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基于谷氨酸棒杆菌NCg11221蛋白的新型细菌表面展示系统

A novel bacterial cell-surface display system based on NCg11221 from *Corynebacterium glutamicum*

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中文摘要:

摘要: 【目的】开发一种新型的大肠杆菌表面展示系统, 为C末端截短NCg11221蛋白作为锚定蛋白提供科学依据, 丰富并优化细菌表面展示系统。【方法】扩增C末端截短NCg11221序列和 β -淀粉酶基因, 构建融合蛋白表达载体。将重组载体PET-NA和空载体PET-28a分别转入Rose tta(DE3) pLysS中, IPTG诱导表达, SDS-PAGE和Western blot鉴定融合蛋白表达情况。将诱导表达菌株进行免疫荧光染色, 荧光显微镜观察和流式细胞分析检测 β -淀粉酶的展示。酶活测定和淀粉水解分析验证被展示 β -淀粉酶的活性。【结果】融合蛋白成功地在大肠杆菌中表达, 有活性的 β -淀粉酶通过与锚定蛋白C末端的融合被展示在了宿主菌表面, 展示 β -淀粉酶的重组菌可以水解利用培养基中的淀粉。【结论】成功开发了一种以C末端截短NCg11221为锚定蛋白的新型大肠杆菌表面展示系统, 并以此系统展示了分子量大小为56 kDa的活性酶, 为该系统在全细胞催化剂或吸附剂等方面的应用奠定了基础。

英文摘要:

Abstract: [Objective] To develop a novel *Escherichia coli* cell surface display system by using C-terminally truncated NCg11221 as the anchoring protein, which greatly enriched or optimized the bacterial displayed systems. [Methods] We amplified the sequence of C-terminally truncated NCg11221 and β -amylase, and constructed the fusion expression vector. Then we transformed the recombinant plasmids PET-NA and PET-28a into Rosetta (DE3) pLysS. The fusion protein expression was induced by IPTG and identified by SDS-PAGE and Western blot analysis. The IPTG induced strains were immunostained and investigated by fluorescence microscope and flow cytometry to detect the displayed β -amylase. Finally, we analyzed the activity of β -amylase and starch hydrolyzation in order to determine whether the displayed β -amylase has the activity or not. [Results] The fusion protein was successfully expressed in *E. coli*, and the active β -amylase was displayed on the cell surface by fusing it to the C terminus of the anchor. The recombinant strain displaying β -amylase can utilize soluble starch in the medium. [Conclusion] A novel *E. coli* surface display system by using C-terminally truncated NCg11221 as the anchor motif was successfully developed. The active enzyme with a molecular size of 56 kDa was displayed on *E. coli* by this system, which provided the basis for the application of the system in whole-cell biocatalyst or biosorbent.

姚文娟, 范文俊, 许小乐, 张伟, 邓小昭. 基于谷氨酸棒杆菌NCg11221蛋白的新型细菌表面展示系统. 微生物学报, 2012, 52(2): 177-183

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