Metabolically engineered E. coli producing phenol

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Many chemicals we use in everyday life are derived from fossil resources. Due to the increasing concerns on the use of fossil resources, there has been much interest in producing chemicals from renewable resources through biotechnology.

Phenol is an important commodity chemical, and is a starting material for the production of numerous industrial chemicals and polymers, including bisphenol A and phenolic resins, and others. At present, the production of phenol entirely depends on the chemical synthesis from benzene, and its annual production exceeds 8 million tons worldwide. Microbial production of phenol seems to be a non-viable process considering the high toxicity of phenol to the cell.

In the paper published online in *Biotechnology Journal*, a Korean research team led by Distinguished Professor Sang Yup Lee at the Department of Chemical and Biomolecular Engineering from the Korea Advanced Institute of Science and Technology (KAIST) reported the successful development of an engineered *Escherichia coli* (*E. coli*) strain which can produce phenol from glucose. *E. coli* has been a workhorse for biological production of various value-added compounds such as succinic acid and 1,4-butanediol in industrial scale. However, due to its low tolerance to phenol, *E. coli* was not considered a viable host strain for the biological production of phenol.

Professor Lee's team, a leading research group in metabolic engineering, noted the genetic and physiological differences of various *E. coli* strains

and investigated 18 different *E. coli* strains with respect to phenol tolerance and engineered all of the 18 strains simultaneously. If the traditional genetic engineering methods were used, this work would have taken years to do. To overcome this challenge, the research team used synthetic small RNA (sRNA) technology they recently developed (Nature Biotechnology, vol 31, pp 170-174, 2013). The sRNA technology allowed the team to screen 18 *E. coli* strains with respect to the phenol tolerance, and the activities of the metabolic pathway and enzyme involved in the production of phenol. The research team also metabolically engineered the *E. coli* strains to increase carbon flux toward phenol and finally generated an engineered *E. coli* strain which can produce phenol from glucose.

Furthermore, the team developed a biphasic extractive fermentation process to minimize the toxicity of phenol to *E. coli* cells. Glycerol tributyrate was found to have low toxicity to *E. coli* and allowed efficient extraction of phenol from the culture broth. Through the biphasic fed-batch fermentation using glycerol tributyrate as an in situ extractant, the final engineered *E. coli* strain produced phenol to the highest titer and productivity reported (3.8 g/L and 0.18 g/L/h, respectively). The strategy used for the strain development and the fermentation process will serve as a framework for metabolic engineering of microorganisms for the production of toxic chemicals from renewable resources.

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