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Metabolic Engineering and Biocatalytic Engineering Using the Cells of Recombinant Microorganisms : Cases of Carotenoids and Aromatic Compounds

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1. Metabolic engineering or biocatalytic engineering?

Two approaches with metabolic engineering and biocatalytic engineering should be considered in the production of a disirable chemical. Here, I would like to mention a few examples of the two approaches in cases of using the cells of recombinant microorganisms. Metabolic engineering for the production of carotenoids has successfully been studied as shown later. In contrast, biocatalytic engineering of carotenoids has been found to be very difficult, although the enzymatic conversion of several carotenoids has recently been feasible with low efficiency[1]. The reason seems to be present in instability of the corresponding enzymes or in water-insolubility of the pigments. On the other hand, biocatalytic engineering for transforming aromatic compounds has attracted much attention by using bacterial cells as shown later, while there are only a few reports in terms of metabolic engineering of aromatic compounds[2].

2. Metabolic engineering of carotenoids

Carotenoids are the most widely distributed class of pigments in nature, displaying yellow, orange, and red color. More than 600 carotenoids have been isolated up to the present. The pigments have recently attracted much attention, due to their beneficial effects on health, e. g. the functions of lycopene and astaxanthin include strong quenchers of singlet oxygen, an involvement in cancer prevention, and enhancers of immune responses. In spite of these diverse effects of carotenoids, the number of physiologically or pharmacologically studied carotenoid species is limited to β-carotene, α-carotene, lycopene, zeaxanthin, lutein, fucoxanthin, and astaxanthin. It is due to a fact that carotenoids abundantly contained in natural sources are restricted to these pigments. Metabolic engineering of carotenoids based on combinatrial biosynthesis of the genes should extend the range of the pigments used for the biological study.

Combinatrial biosynthesis of carotenoids has well been studied using *Escherichia coli* as a host microorganism. *E. coli* does not naturally synthesize carotenoids, but by using various combinations of the carotenogenic genes, recombinant strains accumulating many kinds of carotenoids including unnatural carotenoids have been produced[3-7]. Natural carotenoids available with this combinatrial biosynthesis are β-carotene, α-carotene, lycopene, neurosporene, ζ-carotene, phytoene, zeaxanthin, astaxanthin, canthaxanthin, β-cryptoxanthin, and so on. Examples of unnatural or

very minor carotenoids produced by this method are 7,8-dihydro-β-carotene, 7,8-dihydro-zeaxanthin (parasiloxanthin), 3,4,3',4'-tetradehydrolycopene, 1,1'-dihydroxylycopene, 1,1'-dihydroxy-3,4,3',4'-tetradehydrolycopene, and astaxanthin-β-diglucoside. We have also produced lycopene, β carotene, and astaxanthin using recombinant strains of the non-carotenogenic edible yeast *Candida utilis* [8].

The carotenogenic genes responsible for the synthesis of the above carotenoids have been isolated from carotenogenic bacteria such as the epiphytic bacteria *Erwinia* species[9] (*crtE, crtB, crtI, crtY, crtZ*, and *crtX*), the marine bacterium *Agrobacterium aurantiacum* [10] (*crtB, crtI, crtY, crtZ*, and *crtW*), and the photosynthetic bacteria *Rhodobacter* species[11] (*crtE, crtB, crtI, crtC*, and *crtD*). The first substrate of the enzymes encoded by the above *crt* genes is farnesyl diphosphate (FPP), which is the common precursor not only for carotenoid biosynthesis but also for other isoprenoid compounds such as quinones and sterols. It is thus feasible to direct the carbon flux for the biosynthesis of these isoprenoid compounds partially to the pathway for carotenoid production by the introduction of the carotenogenic genes starting with the *crtE* gene.

It is an important area of metabolic engineering research to increase the carbon flux of the participating central pathway to produce a desirable chemical with superior yield and productivity, by amplifying rate-limiting steps, or by eliminating regulatory mechanisms. In a case of *E. coli*, the expression of the exogenous IPP (isopentenyl diphosphate) isomerase and DXP (1-deoxy-D-xylulose 5-phosphate) synthase genes has been found to be effective to increase carotenoid contents[12,13]. The carotenoid yield has reached 1.6 mg/g (dry weight) of cells. These results imply that IPP isomerase and DXP synthase may be key regulatory step enzymes in the isoprenoid biosynthesis of bacteria such as *E. coli*. On the other hand, in a case of *C. utilis* the expression of the gene coding for the catalytic domain of HMG-CoA reductase has been shown to be effective to increase carotenoid amounts[14]. The carotenoid yield has reached 4.3 mg/g (dry weight) of cells. Partial disruption of the squelene synthase genes, corresponding to the first step for the ergosterol biosynthesis branch, has also appeared to be effective for enhancing carotenoid contents.

3. Biocatalytic engineering of aromatic compounds

The microbial oxidation of aromatic hydrocarbons has been studied for more than 30 years[15]. A multicomponent enzyme arene dioxygenase, which consists of an iron-sulfur protein large (α) subunit and small (β) subunit, ferredoxin, and ferredoxin reductase, introduces both atoms of molecular oxygen into an aromatic nucleus. It usually converts the phenyl ring of aromatic hydrocarbons into the *cis*-cyclohexadienediol. The transformed products are capable of being served as the starting material for many of the early organic syntheses. The hundreds of *cis*-diol metabolites have been synthesized up to date by using bacteria harboring the arene dioxygenase genes[15]. In this symposium, we would like to mention dioxygenation reactions of various polycyclic aromatic

compounds using the cells of recombinant *E. coli* and Actinomycetes *Streptomyces lividans* carrying various arene dioxygenase genes, and also touch on the possibility for obtaining new biotransformed products having hydroxy groups.

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