

CONSTRUCTION OF NOVEL METABOLIC PATHWAYS WITH ARTIFICIAL ENZYMES

Akihiko Kondo, Kobe University & RIKEN
akondo@kobe-u.ac.jp
Tomokazu Shirai, RIKEN
Yutaro Mori, RIKEN
Tomohisa Hasunuma, Kobe University
Christopher J. Vavricka, Kobe University
Michihiro Araki, Kyoto University

Key Words: Artificial Pathway, Artificial Enzyme, Rubber Materials, Alkaloids

Non-fossil raw materials can be utilized for the production of useful compounds by way of microbial "fermentation". Sugars are obtained from carbon fixations of plants or photosynthetic microorganisms, and are used as a carbon source for the biosynthesis of useful target compounds by genetically modified microorganisms. In order for a microorganism to produce enough target compound, techniques for optimal metabolic design must include balance of energy production/consumption, redox pathways, and intracellular carbon flow. With recent innovations in genome analysis technology and information processing technology, computational design tools that can describe more than 1000 genome-scale metabolic reactions to efficiently produce target compounds have been developed worldwide. However, the established tools are not designed to search and create biosynthetic pathways for production of non-natural compounds from fossil resources. We developed BioProV and M-path, new simulation tools that enable metabolic design for the biosynthesis of unnatural compounds. By combining these tools with enzyme engineering technology, we succeeded in expanding the scope of bioproduction targets. The first example is construction of an artificial metabolic pathway to biosynthesize isoprene. Isoprene the raw material for production of synthetic rubber that can be used in automobile tires. Currently, isoprene is industrially produced as a by-product of naphtha pyrolysis. Therefore, by establishing green isoprene production technology, dependence upon petroleum can be reduced. Isoprene is a substance that can exist within cells of many organisms as a monomer of polyisoprene rubber, and also as a structural unit of secondary metabolites. It is difficult to optimize its synthetic pathway due to shortages of intracellular ATP supply, and challenges in the introduction of improved biosynthetic pathways. In nature, isoprene is produced from mevalonic acid through a five-step reaction, but the newly constructed artificial metabolic pathway consists of just two steps from mevalonic acid to isoprene. This results in a three-fold reduction in cellular energy consumption. Furthermore, we succeeded in constructing a highly active enzyme that exhibits 10,000-fold higher isoprene-producing activity relative to natural enzymes. By introducing these artificial metabolic reactions into *Escherichia coli*, efficient artificial isoprene production was achieved. In addition, we have developed a microbial production system for 1,3-butadiene, another alternative source for synthetic rubber. Moreover, rationally engineered enzymes from insects and plants enzymes have resulted in the construction of an artificial pathway to benzyloquinoline alkaloids and downstream opioid analgesics.