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丹参**SmJAZ1**蛋白原核表达及条件优化

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中文摘要：目的：在前期克隆丹参*SmJAZ1*基因的cDNA全长基础上,为研究丹参SmJAZ蛋白的功能,在大肠杆菌BL21(DE3)中诱导表达丹参SmJAZ1蛋白,并对其表达条件进行优化。方法：利用分子克隆的方法将丹参JAZ1基因构建到原核表达载体pET32a上,转化到大肠杆菌BL21(DE3)宿主菌中进行诱导表达。结果：对影响重组蛋白表达的4个因素,即诱导温度、诱导时间、IPTG浓度、及IPTG添加时间进行优化,确定丹参SmJAZ1重组蛋白最适表达条件。IPTG浓度对目的蛋白的表达量没有显著影响;随诱导时间和诱导温度增加,SmJAZ蛋白的表达量增加;而IPTG添加时间对蛋白的表达量有明显影响。结论：丹参JAZ1蛋白在30 ℃温度条件下,重组菌生长2 h(A_{600}=0.9),加入0.1 mmol·L^{-1}浓度的IPTG,诱导20 h后,表达条件最合适。

中文关键词：丹参 JAZ1 原核表达 条件优化

Optimizing expression of recombinant jasmonate ZIM-domain protein from *Salvia miltiorrhiza*

Abstract:Objective: Accumulation of tanshinton in *Salvia miltiorrhiza* are enhanced by exogenous application of jasmonates. The core JA signaling module COI1/JAZ/MYC2 play a central role on control of downstream gene expression in the JA pathway. To obtained the antibody of SmJAZ, SmJAZ recombinant protein was expressed in *Escherichia coli* and optimal expression was performed. **Method:** The full-length *SmJAZ1* ORF was sub-cloned in a prokaryotic expression vector pET32a. The recombinant fusion protein had high expression level in BL21(DE3) strain of *E. coli*, and SDS-PAGE analysis showed its molecular weight was about 24 kDa. **Result:** The induction of *E. coli*[pET32.JAZ1] in different temperature, induction time, IPTG concentrations and IPTG adding time of *E. coli* were performed. The induction time and the induction temperature are positively related trends with SmJAZ1 protein expression, and IPTG concentration had no significant impact in protein expression, whereas IPTG adding time had significant impact on protein expression. **Conclusion:** Shaking the culture at 30 ℃ until the A_{600} is approximately 0.9 (2 h in LB), and add IPTG to a final concentration of 0.1 mmol·L^{-1}, and then the optimal expression of SmJAZ1 recombinant protein were accumulated after the induction time of 20 h.

keywords:*Salvia miltiorrhiza* JAZ1 prokaryotic expression optimizing expression

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