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Toward a Better Understanding of...

The Production of Safranal, Picrocrocin, and Crocin in Synechocystis sp. PCC 6803 and Escherichia coli

Brian Basco, Caleb Ford, Andrew Ng, and Ang Tony Xu

Mentor: Joseph Jez

The current WashU iGEM project focuses on the biosynthesis of the chemicals that are characteristic of the expensive spice saffron. Saffron is highly valued as a spice that adds unmatched flavor, aroma, and color to food. This spice has recently been identified as a potential treatment for diseases ranging from cancer to depression. Unfortunately, the natural form of saffron can reach thousands of dollars per pound due to low yield and the labor-intensive harvesting process. A project was designed to synthesize three chemicals which compose a significant portion of the aroma and coloration of the spice saffron: safranal, picrocrocin, and crocin. Synechocystis sp. PCC 6803 was chosen as the chassis for production of these compounds because of its photosynthetic nature and native production of the substrate, zeaxanthin. It was proposed that the two genes, ZCD and UGTCs2, would be sufficient to make the compounds in this organism. ZCD—native to Crocus sativus—cleaves the ends of zeaxanthin into crocin and hydroxyl-β-cyclocitrinal. These products are then acted on by UGTCs2, to produce our products: safranal, picrocrocin, and crocin. Before attempting to generate these genes in Synechocystis, ZCD and UGTCs2 were cloned into E. coli in conjunction with the genes necessary to produce zeaxanthin. ZCD was successfully cloned into E. coli with the aid of a fusion protein to minimize the presence of inclusion bodies. This result was confirmed by SDS-PAGE followed by trypsin digestion and LC-MS/MS. A flux balance analysis model was also written to predict the optimal conditions for in vivo production of the three target compounds in Synechocystis. It is hoped that with this groundwork laid, future work can produce a biosynthetic alternative to saffron.