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ENGINEERING A SYNTHETIC METABOLIC PATHWAY IN *ESCHERICHIA COLI* FOR CELLULOSE-BASED ISOBUTENE PRODUCTION

S. S. Zaplavnaia[1], I. O. Velegzhaninov[1,2]

[1]*ITMO University, Saint Petersburg*
[2]*Institute of Biology, Komi Science Centre UB RAS, Syktyvkar*

✉ zaplavnayasofiia@gmail.com

Abstract

This project aims to engineer *E. coli* to convert cellulose into isobutene via a synthetic metabolic pathway. By combining cellulase secretion and α-ketoisocaproate dioxygenase expression, the strain could produce isobutene directly from renewable biomass, offering a sustainable alternative to petrochemical synthesis.

The growing global demand for sustainable biofuels has highlighted the urgent need to develop renewable alternatives to fossil-derived hydrocarbons. Among potential candidates, isobutene stands out as a high-value platform chemical used in the production of synthetic high-quality fuel, rubber, plastics, and aviation fuel additives. Traditionally obtained via petrochemical cracking, isobutene can be synthesized biologically, offering a greener production strategy with reduced carbon emissions.

This project focuses on the metabolic engineering of *Escherichia coli* to enable the direct conversion of cellulose into isobutene through a novel synthetic pathway. Since *E. coli* naturally lacks both the capacity to degrade cellulose and the ability to produce isobutene, the strain must be genetically modified at two key levels: (1) extracellular degradation of cellulose into glucose, and (2) conversion of glucose through the leucine biosynthesis pathway into isobutene.

For cellulose degradation, a chimeric gene (*Cel-Tfu*) was designed by fusing the catalytic domain of a cellulase (Cel-CD) from *Bacillus sp.* to the β-1,4-glucosidase gene (*Tfu0937*) from *Thermobifida fusca*). Cel-CD also serves as a secretion carrier, allowing the fused enzyme to be exported outside the cell, where it catalyzes the breakdown of cellulose into glucose [1, 2]. Glucose is then transported into the cell and metabolized via glycolysis to pyruvate, which feeds into the branched-chain amino acid synthesis pathway.

In the second stage, a codon-optimized α-ketoisocaproate dioxygenase (*KICD*) gene from *Rattus norvegicus* is introduced into *E. coli*. KICD catalyzes the conversion of α-ketoisocaproate (KIC), a key intermediate in leucine biosynthesis, into β-hydroxy-β-methylbutyrate (HMB), which spontaneously decomposes into isobutene under physiological conditions [3]. This synthetic route bypasses fermentative pathways and enables non-fermentative, gas-phase production of isobutene, which diffuses out of the bacterial cell.

The project includes the design and construction of recombinant plasmids containing either the *Cel-Tfu* or *KICD* gene, followed by their individual expression in *E. coli*. Each gene product is evaluated separately in terms of transcriptional and translational efficiency, as well as enzymatic activity. Once both constructs are characterized, the genes are co-expressed from a single plasmid to evaluate the functionality of the full synthetic pathway from cellulose to isobutene. The experimental strategy is built around iterative design, expression analysis, and pathway optimization.

The next stages of the project involve optimizing expression conditions, evaluating the volume of isobutene produced, and assessing whether the production level is sufficient for potential industrial applications. Additionally, to improve the yeild of isobutene, overexpression of *leuABCD* operon is suggested focusing on 2-isopropylmalate synthase (*leuA*) modifications as it's expression is down-regulated by KIC concentrations [4].

This research contributes to the development of a sustainable microbial platform for bio-based isobutene production from lignocellulosic feedstocks, paving the way for environmentally friendly alternatives to fossil fuel-based chemical synthesis.

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