行政院原子能委員會 委託研究計畫研究報告

纖維原料水解液高效率發酵菌株之研究及程序開發
Study of the effective celluloase hydrolysate-fermentative microbe and development of the microbe-based process

計畫編號:1022001INER039

受委託機關(構):逢甲大學化工系

計畫主持人: 趙雲鵬

聯絡電話: 04-24517250 ext. 3677

E-mail address: ypchao@fcu.edu.tw

核研所聯絡人員:郭家倫

報告日期:102 年 11月 22日

目 錄

目	錄	I
中	'文摘要	1
英	文摘要	2
壹	、計劃緣起與目的	3
貳	、研究方法與過程	7
	一、建構染色體操作工具箱	7
	二、剃除競爭性的基因	8
	三、強化關鍵基因的表現	9
	四、生產菌之醱酵	10
參	、主要發現與結論	10
	一、剔除競爭代謝路徑	10
	二、工程導引三羧酸循環路徑的碳流方向	11
	三、強化三羧酸循環路徑的關鍵反應步驟	12
	四、增加前驅物草醋酸的生成	13
	五、生產菌之初步醱酵檢測	13
肆	、交老立即	1./

中文摘要

為了發展大腸桿菌之琥珀酸醱酵的技術平台,在今年度的計劃中我們所提議的主要策略在於改造細胞的代謝途徑,以導引碳流至琥珀酸,目前獲致的成果如下:(1)完成基因剃除工具箱的建構,藉此剔除競爭代謝路徑,以減少能源的消耗和碳流量的損失;(2)完成原位鑲箝啟動子工具箱的建構,藉此強化關鍵基因的表現,以導引碳流匯流至琥珀酸;(3)進行先期醱酵條件之探討,結果發現建構菌株可由30g/L葡萄糖轉化生成10g/L琥珀酸,莫爾轉化率達61%。

關鍵字:代謝工程、染色體工程、琥珀酸

Abstract

This study is aimed at to develop a technique platform for fermentative

production of succinic acid in Escherichia coli. In this year, the main

strategy as proposed is to engineer metabolic pathways of E. coli in

order to redirect the carbon flux towards succinate. Our current results

are as follows. (1) The construction of a gene deletion toolbox was

completed. The competing pathways were then removed using this

toolbox to curtail the waste of energy and byproduct production. (2) The

construction of a promoter-insertion toolbox. The key pathways were

enhanced using this toolbox to channel the carbon flux into succinate

node. (3) The preliminary study of succinate fermentation was

conducted. As a consequence, the engineered strain was able to produce

10 g/L succinate from 30 g/L glucose. This result accounts for a molar

conversion yield of 61%.

Keywords: Metabolic engineering, Genomic engineering, succinic acid

2