. coli* 中表达 $\alpha$ -阿拉伯糖苷酶/木糖苷酶对热的稳定性,将 *E. coli* JM109 (DE3) / pAlter-Ex1-xar 菌细胞破碎后的上清经过 70℃ 20 min 的热处理后,可以去除大量菌体蛋白,酶活收率达 70.2%,纯化倍数为 5.26 倍,这一结果表明工业化制备重组酶时可以采用此低成本的方法,重组 $\alpha$ -阿拉伯糖苷酶/木糖苷酶可利用 DEAE-Sephcel 阴离子交换柱层析进一步纯化,收率达 55.8%,纯化倍数为 18.6 倍。由于构建工程菌时在基因表达产物上融合了一个 6 个组氨酸的标签,经  $\text{Ni}^{2+}$  亲和层析柱使酶纯度达到电泳均一。最终通过热处理、DEAE-Sephcel 阴离子柱层析、金属  $\text{Ni}^{2+}$  的亲和层析提纯后,酶达到电泳纯时的提纯倍数为

49.3 倍,收率为 20.4%。SDS-PAGE 法测定 $\alpha$ -阿拉伯糖苷酶/木糖苷酶的分子质量为 85 ku,与理论推算值相吻合。本实验研究为其进一步的酶学性质鉴定和分析工作打下了良好的基础,也为重组酶的大规模工业化提取奠定了基础。

### 参 考 文 献

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## Purification of Recombinant Thermostable Arabinofuranosidase-xylosidase

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**ABSTRACT** The recombinant thermostable arabinofuranosidase-xylosidase from *T. ethanolicus* was purified from culture broth of gene engineering strain *E. coli* JM109 (DE3) / palter-Ex1-xar through following steps: a) heat precipitation; b) ion exchange chromatography on DEAE-sephacel; and c) immobilized metal affinity chromatography. The purified enzyme showed a single band on SDS polyacrylamide gel electrophoresis with a purification of 49.3 fold, and a yield of 20.4%. The optimum activity of arabinofuranosidase was found at pH 6.0 and 80℃, the enzyme had 1 h half-life at 75℃, The optimum activity of xylosidase was found at pH 5.7 and 85℃, the enzyme had 1 h half-life at 84℃. SDS-PAGE analysis showed that the molecular weight of expressed recombinant products was 85.90. It was in good agreement with the molecular mass of enzyme of deduced from the DNA sequence, 85ku.

**Key words** arabinofuranosidase-xylosidase, gene engineering strain, recombinase purification



## 浙江海正集团新型降解塑料聚乳酸进入中试

海正集团有限公司研制的新型生物降解塑料——聚乳酸的研究成果于 2002 年 12 月通过了省级技术鉴定,于 2003 年 6 月进入中试阶段。