Metabolic Engineering of *Escherichia coli* for Isoprenoids Production

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Abstract

The metabolic engineering of natural products has begun to prosper in the past few years due to genomic research and the discovery of biosynthetic genes. While the biosynthetic pathways and genes for some isoprenoids have been known for many years, new pathways have been found and known pathways have been further investigated. Isoprenoids (also known as terpenoids) are a class of secondary metabolites found in all species and constitute the largest class of natural products occurring in nature, with over 30,000 individual compounds identified to date. We present the metabolic engineering of isoprenoids, focusing on lycopene which is an important medicinal and biotechnological compound of natural pigments produced by many microorganisms and plants. Genes enhancing lycopene production in *Escherichia coli* were identified through colorimetric screening of shotgun library clone that was constructed with *E. coli* chromosomal DNA. The *E. coli* had been engineered to produce lycopene, a red colored carotenoid, which enabled to screen genes enhancing lycopene production. Six clones that enhanced lycopene production were isolated. Lycopene production was significantly enhanced with *dxs*, *appY*, *crl*, and *rpoS* among 14 genes in the clones. While *dxs* and *rpoS* have been already reported to enhance lycopene production, *appY* and *crl* were newly identified. A reaction of DXP (1-deoxy-D-xylulose-5-phosphate) synthase encoded by *dxs* is rate-limiting step in synthesis of IPP (isopentenyl pyrophosphate) that is building block of lycopene. Sigma S factor encoded by *rpoS* regulates transcription of genes induced at stationary phase. Genes of *appY* and *crl* encode transcriptional regulators related to anaerobic respiration and formation of curli surface fibers. The *E. coli* harboring plasmid with *appY* produced 2.8mg lycopene/g DCW (dry cell weight) that is the same amount obtained with *dxs* although *appY* is not related to lycopene synthesis pathway directly such as *dxs*. Co-expression of *appY*, *crl* and *rpoS* with *dxs* enhanced lycopene production synergistically. When *appY* was co-expressed with *dxs*, lycopene production was 4.7 mg/g DCW that was 8-fold higher than 0.6 mg/g DCW of lycopene production obtained without expression of both genes.