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Construction of an efficient system for the production of red pepper carotenoids and new derivatives

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Research aims

Capsanthin and capsorubin are useful functional carotenoids with a strong capability of decreasing singlet oxygen. Since they are found only in the fruit of red pepper or pollen grains of the lily, it is difficult to extract them in large amounts. 4-Ketocapsanthin is a novel carotenoid, which has a chemical structure of both capsanthin and astaxanthin. Astaxanthin is used as an anti-oxidant in foods. Therefore, 4-ketocapsanthin is expected to be a 'super anti-oxidant'. We have constructed pathway-engineered *Escherichia coli* cells that can effectively produce an excess of farnesyl pyrophosphate (FPP).[1] In this study, we propose to use this *E. coli* to develop a system for the production of capsanthin, capsorubin, or the novel carotenoid, 4-ketocapsanthin. For this purpose, we will clone and introduce the genes required for the biosynthesis of these carotenoids. The goal of our study is to facilitate a comprehensive examination of the biological activities of these pigments.

Methods

1. Effective production of zeaxanthin

For the efficient production of zeaxanthin (over 10 mg/g DW), we have constructed an *E. coli* strain expressing the *crtE*, *crtB*, *crtI*, *crtY*, and *crtZ* genes from the soil bacterium *Pantoea ananatis* as well as the *Haematococcus pluvialis* gene *idi* (encoding for IPP isomerase).[2]

2. Effective production of antheraxanthin and violaxanthin

To produce antheraxanthin and violaxanthin in the *E. coli*, we introduced the plant zeaxanthin epoxidase (*Zep*) gene into zeaxanthin-producing *E. coli*.

3. Effective production of capsanthin and capsorubin

To produce capsanthin and capsorubin in the *E. coli*, we will introduce the capsanthin-capsorubin synthase (*Ccs*) gene from *Capsicum annuum* or *Lilium lancifolium* into the antheraxanthin- and violaxanthin-producing *E. coli*.

4. Effective production of 4-ketocapsanthin

To produce 4-ketocapsanthin in the *E. coli*, we will introduce the *crtW* (carotenoid 4,4′-ketolase) gene from marine bacterium *Brevundimonas* sp. strain SD212 into the capsanthin- and capsorubin-producing *E. coli*.

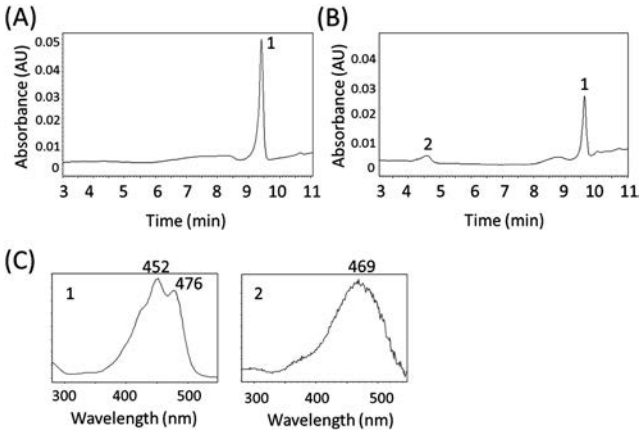

Fig. 1. Analysis of products from genetic engineered *E. coli*.
(A) HPLC chromatogram of the recombinant *E. coli* M12 (pET-MpZEP), (B) HPLC chromatogram ot the recombinant *E. coli* M12 (pET-MpZEP/GDH+pRSF-ferredoxin/FNR), (C) Absorption spectra of individual peaks. 1, zeaxanthin; 2, novel carotenoid.

Results

1. Effective production of zeaxanthin

We constructed the plasmid pACHP-Zea, which contains the *crtE*, *crtB*, *crtI*, *crtY*, and *crtZ* genes from *Pantoea ananatis* along with the *idi* gene from *Haematococcus pluvialis*, and introduced it into *E. coli* BL21 (DE3) cells. The productivity of these recombinant *E. coli* was 2–3 fold higher than that of recombinant *E. coli* bearing the *crtE*, *crtB*, *crtI*, *crtY*, and *crtZ* genes but not the *idi* gene (plasmid pACCAR25ΔcrtX.[3] The zeaxanthin-producing *E. coli* strain carrying the plasmid pACHP-Zea is called M12.

2. Effective production of antheraxanthin and violaxanthin

We cloned the zeaxanthin epoxidase gene (*MpZEP*) from liverwort *Marchantia polymorpha*, to construct the plasmid pET-MpZEP. This plasmid was introduced into the *E. coli* M12 strain. Neither antheraxanthin nor violaxanthin were present in this *E. coli* M12 (pET-MpZEP) strain. Previous reports demonstrate that functional zeaxanthin epoxidase requires O_2, ferredoxin, ferredoxin reductase (FNR), FAD, and NADPH. Since *E. coli* are known to lack these co-factors, except for O_2, we cloned and introduced the genes expressing ferredoxin-FNR and an NADPH-recycling enzyme (glucose dehydrogenase; GDH) into the recombinant

E. coli M12 (pET-MpZEP) strain. HPLC analysis could detect neither antheraxanthin nor violaxanthin in the resultant recombinant *E. coli* M12 (pET-MpZEP/GDH+pRSF-ferredoxin/FNR) strain. Instead, a peak representing a novel carotenoid was found (Fig. 1). Currently, we are in the process of preparing this compound for NMR analysis.

3. Effective production of capsanthin and capsorubin

We have cloned the capsanthin-capsorubin synthase (*Ccs*) gene from *Capsicum annuum*. After the construction of antheraxanthin- and violaxanthin-producing *E. coli*, we propose to introduce the *Ccs* gene in this strain.

4. Effective production of 4-ketocapsanthin

We have cloned the *crtW* (carotenoid 4,4′-ketolase) gene from the *Brevundimonas* SD212 strain. After the construction of capsanthin- and capsorubin-producing *E. coli*, we will introduce the *crtW* gene in this strain.

Conclusions

In this study, we have constructed a recombinant *E. coli* strain that can efficiently produce zeaxanthin. The combination of zeaxanthin epoxidase, ferredoxin-FNR, and NADPH-recycling led to the unexpected production of a novel carotenoid, although antheraxanthin and violaxanthin were not detected. Structural identification of the novel carotenoid using NMR analysis is expected to provide insight into its biosynthesis.

The production of 4-ketocapsanthin from the cloned *Ccs* and *crtW* genes will be successfully achieved in the *E. coli*, after we carry out the production of antheraxanthin and violaxanthin.

References

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