NU Tech
Technology Partnership of Nagoya University, Inc.

(B-42)

Novel Green Method to Convert Benzene to Phenol

Background

Direct oxidation of unactivated carbon–hydrogen bonds has been a long-standing challenge in synthetic chemistry. The cumene process is widely used for synthesizing phenol from benzene, yet high-energy-consuming. Green alternatives of the conventional process have been long sought in the industries.

Technology Overview

Nagoya University researchers have succeeded in developing an *Escherichia coli* whole-cell biocatalyst for the direct hydroxylation of benzene into phenol. This *in vivo* method enables wild-type cytochrome P450BM3 (P450BM3) expressed in *E.coli* to activate and hydroxylate its non-native substrates, such as benzene.

The high substrate specificity of P450BM3 generally results in very low catalytic activity towards its non-native substrates. To alter the substrate specificity of wild-type P450BM3 and make it work in living bacteria, the researchers developed a unique reaction system which induces the substrate misrecognition of the enzyme. They developed inert mimics of native substrates ('decoy molecules') to activate P450BM3. One of the decoy molecules, *N*-heptyl-L-prolyl-L-phenylalanine (C7-Pro-Phe), permeates into the cell and strongly activates intracellular P450BM3, achieving 59% conversion of benzene to phenol.

Advantage:

- Process at room temperature and atmospheric pressure.
- C7-Pro-Phe permeates into *E. coli* and activates P450BM3 in the cell.
- Phenol yield up to 59% and further hydroxylation to hydroquinone yield up to 16% in 5 hour-process. (Benzene conversion rate 75%)
- No supplementation of P450 cofactor, or NADPH, in the medium required.
- Applicable to other bacteria and substrates.
- Applicable to mutated P450s.

Figure

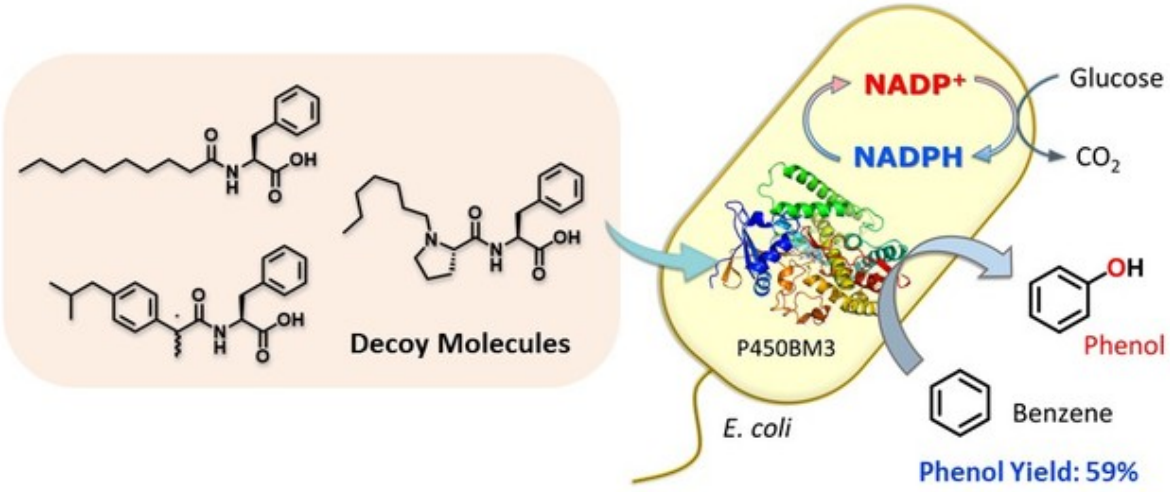

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Fig.1. Phenol production using bacteria
Whole-cell biocatalyst utilizing *E. coli* for benzene hydroxylation activated by decoy molecules.

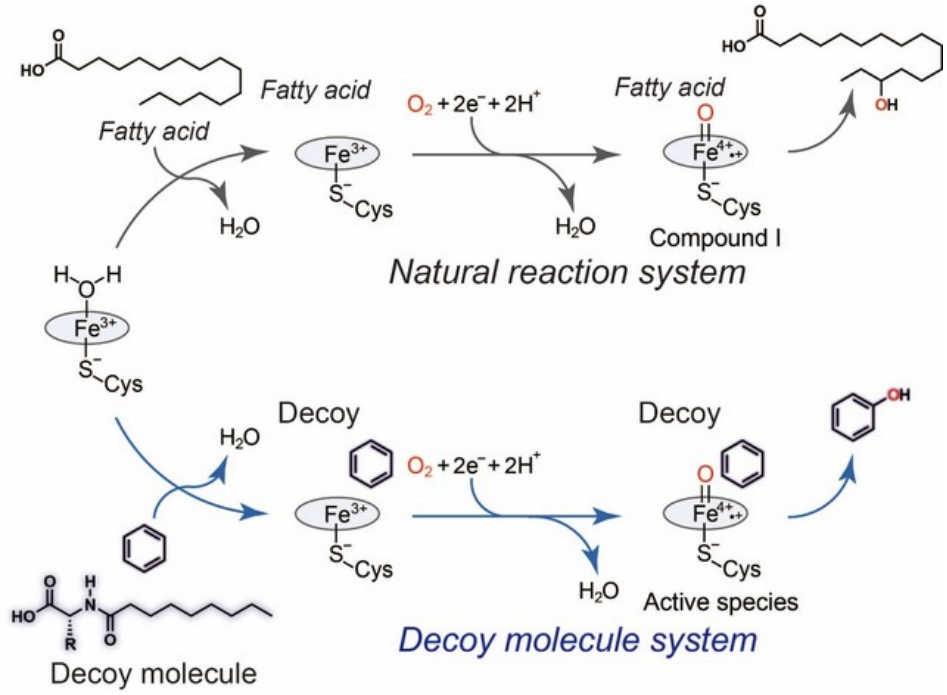

Fig.2. Hydroxylation of benzene using Decoy molecule system
General catalytic cycle of P450BM3 (black) and a plausible catalytic cycle (blue) for benzene hydroxylation catalyzed by P450BM3 with assistance of the decoy molecule.

Further Details

Masayuki Karasawa, Joshua Kyle Stanfield, Sota Yanagisawa, Osami Shoji Prof and Yoshihito Watanabe. Whole-Cell Biotransformation of Benzene to Phenol Catalysed by Intracellular Cytochrome P450BM3 Activated by External Additives. Angew Chem Int Ed Engl. 2018 Sep 17;57(38):12264-12269. doi: 10.1002/anie.201804924.

Seeking

Licensing and collaborative research and development

IP Status

Patent application filed

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