

合浦珠母贝矿化基因Pearlin重组蛋白的表达条件优化

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摘要

为了获得稳定高表达的合浦珠母贝 (*Pinctada fucata*) 壳质蛋白Pearlin基因的重组融合蛋白, 该研究从合浦珠母贝外套膜总RNA中扩增得到Pearlin的cDNA, 将其ORF克隆至原核表达载体pET32a构建得到重组质粒pET32a-Pearlin, 并转化表达宿主菌大肠杆菌 (*E. coli*) BL21 (DE3), 得到的原核重组融合蛋白pET32a-Pearlin约为34.19 kD。对重组融合蛋白表达所需IPTG诱导浓度、培养温度、培养基pH及IPTG诱导时间、时机进行了优化。结果显示, IPTG浓度在0.6~1.4 mmol·L⁻¹范围内诱导效果最佳; 在IPTG浓度为1 mmol·L⁻¹、相同培养时间(6 h)下, 37 °C表达蛋白最多; 重组蛋白在pH分别为6.0、7.0、8.0的培养基中表达量变化不大; 在IPTG诱导浓度一定的条件下, 最佳诱导时间为4~6 h; 在IPTG诱导浓度和诱导时间一定的条件下, 在转接3~4 h后进行IPTG诱导蛋白表达量较理想。对重组融合蛋白pET32a-Pearlin的可溶性进行检测, 发现在不同的诱导条件下, 融合蛋白都主要以包涵体形式存在。

关键词: 合浦珠母贝, Pearl in, 原核表达, 表达条件优化, 包涵体

Abstract :

To obtain high and stable expression recombinant fusion protein of Pearl in, we constructed the recombinant vector by using the open reading frames (ORF) of Pearl in cloned from the mantle tissue of pearl oyster (*Pinctada fucata*) and optimized the expression conditions. The Pearl in ORF was cloned into the vector pET32a and the plasmids of pET32a-Pearlin were obtained and then transformed into *E. coli* BL21(DE3). His-tagged insoluble fusion protein was highly expressed and the molecular weight of the fusion protein was about 34.19 kD. We optimized the conditions for IPTG concentrations, induction duration and timing, and temperature and pH of the medium. The results showed that the optimal IPTG concentration ranged from 0.6 mmol·L⁻¹ to 1.4 mmol·L⁻¹ and the best temperature was 37 °C when IPTG concentration was 1.0 mmol·L⁻¹ and incubation time was 6 h. The expression levels of recombinant protein did not change significantly when the pH of medium was 6.0, 7.0 and 8.0. When IPTG concentration was kept constant (1.0 mmol·L⁻¹), 4~6 h induction was optimal; when IPTG concentration (1.0 mmol·L⁻¹) and induction duration (6 h) were kept constant, the best starting time for induction was 3~4 h after transformation. Solubility test indicates that fusion protein pET32a-Pearlin was mainly in the form of inclusion body.

Key words: pearl oyster Pearl in prokaryotic expression optimization of expression condition inclusion body

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