Microbial upcycling of food wastes into novel Carotenoid and bioplastics

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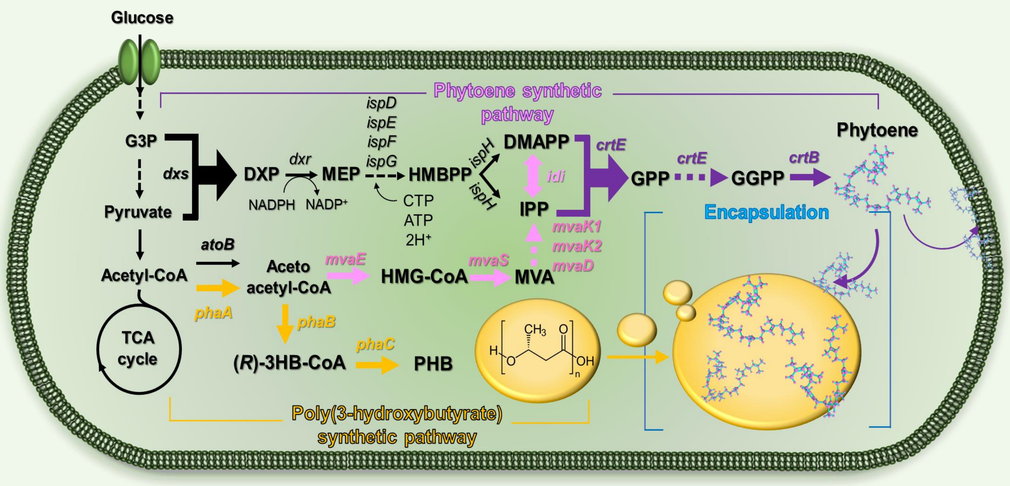
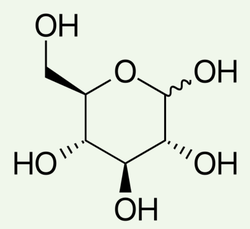
Abstract

Increasing demands on the carotenoids have focused attention on the need to develop industrially feasible bioprocess to make more things naturally and safely. Although many efforts have been devoted to developing efficient ways to increase carotenoid stability and productivity, noticeable progress has not yet been reported. Here we report the development of microbial production of carotenoids and bioplastics. First, the E. coli strain was engineered to produce phytoene through the introduction of phytoene biosynthetic pathway from *Deinococcus radiodurans* R1 strain. Next, the phytoene-producing *E. coli* strain was further engineered to co-produce bioplastics together with highly stabilized novel carotenoids. This method not only provides a way to produce high-value compounds in an environmentally friendly manner but can also be a promising platform technology for valorising food waste into high-value compounds [This work was supported by the Ministry of Environment's waste resource energy recycling professional training project (YL-WE-22-001).

Rational metabolic engineering host strain | **Enzymatic saccharification of kimchi cabbage wastes** | **Fermentation process**



Cellulolytic enzyme mixture



Fermentable sugar

Results

1) Construction of phytoene producing *E. coli*

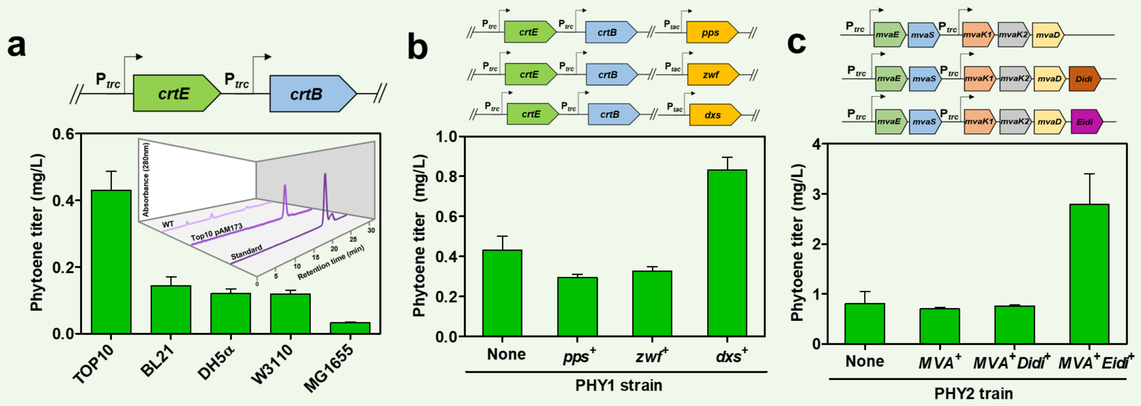
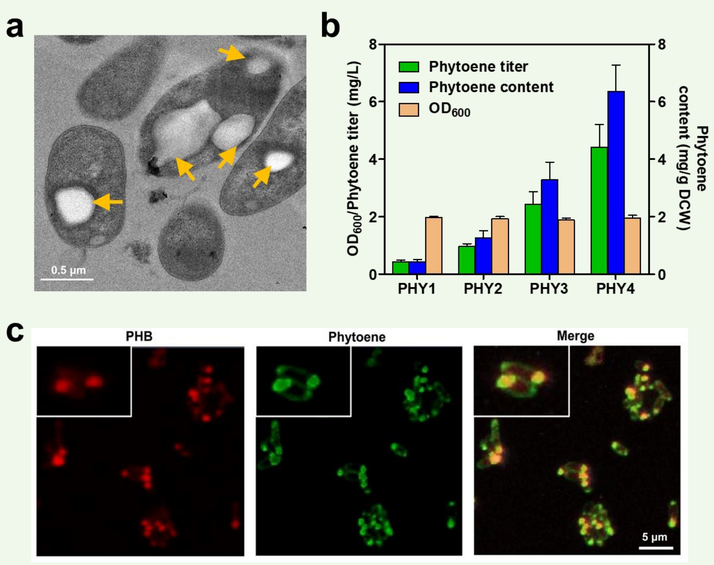


Fig. 1. Rational metabolic engineering for overproduction of phytoene. (a) Screening of *E. coli* strain to choose suitable phytoene production. Inset image indicate HPLC chromatogram of the phytoene extracted from wild-type, *E. coli* TOP10 expressing *crtB* and *crtE* genes, and phytoene standard. (b) The effect of overexpressing genes of *pps*, *zwf*, and *dxs* on phytoene production (c) The effect of combinatorial expression of MVA synthetic genes with type II *Didi* or type I *Eidi* on phytoene production.

- **The *E. coli* TOP10 strain expressing the *crtE* and *crtB* genes, named PHY1, used as a base strain.**
- ***E. coli* TOP10 strain (named as PHY3) expressing MVA genes with *Eidi* showed a 6.1-fold increase phytoene production up to 2.8 ± 0.6 mg/L compared to that of obtained in a base strain (0.43 ± 0.06 mg/L).**

2) Enhanced production by employing PHB synthesis in *E. coli*



- **Transmission electron microscopic (TEM) analysis showed that 3HB granules (1-3 granules per cells) were successfully formed within the cells by employing PhaCAB from *Cupriavidus necator* H16 in PHY3 strain (named as PHY4).**
- **The PHY4 strain produced 4.42 ± 0.79 mg/L of phytoene titer and 6.36 ± 0.95 mg/g DCW of phytoene content, which showed the best production yield.**
- **By analyzing confocal images, most intracellular phytoene was co-localized onto 3HB granules (orange color in merged image) rather than cell membranes.**

Fig. 2. The formation of poly(3-hydroxybutyrate) (3HB) in PHY4 strain. (a) Transmission electron microscopic (TEM) image of PHY5 strain. (b) Comparison of phytoene titer, content, and cell density in the phytoene producing strains depending on metabolic engineering steps. (c) Confocal laser scanning microscopic (CLSM) analysis after nile red staining.

3) Phytoene production using kimchi cabbage waste (KCW) hydrolysates

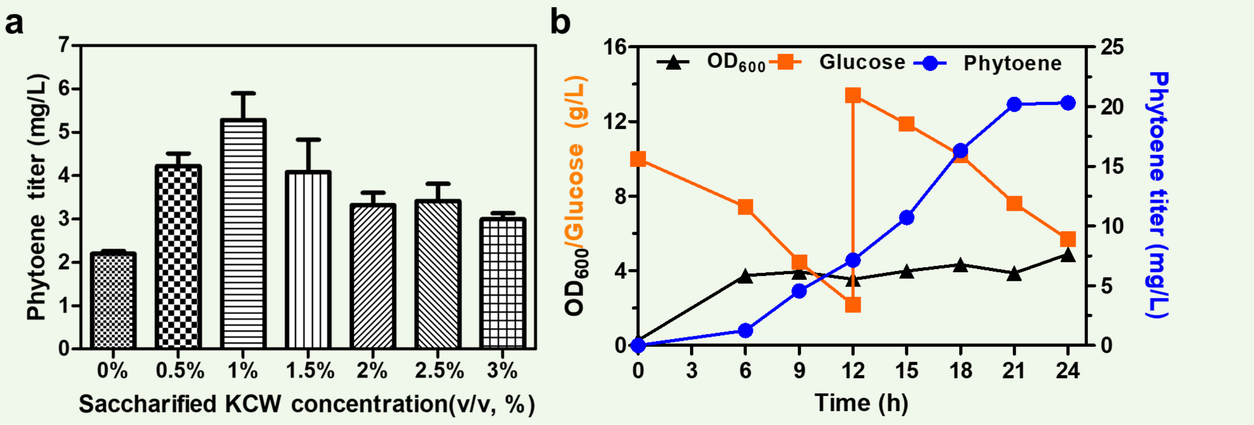


Fig. 4. Microbial phytoene production using a metabolically engineered *E. coli* from KCW hydrolysates. (a) The effect of the concentration of KCW hydrolysates on phytoene production. (b) Fed-batch fermentation profile of PHY4 strain. For induction, 1 mM ITPG was added at the OD_{600} of 0.6~0.8. Data represent the means and standard deviations (n = 3)

- **PHY4 stain showed the best phytoene production (5.28 ± 1.06 mg/L) when cultivatied in modified MR medium supplemented with 1% of KCW hydrolysate.**
- **During fermentation process, PHY4 strain produced 20.3 mg/L of phytoene from 26.9 g/L of glucose derived from KCW hydrolysate.**

4) Evaluation of light stability for PHB-encapsulated phytoene

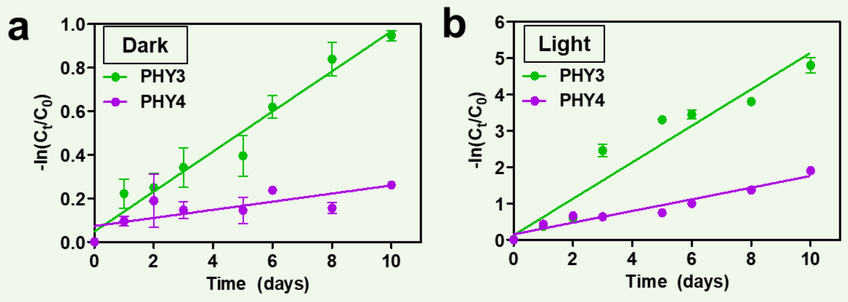


Fig. 4. First-order degradation kinetics of phytoene produced by PHY3 or PHY4 strain during storage for 10 days. (a) dark and (b) light conditions (squared). Data represent the means and standard deviations (n = 3)

- **The degradation rate constant (k; day^{-1}) of phytoene in PHY4 under dark condition was 0.0199, which was approximately 4.6-fold lower than that in PHY3 (0.091).**
- **A half-life of phytoene in PHY4 under dark and light conditions was 34.8 days (dark) and 4.3 days (light), respectively, whereas PHY3 was determined by 7.6 days (dark) and 1.4 days (light).**

Conclusion

- ✓ In this study, we reported for the first time the development of valorization method for production of a novel colorless carotenoid, phytoene using PHB-producing *E. coli*.
- ✓ Phytoene- and PHB-producing *E. coli* strain could efficiently utilize hydrolyzed KCWs as the carbon source and produced 20.3 mg/L of phytoene with a yield and productivity of 0.75 mg/g glucose and 0.84 mg/L/h, respectively.
- ✓ Moreover, co-production of PHB showed remarkable protection for intracellular phytoene under light exposure

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