# EXPLOITING FATTY ACID METABOLIC PATHWAY FOR PRODUCTION OF SHORT CHAIN FATTY ACIDS IN *E. coli*

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Keywords**:** Thioesterase, Short chain fatty acids, Butyric acid, *Escherichia coli*, Fed-batch fermentation

Worldwide demand of sustainable fuels and chemicals has encouraged researchers for microbial synthesis of short chain fatty acids (SCFAs), such as such as butyric acid (C4), as they are attractive precursors to replace petroleum-based fuels and chemicals.

In this study, we explored the fatty acid metabolism for production of butyric acid in *E. coli* with the help of three thioesterases, i.e., TesAT from *Anaerococcus tetradius*, TesBF from *Bryantella formatexigens* and TesBT from *Bacteroides thetaiotaomicron*.

We found that *E. coli* strain transformed with gene for TesBT and grown in presence of 8 g/L glucose produced maximum butyric acid titer at 1.46 g/L, followed by that of TesBF at 0.85 g/L and TesAT at 0.12 g/L, showing that these thioesterases were efficiently converting short chain fatty acyl-ACP into corresponding acid. The titer of butyric acid varied significantly depending upon the strain genotype and plasmid copy number. Deletion of genes involved in initiating the fatty acid degradation such as fatty acyl-CoA synthetase and acyl-CoA dehydrogenase and overexpression of FadR, which is a dual transcriptional regulator, exerts negative control over fatty acid degradation pathway, reduced up to 30% of butyric acid titer. This observation suggested that β-oxidation pathway is working synergistically with fatty acid synthesis pathway in production of butyric acid. Moreover, accelerating the fatty acid elongation cycle by overexpressing acetyl-CoA carboxyltransferase (Acc) and 3-hydroxy-acyl-ACP dehydratase (FabZ) or by deleting FabR, the transcription suppressor of elongation, did not improve the butyric acid titer, rather favored the long chain fatty acid production. Use of chemical inhibitor cerulenin, which limits the fatty acid elongation cycle, increased the butyric acid titer by 1.7-fold in case of TesBF, while it had adverse impact in case of TesBT. *In vitro* enzyme assay showed that cerulenin also inhibited the short chain specific thioesterases, though inhibitory concentration varied according to the type of thioesterase used.

Further improvement in butyric acid was achieved by process optimization. Owing to the same pathway for both cell growth and butyric acid production, a balance was achieved between the two by growing the cells under nutrient and oxygen limiting condition. Keeping these factors in mind, a fed-batch cultivation strategy was devised for production of butyric acid in phosphorous and carbon limiting condition. Finally, we obtained 14.3 g/L of butyric acid and 17.5 g/L of total free fatty acid.

The strategy used in this study resulted in highest reported titers of butyric acid and free fatty acids in engineered *E. coli* and could be used to replace the traditional chemical methods for production of butyric acid.