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生物化学工程与技术

定点突变的谷氨酰胺合成酶的表达条件和在酶法合成谷氨酰胺中的应用

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摘要

针对酶法生产谷氨酰胺中高浓度铵盐条件下的谷氨酰胺合成酶（GS）腺苷酰化问题，从谷氨酸棒杆菌Corynebacterium glutamicum ATCC 14067调取编码GS的基因glnA，将GS的腺苷酰化位点Tyr405定点突变为Phe405，并在大肠杆菌中表达突变后的GS，优化产酶条件。用突变的GS在摇瓶中进行酶催化过程，通过补加酶催化底物谷氨酸钠和氯化铵，可以提高定点突变的GS生产谷氨酰胺的能力，谷氨酰胺产量达到16.8 g·L^{-1} ;在5 L反应器规模的酶催化生产谷氨酰胺过程中，通过调控底物补加方案和反应条件，谷氨酰胺的产量达到34.2 g·L^{-1} ，谷氨酰胺对谷氨酸的摩尔转化率为96.3%。

关键词

谷氨酰胺 酶催化 谷氨酰胺合成酶 腺苷酰化位点

分类号

Expression of site-directed mutant glutamine synthetase and enzymatic glutamine production

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Abstract

To solve putative adenylation of glutamine synthetase (GS) in the enzymatic production of glutamine at a high concentration of ammonium salt, glnA encoding GS was cloned from Corynebacterium glutamicum ATCC 14067 and mutated by overlapping primer to replace the putative adenylation site of GS, Tyr405 by Phe405.The mutant GS was expressed in E.coli and the conditions of GS production were optimized.In the process catalyzed by the mutant GS,glutamine production was increased by adding monosodium glutamate and ammonium chloride in shaking flasks and glutamine yield reached 16.8 g·L^{-1} .By controlling the enzymatic conditions in 5 L bioreactor, glutamine yield reached 34.2 g·L^{-1} with a molar conversion of 96.3% to glutamate.

Key words

glutamine enzymatic catalysis glutamine synthetase adenylylation site

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