Секция «3.14 Молекулярная биология»

Synthetic Pathway Engineering in Escherichia coli for Cellulose-Driven Isobutene Biosynthesis

Научный руководитель – Велегжанинов Илья Олегович

Заплавная София Сергеевна

Студент (магистр)

Национальный исследовательский университет ИТМО, Санкт-Петербург, Россия

E-mail: sszaplavnaya@itmo.ru

The growing demand for sustainable biofuels has raised interest in microbial platforms capable of converting biomass into value-added chemicals such as isobutene which is still produced from petrochemical feedstocks. Despite the inability of *E. coli to* naturally degrade cellulose or produce isobutene, it can be genetically engineered to exhibit a needed phenotype and perform both cellulose-untilizing and isobtene producing functions.

Building on the secretable cellulase strategy described by Gao et al., where a chimeric gene was designed by fusing the cellulase from *Bacillus sp.* to the β-1,4-glucosidase from *Thermobifida fusca*, we are developing a chimeric enzyme enabling extracellular cellulose hydrolysis [1]. In contrast to earlier designs, we are focusing on secretable endoglucanase (*CelA*) and β-1,4-glucosidase (*BglB*) genes derived from *Streptomyces spp.*, a genus known for its secondary metabolites production with a large part of its species demonstrating strong cellulolytic activity [2]. Codon-optimized gene sequences will be assembled into a chimeric construct *Cel-Bgl* to ensure efficient secretion and cellulose breakdown into glucose.

The released glucose will enter glycolysis and be converted to pyruvate, feeding into the branched-chain amino acid pathway. A codon-optimized α-ketoisocaproate dioxygenase (*KICD*) gene from *Rattus norvegicus*, when introduced into *E. coli*, will catalyze the conversion of α-ketoisocaproate into β-hydroxy-β-methylbutyrate (HMB), which spontaneously decomposes into isobutene [3]. The gaseous product diffuses from the culture into the air space of the flask.

To ensure stability, transcriptional strength and low metabolic burden, expression conditions are being optimized by comparing gene and protein expression from three vectors: high-copy pUC19 and low-copy pET28(a)+ plsamids, and a novel construct comprised of modified pET28(a)+ in which the T7 promoter and lac operator are replaced with the constitutive promoter J23118 offering prospects for an efficient protein expression at a predictable rate with no need for IPTG addition which can exert inhibitory effects on bacteria.

This project establishes a sustainable microbial platform by engineering *E. coli* to couple *Streptomyces*-derived chimeric cellulases for efficient cellulose hydrolysis with a synthetic KICD-mediated pathway for isobutene biosynthesis, enabling the conversion of lignocellulosic waste into a high-value gaseous bio-based chemical.

Источники и литература

1) Gao D, Luan Y, Wang Q, Liang Q, Qi Q. Construction of cellulose-utilizing Escherichia coli based on a secretable cellulase. Microb Cell Fact. 2015 Oct 9;14:159. doi: 10.1186/s12934-015-0349-7.

2) Mustila H, Kugler A, Stensjö K. Isobutene production in Synechocystis sp. PCC 6803 by introducing α-ketoisocaproate dioxygenase from Rattus norvegicus. Metab Eng Commun. 2021 Jan 23;12:e00163. doi: 10.1016/j.mec.2021.e00163.

3) Tikariha H, Pavagadhi S, Mayalagu S, Poh MCH, Swarup S. Hybrid Genome Assembly for Predicting Functional Potential of a Novel Streptomyces Strain as Plant Biomass Valorisation Agent. Indian J Microbiol. 2021 Sep;61(3):283-290. doi: 10.1007/s12088-021-00935-5. Epub 2021 Apr 22.