S06-6 Synthetic biology in benzylisoquinoline alkaloid biosynthesis

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It is difficult to produce alkaloids on a large scale under the strict control of secondary metabolism in plants, and they are too complex for cost-effective chemical synthesis. Recently, some attempts to reconstruct entire plant biosynthetic processes have been examined in microbial systems. Benzylisoquinoline alkaloids (BIAs), biosynthetic pathway would be the good model for such reconstruction, since major biosynthetic genes have been isolated and characterized. To produce BIAs in microbes, we first modified the BIA pathway. We simplified the first steps and reconstructed the pathway in *Escherichia coli* with cDNAs of monoamine oxidase from *Micrococcus luteus* and norcoclaurine synthase (NCS), norcoclaurine 6-O-methyltransferase (6OMT), coclaurine-N-methyltransferase (CNMT), and 3'-hydroxy-N-metylcoclaurine-4'-O- methyltransferase (4'OMT) from *Coptis japonica*. Using this reconstituted microbial and plant enzyme system, we synthesized (*S*)-reticuline, the key intermediate in BIA biosynthesis, from dopamine by crude enzymes from transgenic *E. coli*. Furthermore, we synthesized an aporphine alkaloid, magnoflorine, or a protoberberine alkaloid, scoulerine, from dopamine via reticuline using different combination cultures of transgenic *Escherichia coli* and *Saccharomyces cerevisiae* cells. These results indicate that microbial systems that incorporate plant genes can not only enable the mass production of scarce BIAs, but may also open up new pathways for the production of novel BIAs. The potentials of reconstruction of secondary metabolic pathway in microbe would be discussed.