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Production of non-natural products by microbial cell factories using artificial metabolic pathways

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PURPOSE OF THE ABSTRACT

We have developed rapid cell factory construction technology (biofoundry platform), which is an integrated system (DBTL) of advanced technologies such as metabolic design system (Design), rapid breeding technology using advanced genome engineering technologies (Build), rapid and accurate metabolic evaluation technology (Test), and machine learning or mathematical modelling for further improvement and a new metabolic pathway design (Learn). The biofoundry platform, a fusion of biotechnology and digital technology, is expected to be a major force not only in the construction of cell factories but also in the development of various biotechnologies.

In the biofoundry platform, to design and construct microorganisms that produce useful compounds, it is essential to have the technology to optimally design "metabolism," which includes not only the flow of carbon within the cell, but also the production and consumption of energy and the balance of redox. The limitation of the previous design tools is that it cannot design artificial metabolic pathways for the production of non-natural compounds that are not produced by living organisms, because they use only natural metabolic pathways existing in KEGG. Therefore, the BioProV method was newly devised to describe only chemical reaction patterns from the databases of metabolic and enzymatic reactions, such as KEGG and BRENADA. In this methods, similar chemical reaction patterns were reclassified as a single chemical reaction and trained by a computer. Then, the precursors were reverse synthesized from the target compounds in a random and exhaustive manner. The simulation is successful when the reverse-synthesized precursor contains a compound that is known to exist in vivo. With this BioProV, it is now possible to design artificial metabolic pathways to synthesize non-natural compounds. However, since we focused only on chemical reaction patterns, we need a technology to rapidly create artificial enzymes with specificity that does not exist in nature. Here, the important thing in the construction of artificial metabolic pathways became the problem of enzyme engineering: how to quickly create highly active artificial enzymes with the desired reaction specificity.

As an example of microbial production of non-natural compounds by designing artificial metabolic pathways, I describe the case of butadiene. Using BioProV, an artificial metabolic pathway design tool, we found a pathway to produce 1,3-butadiene via a two-step decarboxylation reaction using cis,cis-muconic acid (ccMA), as a starting compound. The next challenge is to create an artificial enzyme that performs a two-step decarboxylation reaction of cis,cis-muconic acid (ccMA), which does not exist in nature. We modified the substrate specificity of ferulic acid decarboxylase (FDC), which has very wide substrate specificity, to create an artificial enzyme that converts ccMA to 1,3-butadiene and incorporated to create an artificial metabolic pathway for 1,3-butadiene production in Escherichia coli. Using this E, coli which has artificial metabolic pathway with artificial enzyme, we have succeeded to produce 1,3-butadiene from glucose.

As shown in this example, it is very important to rapidly create artificial metabolic pathways with artificial enzymes to construct microbial cell factories for the production of non-natural products by using biofoundry platform. To solve this problem, we are now constructing the new enzyme and pathway database for biofoundry platform. I will describe the current progress of our approach.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

Biofoundry | Engineering Biology | Artificial Enzyme | Metabolic Pathway

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