442e Engineering Microorganisms for Plant Estrogen Production

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Flavonoids are plant derived estrogens with potent pharmacological properties. We present the simultaneous expression of a gene ensemble which consists of 4CL-2 (4-coumarate:CoA ligase), chsA (chalcone synthase), and chiA (chalcone isomerase) in Escherichia coli and the baker's yeast Saccharomyces cerevisiae that led to the biosynthesis of flavanones, the precursors of the vast majority of flavonoids, from phenylpropanoid acids. Further incorporation of FSI or FSII (flavone synthases), FHT (flavanone 3*f*"-hydroxylase), FLS (flavonol synthase), and 7-OMT1A (7-O-methyltransferase) into the recombinant microorganisms resulted in the biosynthesis of various phytoestrogen molecules. More specifically, the microbial factories generated the flavones chrysin (4mg/L), apigenin (3.5mg/L), luteolin (2.6mg/L), genkwanin (0.2mg/L) and the flavonol kaempferol (0.045mg/L). In general, the yeast recombinant strains proved to be superior to E. coli as they were able to generate up to 1000-fold more flavonoid compounds. Finally, we also present the functional expression of a P450 monooxygenase, flavonoid 3'5'hydroxylase, in E. coli that was achieved by modifying its N-terminal as well as by fusing it at its C-terminus with a P450 reductase gene. Introduction of the chimeric enzyme into E. coli recombinant strains allowed the biosynthesis of 3' and 3'5'-hydroxylated flavonoids, such as quercetin and myricetin.