

Lauroyl-Acyl Carrier Protein Thioesterase: a Key Enzyme for Regulation of Medium-Chain Fatty Acid Synthesis in *E. Coli*

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Abstract. Long carbon chain nylon is a high-performance, high price of chemical material, due to its unique thermal, physical, chemical and mechanical properties superior to other materials, for only specified use in automotive fuel lines and brake system of high specification materials. Chemical process using high active catalyst (Et₂AlCl) water explosion as a major potential risk factors, the continued use of the risk is still high. Therefore, this study will focus in the safety of long carbon-chain aliphatic dicarboxylic acids nylon materials green process development, in order to develop a series of relatively long carbon chain aliphatic dicarboxylic acids nylon materials in response to the majority of the market required.

Keywords: long carbon chain nylon, long carbon-chain aliphatic dicarboxylic acids, green process development

1. Introduction

Goal of this study is to develop a biological method development of nylon materials, microbes as a cell production factory; the key issue is to explore ways to enhance the content of in vivo ω -oxidation reaction (ω -oxidation) substrate specificity (lauric acid), dodecanedioic acid for nylon materials - biological metabolic paths to explore the long chain nylon raw materials- α , ω -dicarboxylic acid biosynthesis.

2. Material and Methods

2.1. Materials and cultivation condition

The *E. coli* strain was purchased from Yeastern Biotech Co. (Taiwan) and cultured with a rich medium (LB) at 37 °C. The standard of methyl caprate, methyl laurate, methyl myristate, Methyl pentadecanoate, methyl palmitate, methyl palmitoleate, methyl stearate, methyl oleate and methyl linoleate were purchased from Sigma Co. (USA).

2.2. Construction of lauroyl-ACP thioesterase genes

The *E. coli*-codon-optimized genes (BTE and FatB3) from *Umbellularia californica* and *Cocos nucifera* were obtained through artificial oligonucleotide synthesis from GeneScript (USA) and transformed into *E. coli*. The resulting chimeric genes were re-isolated as BamHI/SalI and EcoRI/HindIII fragments and cloned into the pET24a vector. Kanamycin was used as the selection markers in *E. coli*. The β -oxidation mutants was modified the acyl-CoA synthetase by double crossover of the lauroyl-ACP thioesterase genes to form a new recombinant strain.

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2.3. Lipid extraction and gas chromatography analysis

The total lipids were estimated as fatty acid methyl esters (FAME) by the direct transesterification method. Fatty acid methyl esters were analyzed by a gas chromatography (Agilent VARIAN 3900, USA) equipping with a flame ionization detector (FID) and a Stabilwax column. Nitrogen (1.5 mLmin^{-1}) was used as the carrier gas. Temperature was programmed incrementation from $130 \text{ }^\circ\text{C}$ to $180 \text{ }^\circ\text{C}$ with a $10 \text{ }^\circ\text{C min}^{-1}$ and thereafter to $210 \text{ }^\circ\text{C}$ with a $15 \text{ }^\circ\text{Cmin}^{-1}$. Injector and detector were maintained at $220 \text{ }^\circ\text{C}$ and $250 \text{ }^\circ\text{C}$, respectively. FAME contents were determined from their corresponding peak areas using Methyl pentadecanoate as the internal standard. The data presented are the average of three estimations.

3. Result and Discussions

3.1. Effect of lauroyl-ACP thioesterase of BTE on redirecting of fatty acid composition

In this feasibility assessment study, lauryl acid production pathway regulation of *E. coli* by insertion an artificial DNA fragment selected from *Umbellularia californica* (*E. coli* codon usage) to wild type and β -oxidation mutants (*), shown in Fig. 1. To construct a transgenic *E. coli* of biological fatty acid metabolic pathway control, regulation of lauryl acid production. The modified *E. coli* was cultured then analyzes the fatty acid composition of the cell lysed, the results shown in Fig. 2. The data indicated that C16: 0 fatty acid ratio presented reduced, and improved the C12: 0 fatty acid ratio of the modified *E. coli*.

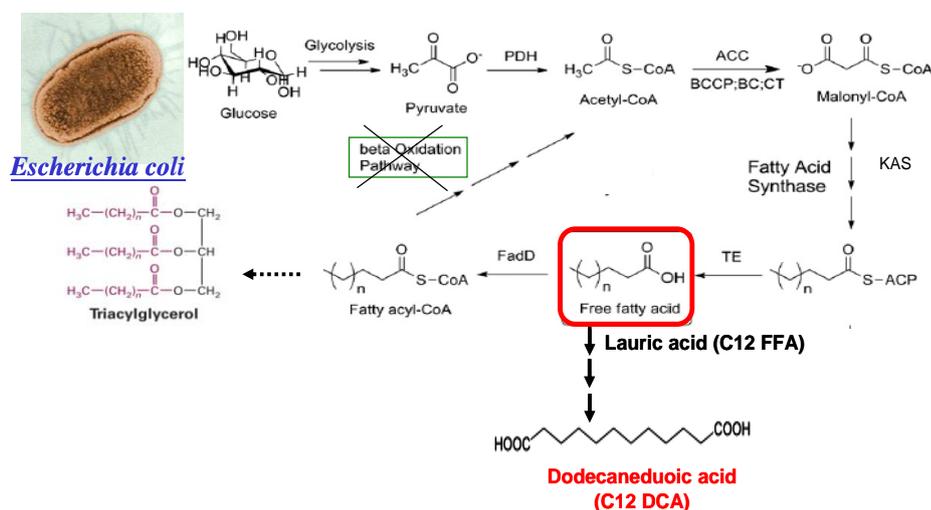


Fig 1. The fatty acid biosynthesis pathway in *E. coli*

3.2. Effect of lauroyl-ACP thioesterase of FatB3 on redirecting of fatty acid composition

In this feasibility assessment study, lauryl acid production pathway regulation of *E. coli* by insertion an artificial DNA fragment selected from *Cocos nucifera* (*E. coli* codon usage) to wild type and β -oxidation mutants (*), shown in Fig. 1. To construct a transgenic *E. coli* of biological fatty acid metabolic pathway control, regulation of lauryl acid production. The modified *E. coli* was cultured then analyzes the fatty acid composition of the cell lysed, the results shown in Fig. 3. The data indicated that C16: 0 fatty acid ratio presented reduced, and improved the C12: 0 fatty acid ratio of the modified *E. coli*.

3.3. Effect of lauroyl-ACP thioesterase of BTE and FatB3 on redirecting of fatty acid composition

In this feasibility assessment study, lauryl acid production pathway regulation of *E. coli* by insertion two artificial DNA fragments selected from *Cocos nucifera* and *Umbellularia californica* (*E. coli* codon usage) to wild type and β -oxidation mutants (*), shown in Fig. 1. To construct a transgenic *E. coli* of biological fatty acid metabolic pathway control, regulation of lauryl acid production. The modified *E. coli* was cultured then analyzes the fatty acid composition of the cell lysed, the results shown in Fig. 4. The data indicated that C16: 0 fatty acid ratio presented reduced, and improved the C12: 0 fatty acid ratio of the modified *E. coli*.

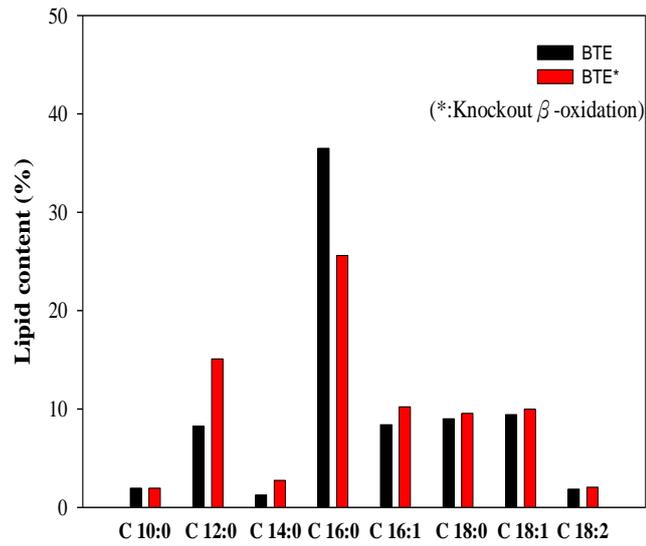


Fig. 2 : The fatty acid composition of the modified *E. coli* of BTE insertion

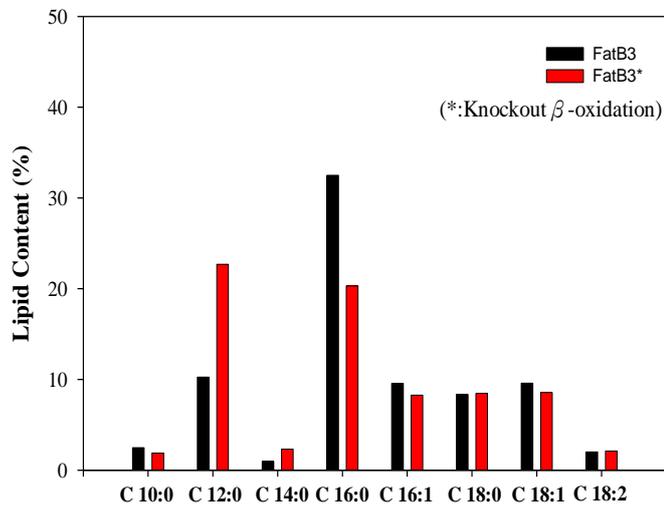


Fig. 3 : The fatty acid composition of the modified *E. coli* of FatB3 insertion

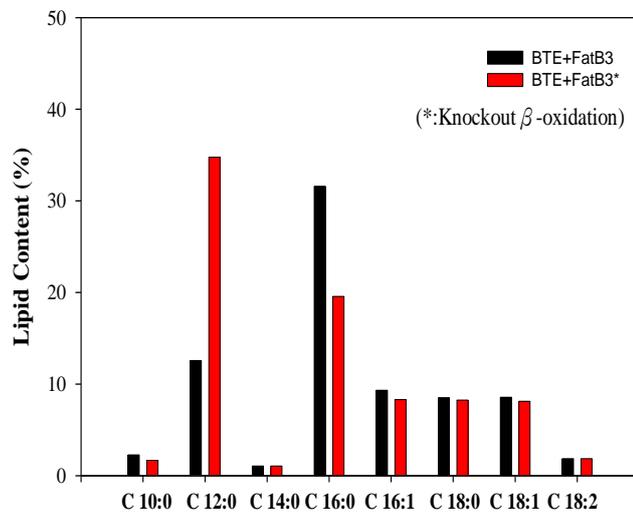


Fig. 4: The fatty acid composition of the modified *E. coli* of BTE and FatB3 insertion

4. Acknowledgements

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5. References

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