Development of a Recombinant *Escherichia coli* strain for Overproduction of Plant Pigment, Anthocyanin

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Supplementary Information

Content

1. Supplementary Figure S1.
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Figure S1. Cyanidin 3-*O*-glucoside (C3G) concentration depicted in color intensities of different background strains harboring anthocyanin pathway enzymes in fusion construct and overexpression of UDP glucose pathway enzymes. Combining 2 modifications of *e. coli* efflux pumps: tolC knockout preventing the efflux of catechin and yadH overexpression facilitating the efflux of C3G, resulted in a strain with highest C3G production.

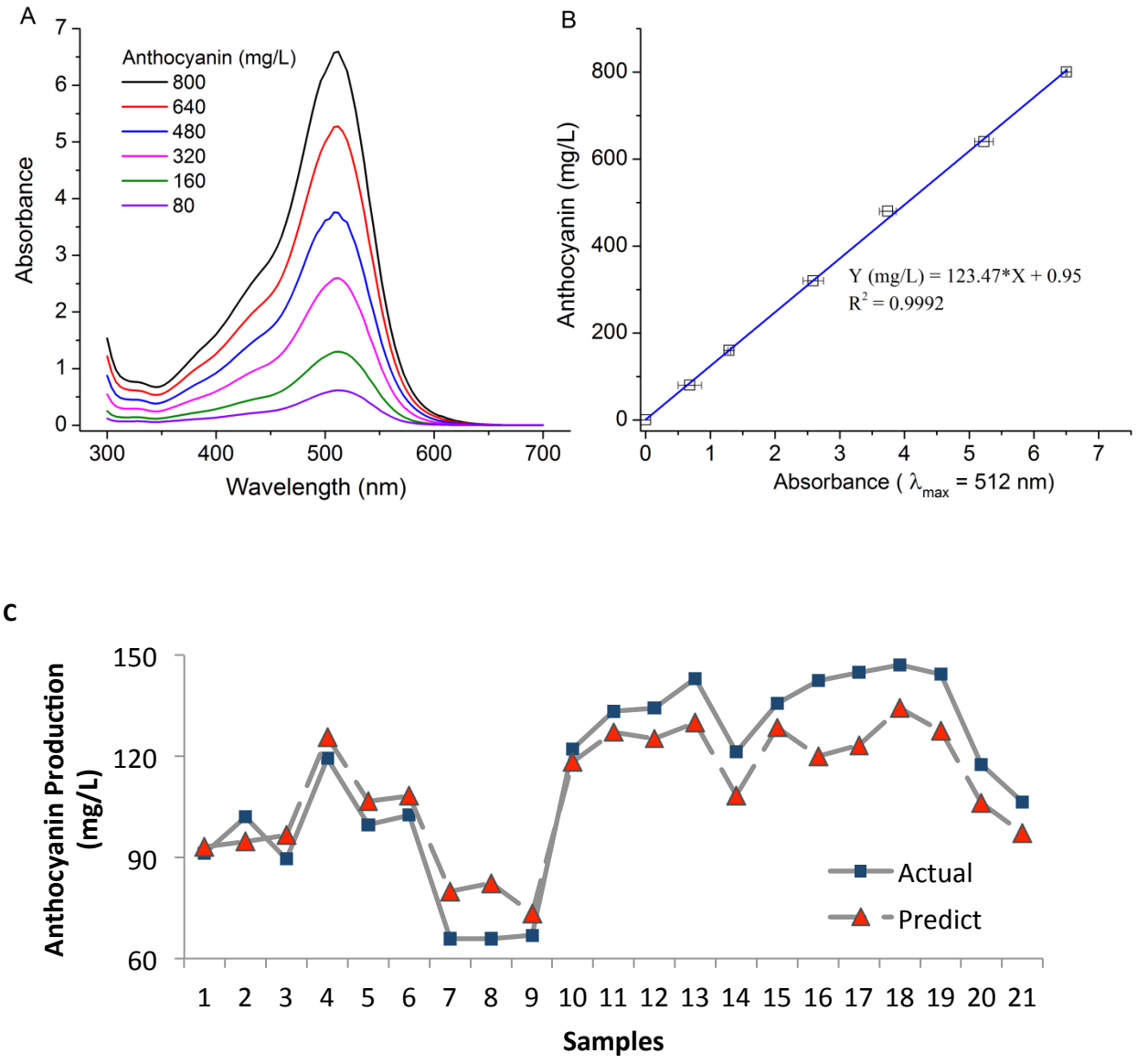

Figure S2. Cyanidin 3-*O*-glucoside (C3G) colorimetric assay development. (***A,B***) C3G calibration curve measured from 80mg/L to 800mg/L at an absorbance of 512nm. (***C***) Comparing predicted titers of C3G using calibration curve to the actual production measured using HPLC of 21 different samples.

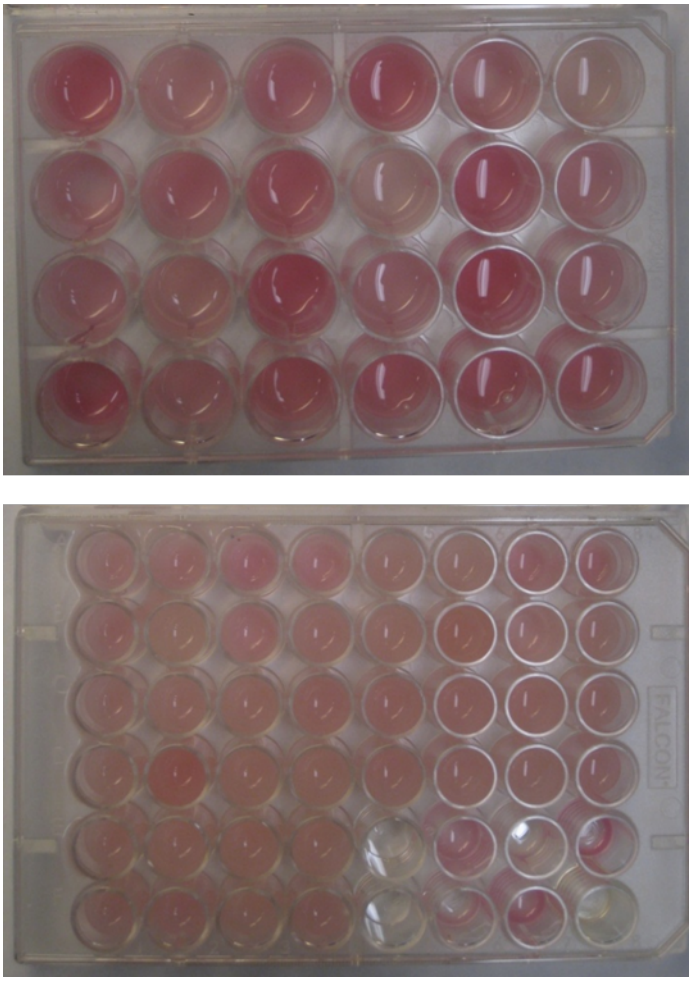

Figure S3. Examples of 24- and 48-well plates used for fermentation parameters optimization. Parameters optimized include induction OD, IPTG, sodium ascorbate, 2-oxoglutarate, orotic acid and glutamate concentrations.

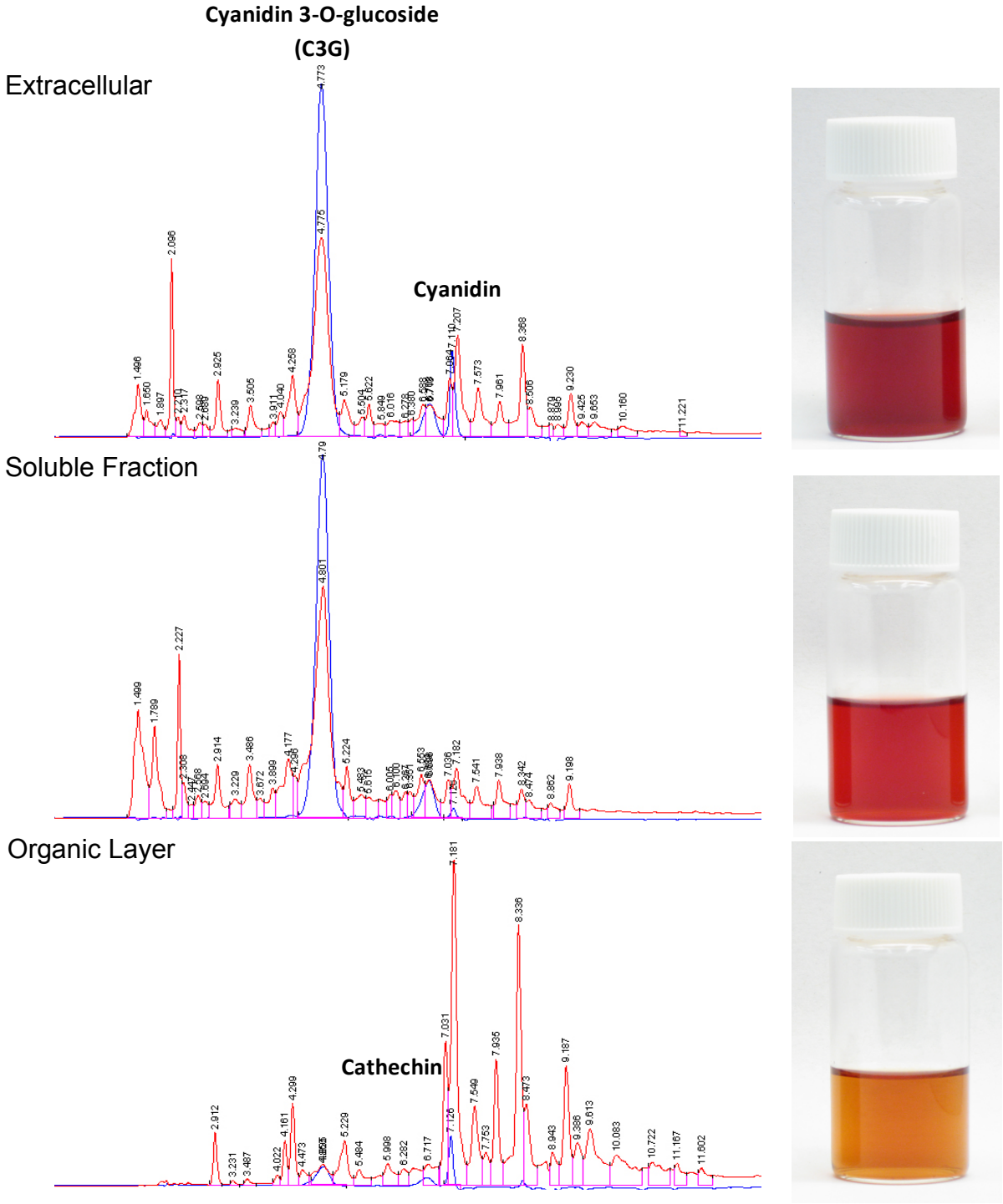

Figure S4. HPLC chromatogram of cell culture before and after extraction with ethyl acetate. After extraction, C3G remained mostly in the soluble fraction while most of the cyanidin and unknown hydrophobic compounds including catechin were extracted into the organic layer.