Best Practice

November 2025

Metabolic engineering of *Pseudomonas putida* for efficient production of PHA from lignin derivatives

Polyhydroxyalkanoates (PHAs) are biodegradable biopolymers that offer a sustainable alternative to conventional petrochemical-based plastics. However, their widespread adoption is hindered by high production costs. *Pseudomonas putida* (strain KT2440), a soil bacterium with natural aromatic catabolic pathways, is a promising microbial chassis for converting lignin-derived compounds into medium-chain-length PHA (mcl-PHA). This study aimed to enhance this bioconversion process and reduce production costs through genetic engineering and bioprocess optimization.

Project activity

A genome-reduced derivative, *P. putida* (strain KTU-U27)—previously shown to exhibit superior PHA productivity compared to its parental KT2440 strain—was further engineered to improve mcl-PHA synthesis from the lignin-derived aromatic compound p-coumaric acid (p-CA). The following genetic modifications were introduced:

- Inhibition of PHA degradation by deleting the phaZ gene, which encodes the enzyme responsible for intracellular PHA breakdown.
- Suppression of fatty acid β-oxidation through deletion of fadBA1 and fadBA2 genes.
- Enhanced PHA biosynthesis via overexpression of phaC1 and alkK, key genes in the PHA production pathway.
- Enhanced resistance to substrate toxicity by overexpressing the ttg2ABCDE efflux operon and the vacJ genes.

agriloop

Bioprocess optimization of the carbon-to-nitrogen (C/N) ratio and high-density fed-batch fermentation further improved PHA productivity.

Under optimized conditions (initial C/N ratio of 8:4), the final engineered strain (KTU-U27▵Z2BA-P46C1K-P46TJ) achieved:

- Cell dry weight: 2050 mg/L
- PHA content: 82.19 wt%
- PHA yield: 1685 mg/L

Key findings and recommendations

This study demonstrates that an integrated strategy—combining genome reduction, metabolic pathway engineering, and bioprocess refinement—can substantially improve the bioconversion efficiency of lignin-derived aromatics to mcl-PHA.

The engineered strain significantly improved the performance of *P. putida* KTU-U27, achieving the highest reported yield of mcl-PHA using p-CA as the sole carbon source.

The enhanced *P. putida* KTU-U27 platform provides a scalable, cost-effective solution for sustainable PHA production from lignin, improving the viability of bio-based plastics.

References

Systems metabolic engineering of genome-reduced *Pseudomonas putida* for efficient production of polyhydroxyalkanoate from p-coumaric acid. J. Agric. Food Chem. 73(21):12899–12907.Chen Y, Liu Y, Meng Y, Jiang Y, Zhang X, Liu H, Reis MAM, Qi Q, Yang C, Liu R, 2025.

Contact

- **Chao Yang,** Key Laboratory of Molecular Microbiology and Technology for Ministry of Education
yangc20119@nankai.edu.cn

For more information about the AgriLoop Project, visit:
agriloop-project.eu

Funded by the European Union

中华人民共和国科学技术部
Ministry of Science and Technology of the People's Republic of China

UK Research and Innovation

Project funded by
Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra
Swiss Confederation
Federal Department of Economic Affairs, Education and Research EAER
State Secretariat for Education, Research and Innovation SERI

This project has received funding from the European Union's Horizon Europe research and innovation programme under the grant agreement No. 101081776, the UK Research and Innovation (UKRI) fund under the UK government's Horizon Europe funding guarantee, the Swiss State Secretariat for Education, Research and Innovation (SERI) and from the National Key Research and Development Program supported by the Ministry of Science and Technology of the People's Republic of China (No. 2023YFE0104900). Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of neither of the aforementioned Funding authorities. Neither the European Union, the United Kingdom, the Swiss Confederation or the People's Republic of China nor the European Commission, UKRI, SERI or NKRDPC can be held responsible for them.