

Metabolically Engineered *Escherichia coli* for High-Level Production of Astaxanthin

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Astaxanthin is a reddish keto-carotenoid which acts as powerful antioxidant. Here, we report metabolically engineered *Escherichia coli* for high-level and high productivity astaxanthin production. First, the *crt* genes (*crtE*, *crtY*, *crtI*, *crtB*, and *crtZ*) from *Pantoea ananatis* and the truncated *BKT* gene (*trCrBKT*) from *Chlamydomonas reinhardtii* were introduced. Eight different fusion tags were tested on *trCrBKT* for stable expression and efficient guiding to the membrane. The signal peptide (*OmpF* and *TrxA*) were tagged to *trCrBKT* and 12.90 mg/L of astaxanthin was produced, 2.08-fold higher than without tags. Culture condition optimization increased the production up to 332.23 mg/L by fed-batch cultivation. Further improvement by overexpressing the *ispD* and *ispF* genes, identified by *in silico* FVSEOF, produced 377.10 mg/L of astaxanthin with 9.20 mg/L/h productivity. The titer increased to 432.82 mg/L with a productivity of 9.62 mg/L/h when IPTG was reduced to from 1 mM to 0.5 mM. *Hok/sok* system was introduced to improve plasmid stability and this strain produced 385.04 mg/L of astaxanthin with a productivity of 7.86 mg/L/h. (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557)