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

Заплавная С. С. (ИТМО)**, Велегжанинов И. О.** (ИТМО), **Эльдиб А.А.** (ИТМО)
Научный руководитель – ассистент Эльдиб Ахмед Абделкадер Мохамед Отман (ИТМО)

Введение. The growing demand for sustainable biofuels has led to extensive research into microbial platforms for converting renewable biomass into valuable chemicals. Isobutene, as a key industrial compound, is traditionally derived from petrochemical sources. However, engineered microorganisms present a promising alternative for its production. *Escherichia coli* lacks the natural ability to degrade cellulose or synthesize isobutene, necessitating genetic modifications. This study aims to develop an *E. coli* strain capable of extracellular cellulose degradation and subsequent isobutene biosynthesis through a synthetic metabolic pathway.

Основная часть. To hydrolyze cellulose, β-1,4-glucosidase (*Tfu0937*) from *Thermobifida fusca* will be fused to the catalytic domain of the alkaline cellulase with endo-glucanase activity (*Cel-CD*) from *Bacillus sp.* forming a functional heterologous cellulase (*Cel-Tfu*) [1]. Catalytic domain of the cellulase has been shown to possess the function of a carrier protein which will transport the fusion enzyme into the extracellular space, where it will degrade the cellulose from the nutrient medium resulting in glucose.

Glucose, the main carbon source for *E. coli*, enters glycolysis where it's converted into pyruvate which is then directed toward the amino acid biosynthesis pathway and serves as a precursor for l-leucine biosynthesis. The codon-optimized α-ketoisocaproate dioxygenase (*KICD*), identified in *Rattus norvegicus*, will play a crucial role in this process by converting α-ketoisocaproate (KIC), a direct precursor of l-leucine, into isobutene by catalyzing decarboxilation and oxygenation of KIC into an unstable β-hydroxy-β-methylbutyrate (HMB), which non-enzymatically decomposes into isobutene [2]. The produced isobutene will exit the cell into the gas-tight vials and will be extracted using gas-tight syringe, its purity and concentration will then be analyzed using gas chromatography.

Thus, expression in *E. coli* of both mutant *Cel-Tfu* and codon optimized *KICD* genes will create a synthetic pathway enabling the bacteria to utilize cellulose as a main carbon source and convert it into isobutene.

Выводы. The engineered *E. coli* strain presents a novel approach for converting cellulose into isobutene, combining enzymatic hydrolysis and metabolic engineering. This research lays the foundation for bio-based isobutene production, offering a renewable alternative to fossil-derived methods.

Список использованных источников:

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