Bio-based p-xylene oxidation into terephthalic acid by engineered E. coli

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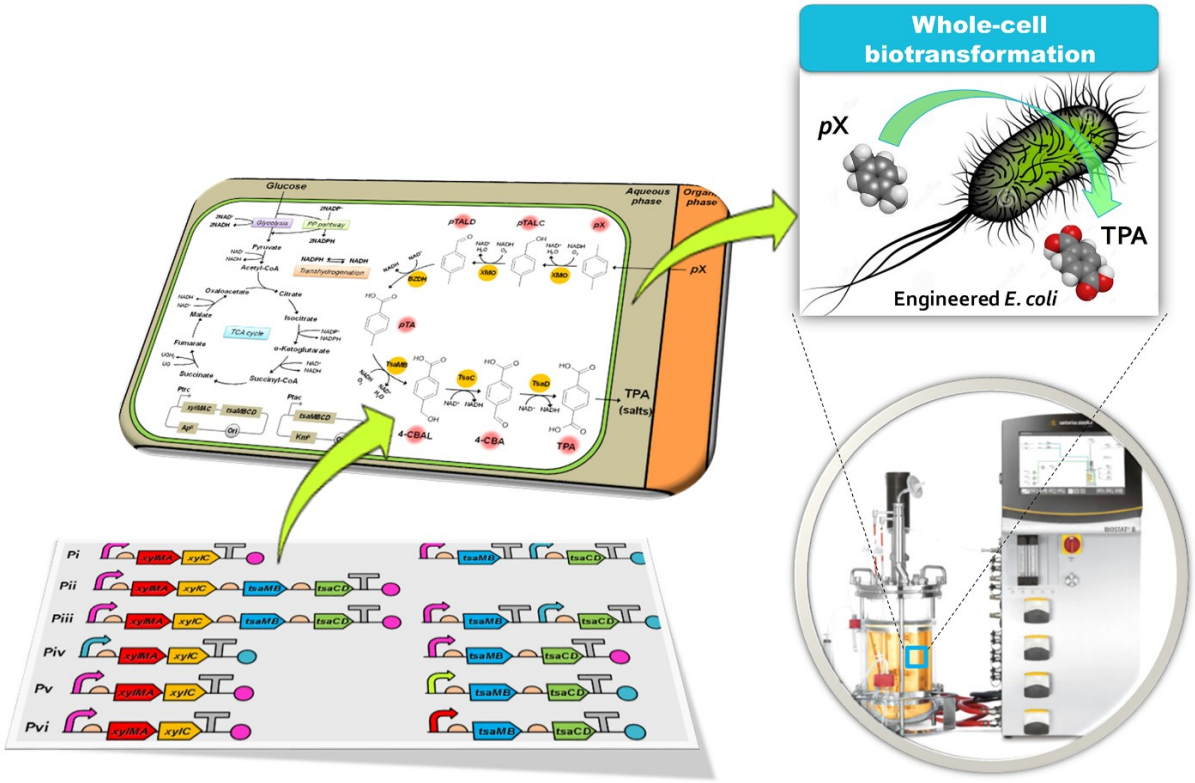

This schematic diagram shows the overall conceptualization of how metabolically engineered *E. coli* produced TPA from pX. The engineered *E. coli* was developed through reconstituting a synthetic metabolic pathway for pX conversion to TPA and optimized for increased TPA yield and byproduct elimination. Two-phase partitioning fermentation system was developed for demonstrating the feasibility of large-scale production of TPA from pX using the engineered *E. coli* strains, where pX was supplied in the organic phase and TPA was produced in the aqueous phase. Credit: KAIST

KAIST researchers have established an efficient biocatalytic system to produce terephthalic acid (TPA) from p-xylene (pX). It will allow this industrially important bulk chemical to be made available in a more environmentally-friendly manner.

The research team developed metabolically engineered *Escherichia coli* (*E. coli*) to biologically transform pX into TPA, a chemical necessary in the manufacturing of polyethylene terephthalate (PET). This biocatalysis system represents a greener and more efficient alternative to the traditional chemical methods for TPA production. This research, headed by Distinguished Professor Sang Yup Lee, was published in *Nature Communications* on May 31.

The research team utilized a metabolic engineering and synthetic biology approach to develop a recombinant microorganism that can oxidize pX into TPA using microbial fermentation. TPA is a globally important chemical commodity for manufacturing PET. It can be applied to manufacture plastic bottles, clothing fibers, films, and many other products. Currently, TPA is produced from pX oxidation through an industrially well-known chemical process (with a typical TPA yield of over 95 mol%), which shows, however, such drawbacks as intensive energy requirements at high temperatures and pressure, usage of heavy metal catalysts, and the unavoidable byproduct formation of 4-carboxybenzaldehyde.

The research team designed and constructed a synthetic metabolic pathway by incorporating the upper xylene degradation pathway of *Pseudomonas putida* F1 and the lower p-toluene sulfonate pathway of *Comamonas testosteroni* T-2, which successfully produced TPA from pX in small-scale cultures, with the formation of p-toluate (pTA) as the major byproduct. The team further optimized the pathway gene expression levels by using a synthetic biology toolkit, which gave the final engineered *E. coli* strain showing increased TPA production and the

complete elimination of the byproduct.

Using this best-performing strain, the team designed an elegant two-phase (aqueous/organic) fermentation system for TPA production on a larger scale, where pX was supplied in the organic phase. Through a number of optimization steps, the team ultimately achieved production of 13.3 g TPA from 8.8 g pX, which represented an extraordinary yield of 97 mol%.

The team has developed a microbial biotechnology application which is reportedly the first successful example of the bio-based production of TPA from pX by the microbial fermentation of engineered *E. coli*. This bio-based TPA technology presents several advantages such as ambient reaction temperature and pressure, no use of heavy metals or other toxic chemicals, the removable of byproduct formation, and it is 100% environmentally compatible.

Professor Lee said, "We presented promising biotechnology for producing large amounts of the commodity chemical TPA, which is used for PET manufacturing, through metabolically engineered gut bacterium. Our research is meaningful in that it demonstrates the feasibility of the biotechnological production of bulk chemicals, and if reproducible when up-scaled, it will represent a breakthrough in hydrocarbon bioconversions."

More information: Zi Wei Luo et al, Biotransformation of p-xylene into terephthalic acid by engineered Escherichia coli, *Nature Communications* (2017). DOI: 10.1038/ncomms15689

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