

196g Biosynthesis of Plant-Specific Flavanols and Anthocyanins in Escherichia Coli

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Flavonoids belong to the group of plant polyphenols. The six major subclasses of flavonoids are flavones, flavonols, flavanones, flavanols, anthocyanidins, and isoflavones. Recently, interest in the possible health benefits of flavonoids has increased due to their potent antioxidant and anti-radical scavenging activities observed in vitro. We have accomplished the heterologous biosynthesis of anthocyanins and flavanols in *E. coli* by constructing three sets of artificial gene clusters. The first includes four genes of heterologous origins: Mdf3H encoding flavanone 3 β -hydroxylase from *Malus domestica*, dfr encoding dihydroflavonol reductase from *Anthurium andraeanum*, MdANS encoding anthocyanidin synthase from *Malus domestica*, and PGT8 encoding anthocyanidin 3-O-glucosyltransferase from *Petunia x hybrida*. The dfr, PGT8, Mdf3H, and MdANS genes were expressed in *E. coli* strain JM109 and we were successful in producing both cyanidin and pelargonidin 3-O-glucoside (about 10 μ g/ml) by feeding naringenin (1mM). The second artificial gene cluster includes three genes of heterologous origins: Mdf3H, dfr, and lar encoding leucoanthocyanidin reductase from *Desmodium uncinatum*. The expression of the gene cluster in *E. coli* strain BL21Star produced afzelechin (about 0.25mg/L) and catechin (about 2.5mg/L) by feeding naringenin (0.2 mM) and eriodictyol (0.1 mM), respectively. A third gene cluster was constructed to produce flavanones, the precursors of the vast majority of flavonoids. This cluster includes the genes 4CL2 encoding 4-coumaroyl:CoA ligase from *Petroselinum crispum*, CHS-A encoding chalcone synthase from *Petunia x hybrida*, and CHI encoding chalcone isomerase also from *Medicago sativa*. Combination of the flavanone and flavanol gene clusters, allowed us to produce afzelechin and catechin from coumaric acid and caffeic acid, respectfully, in shake flask experiments in M9 minimal media.