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1 Section: Microbial and Enzyme Technology

2 **High versus low level expression of the lycopene biosynthesis genes**

3 **from *Pantoea ananatis* in *Escherichia coli***

4

5 Christoph Albermann

6

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11 **Abstract**

12 The heterologous synthesis of lycopene in non-carotogenic *Escherichia coli* required the  
13 introduction of the biosynthesis genes *crtE*, *crtB*, and *crtI*. Recombinant *E. coli* strains,  
14 expressing each lycopene biosynthesis gene from *Pantoea ananatis* using multi-copy plasmid  
15 or single-copies after stable chromosomal integration, were cultivated and the formation of  
16 lycopene was investigated. The different expression conditions significantly influenced the  
17 lycopene formation as well as the growth behaviour. High plasmid expression levels of *crtI*  
18 with a single copy background of *crtE* and *crtB* in *E. coli* led to a predominate synthesis of  
19 tetrahydrolycopene at 253  $\mu\text{g g}^{-1}$  (cdw).

20

21 Key words: chromosomal integration, heterologous carotenoid production, lycopene,  
22 tetrahydrolycopene

23

## 24 **Introduction**

25 Carotenoids are ubiquitous with important nutritional and physiological relevance for plants,  
26 algae and bacteria. Carotenoids have gained industrial importance as colorant for cosmetic-,  
27 food-, and animal feed-products. Recent reports on carotenoids have further demonstrated that  
28 carotenoids have manifold beneficial effects on human health (Nagao 2009). The simplest C<sub>40</sub>  
29 carotenoid, lycopene, is an effective antioxidant and has promising biological activity for the  
30 prevention of several types of cancer, including breast cancer and prostate cancer (Seren et al.  
31 2008). The genes responsible for the synthesis of lycopene have been isolated from the  
32 epiphytic bacterium, *Erwinia*, and plants, and the functions of the carotenogenic genes have  
33 been identified (Hundle et al. 1991; Misawa et al. 1990; To et al. 1994; Albrecht et al. 1995;  
34 Sandmann 1994). The first substrate of the enzymes encoded by the *Erwinia* carotenoid  
35 biosynthesis gene cluster is farnesyl pyrophosphate (FPP) which is the common precursor for  
36 the biosynthesis of carotenoids and also of numerous other isoprenoids. The biosynthesis of  
37 lycopene in *E. coli* requires the expression of the genes *crtE*, *crtB*, and *crtI*, which utilize the  
38 *E. coli* isoprenoid precursor FPP. CrtE is a geranylgeranyl synthase that catalyzed the  
39 condensation of FPP and IPP to GGPP and CrtB is a phytoene synthase (Sandmann &  
40 Misawa 1992). The phytoene desaturase (CrtI) catalyzed the introduction of double bonds  
41 leading to an extended conjugated double bond system. The insertion of four double bonds  
42 into phytoene by CrtI from *Pantoea ananatis* (formerly *Erwinia uredovora*) results in the  
43 formation of lycopene (Sandmann 2009).

44 Due to the increasing demand for carotenoids, improvements have been made in the  
45 formation of carotenoids by recombinant bacteria, yeasts, and plants expressing carotenoid  
46 biosynthesis genes or gene clusters (Das et al. 2007). In particular, the non-carotenogenic *E.*  
47 *coli* has been widely used as a host for improved carotenoid productivity by transformation  
48 with appropriate biosynthesis genes, and methods were developed for increasing the  
49 biosynthesis of isoprenoid precursors in *E. coli* (Harada & Misawa, 2009).

50 In this study, the formation of lycopene by heterologous *E. coli* strains expressing each  
51 biosynthesis gene from *P. ananatis* either from a multi-copy plasmid or from single copy  
52 inserted into the chromosome is presented.

53

54

## 55 **Material and methods**

### 56 **Bacterial strains, growth conditions, and media**

57 The bacterial strains used were *Escherichia coli* BW25113 lac<sup>+</sup> and *E. coli* DH5 $\alpha$  (Table 1).  
58 If not stated otherwise, they were grown at 37 °C in LB medium. Antibiotics used were:  
59 100  $\mu$ g ampicillin/ml, 50  $\mu$ g chloramphenicol/ml and 50  $\mu$ g kanamycin/ml. Difco MacConkey  
60 agar was purchased from Nordwald (Hamburg, Germany). Carotenoid standards were  
61 provided by the Institute of Technical Biochemistry, University of Stuttgart. All other  
62 chemical and reagents are from Sigma-Aldrich or Roth.

### 63 **Cloning of the biosynthesis genes in *E. coli***

64 In order to create an artificial lycopene gene cluster for expression in *E. coli*, the following  
65 cloning strategy was used. Modified PCR primers were used to create a *Bgl*III restriction site  
66 upstream the start-codon of the gene, plus an artificial RBS and a *Bam*HI or a *Bgl*III site down-  
67 stream of the stop-codon. *crtB* was amplified from plasmid pCAR16 (Misawa et al. 1990)  
68 using the primers: GATGCTGGAAGATCTCATATGAATAATCCGTCG and  
69 CTTCAGGCGAGGATCCGGGAAAGACATGG. The resulting PCR product was digested  
70 by the restriction enzymes *Bgl*III plus *Bam*HI and were ligated into the *Bam*HI-hydrolyzed  
71 pCAS30, resulting in plasmid pCAS56. *crtI* was amplified from plasmid pCAR16 using the  
72 primers: CATTGCAAGATCTTATGAATTCTCATCGTTAAAGAGCGAC and  
73 CGTTTAGATCTCATGATTGAGTAACGACGGATTATTCA. The resulting PCR product

74 was digested by *Bgl*III was ligated into the *Bam*HI-hydrolyzed pCAS56, resulting in plasmid  
75 pCAS58. Restriction digestions and DNA sequencing were performed for all constructs to  
76 verify the orientations and sequences of the cloned biosynthesis genes. Takara PrimeStar  
77 DNA polymerase (Lonza, Belgium) was used for amplification. The chromosomal integration  
78 of recombinant *crt*-gene expression cassettes and elimination of antibiotic resistance gene  
79 cassettes using plasmid pCP20 (Cherepanov & Wackernagel 1995) were conducted as described  
80 by Albermann et al. (2010).

81

### 82 **Cultivation of *E. coli* cultures and analysis of carotenoids**

83 Recombinant, lycopene-producing *E. coli* strains were cultivated at 30°C in 50 ml LB  
84 medium. Plasmid carrying strains were cultivated in the presence of a corresponding  
85 antibiotic. IPTG was added at an OD<sub>600</sub> of 0.3. Cultures with strains that carry plasmid  
86 pJOE5559 (unpublished work) were also induced with L-rhamnose at 1.2 mM. Samples (2  
87 ml) were withdrawn from cultures after 24h. The cells were harvested by centrifugation,  
88 washed with cold water, and subsequently extracted with acetone (500 µl) for 15 min at 50°C.  
89 Insoluble components of the extract were removed by centrifugation (20,000 x g). HPLC  
90 analysis was performed on Dionex HPLC Instrument installed with a Chromeleon Software,  
91 Gina autosampler, P580 pumps, and detection with a UV lamp. Products were analyzed by  
92 loading 20 µl supernatant onto a C30-reverse-phase HPLC column (250 mm x 4.6 mm, 5 µm,  
93 YMC-Europa GmbH, Dinslaken, Germany) attached with a guard column containing matrix  
94 of same material as the column. The solvents used (at 1 ml/min) were A: *tert*-butyl ether  
95 (MTBE)/methanol/water (19/80/1, by vol.) and B: MTBE/methanol/water (90/9/1, by vol.).  
96 Gradient conditions: equilibration conditions at 60 % B (v/v); 0 to 40 min linear gradient from  
97 60% B to 100 % B (v/v); 40 to 45 min 100 % B (v/v). Standard curves correlating areas to  
98 concentration of lycopene and tetrahydrolycopene (TDHL) was used to determine the

99 quantity of these carotenoids in the samples. HPLC-MS analysis was carried out on a HPLC  
100 coupled to a UV-detector and a mass spectrometer (LCMS-2010EV/dual ion source  
101 DUIS2010, Shimadzu, Germany), using identical conditions as described above.

102 For the semi-preparative isolation of TDHL, *E. coli* BW-EB pJF-crtI was cultivated in 400 ml  
103 minimal medium (3 g  $\text{KH}_2\text{PO}_4 \text{ l}^{-1}$ , 12 g  $\text{K}_2\text{HPO}_4 \text{ l}^{-1}$ , 5 g  $(\text{NH}_4)_2\text{SO}_4 \text{ l}^{-1}$ , 0.3 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O} \text{ l}^{-1}$ ,  
104 0.015 g  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O} \text{ l}^{-1}$ , 0.1 g  $\text{NaCl} \text{ l}^{-1}$ , 5 g Glucose monohydrate  $\text{ l}^{-1}$ , 15 ml  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$   
105 /sodium citrate  $\text{ l}^{-1}$  (from a solution of 7.5 g  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O} \text{ l}^{-1}$  and 100 g sodium citrate  $\text{ l}^{-1}$ ), 7.5  
106 mg thiamine  $\text{ l}^{-1}$ , 33 ml trace elements  $\text{ l}^{-1}$ ) at 30°C. The culture was induced with 0.5 mM  
107 IPTG at  $\text{OD}_{600}$  of 0.3. After 24 h, the cells were harvested by centrifugation, washed with cold  
108 water, and subsequently extracted twice with 20 ml acetone by vigorous shaking. Insoluble  
109 components of the extract were removed by centrifugation (20,000 x g). Acetone was  
110 removed under a stream of nitrogen and the residual material was dissolved in MTBE. TDHL  
111 was isolated by HPLC (several runs) using the same device as for analysis. The mobile phase  
112 was MTBE/methanol/water (90/9/1, by vol.) at 1.2 ml  $\text{min}^{-1}$  for 20 min isocratic; 200  $\mu\text{l}$  as  
113 injection volume. TDHL fractions were collected, dried under a stream of nitrogen and  
114 subsequently dissolved in acetone. TDHL content in acetone solution was quantified by  
115 measuring the absorbance at 508 nm. Concentration of TDHL was determined using an  
116 extinction coefficient ( $5933 \text{ (g/ml)}^{-1} \text{ cm}^{-1}$ , Unrean et al. 2010).

117

118

119 **Results and discussion**

120 To investigate the effect of single- and multi-copy expression of *crtE*, *crtB*, and *crtI* from *P.*  
121 *ananatis* on lycopene biosynthesis in *E. coli*, strains with all different combinations of two  
122 chromosomal and one plasmid-encoded *crt*-gene were constructed. The expression of each  
123 gene is controlled by an IPTG-inducible *tac*-promoter. The chromosomal insertion of the  
124 heterologous expression cassettes were conducted by a method described recently  
125 (Albermann et al. 2010). This method is based on the  $\lambda$ -Red-recombineering technique  
126 (Datsenko & Wanner 2000; Zhang et al. 1999). The method allows for a fast and accurate  
127 screening of the recombination event by differential MacConkey agar medium. The following  
128 strains were received after integration of two individual *crt*-expression cassettes into the  
129 chromosome of *E. coli* BW25113 *lac*<sup>+</sup>: *E. coli* BW-EB, *E. coli* BW-EI, *E. coli* BW-BI (table  
130 1). To allow the biosynthesis of lycopene in these strains, each of the strains was transformed  
131 with the multi-copy expression vector pJF119 $\Delta$ N carrying the respective *crt*-gene, so that  
132 each strain carries the heterologous genes *crtE*, *crtB*, and *crtI*, resulting in *E. coli* BW-EB  
133 pJF119-*crtI*, *E. coli* BW-EI pJF119-*crtB*, and *E. coli* BW-BI pJF119-*crtE*, respectively.  
134 These strains were cultivated in LB medium and the cultures were induced with different  
135 concentrations of IPTG (0 – 1 mM). *E. coli* BW-Lyco, that contains all three lycopene  
136 biosynthesis genes on the chromosome, *E. coli* BW-Lyco pJF119 $\Delta$ N, and *E. coli* BW25113  
137 *lac*<sup>+</sup> pJF119-*crtE*BI were used as strains for comparison.

138 The lycopene content per volume as well as per g cell dry wt are given in Figure 1. *E. coli*  
139 BW-Lyco, which expresses *crtE*, *crtB*, and *crtI* from individual chromosomal copies, had the  
140 highest lycopene formation ( $3.9\pm 0.1$  mg l<sup>-1</sup>;  $1.65\pm 0.12$  mg g<sup>-1</sup> cdw). The enhanced expression  
141 of *dxs*, *idi*, and *ispA* by *E. coli* BW-Lyco pJOE5559 resulted in the formation of  $8.72\pm 0.21$   
142 mg l<sup>-1</sup>,  $4.45\pm 0.32$  mg g<sup>-1</sup> cdw lycopene showing that the enzyme activities of CrtE, CrtB, and  
143 CrtI expressed by single chromosomal gene copies is sufficient for the conversion of FPP to

144 lycopene even under conditions with increased supply of isoprenoid precursors. The induction  
145 of these strains with IPTG had no significant effect either on growth or on lycopene  
146 formation. *E. coli* BW-Lyco pJF119 $\Delta$ N, carrying an additional *lacI* gene on the plasmid,  
147 showed an increase in lycopene formation after increasing the inducer concentration showing  
148 that the expression of the chromosomal *lacI* is not sufficient to repress the tac-promoter under  
149 the given conditions. A higher LacI concentration, as given in *E. coli* BW-Lyco pJF119 $\Delta$ N,  
150 led to an IPTG-inducible expression of the heterologous genes.

151 Expression of lycopene biosynthesis genes by an artificial, plasmid encoded *crtEBI*-operon  
152 (*E. coli* BW pCAS58) yielded  $1.77\pm 0.23$  mg l<sup>-1</sup>,  $0.84\pm 0.11$  mg g<sup>-1</sup> (cdw) lycopene without  
153 induction. The amount of lycopene decreased upon induction by IPTG. The plasmid  
154 expression of *crtE* by *E. coli* BW-BI pJF119-*crtE* under different inducer concentrations led  
155 to a similar amount of lycopene per cell ( $1.35\pm 0.08$  mg g<sup>-1</sup> cdw) but the total biomass  
156 decreased due to the high expression of *crtE*. The higher lycopene content in *E. coli* BW-BI  
157 pJF119-*crtE* compared to *E. coli* BW-Lyco pJF119 $\Delta$ N in cultures without induction indicates  
158 that the CrtE catalyzed reaction is the most limiting step in the conversion of FPP to lycopene.

159 The *crtB* plasmid expression by *E. coli* BW-EI pJF119-*crtB* led to a decrease in growth as  
160 well as on lycopene formation. The CrtB product phytoene was not detected in the cell  
161 extract. Growth retardation may occur due to the interference of CrtB with the formation of  
162 prenylquinones in *E. coli* (Neudert et al. 1998), but the low biomass and lycopene amount per  
163 cell in the stationary phase (after 24 h) in induced cells (> 0.1 mM IPTG) suggests that the  
164 CrtB protein also being toxic or a protein burden to *E. coli*. This is also supported by the  
165 observation that *E. coli* BW-EI pJF119-*crtB* pJOE5559, which showed increased formation of  
166 FPP by the additional copies of *dxs*, *idi*, and *ispA*, had reduced growth under high *crtB*  
167 expression conditions (data not shown).



168 A further effect on the plasmid overexpression of a single *crt*-gene was observed by the  
169 expression of *crtI*. Cultivation of *E. coli* BW-BE pJF119-*crtI* yielded, beside lycopene,  
170 predominantly in the formation of 3,4,3',4'-tetrahydrolycopene (TDHL) (Fig. 2), which  
171 was identified by LC-MS analysis ( $M^+$  532) and UV/visible spectroscopy (Fig. 2H). For the  
172 quantification of TDHL, *E. coli* BW-BE pJF119-*crtI* was cultivated in minimal medium and  
173 the extracted TDHL was isolated by semi-preparative HPLC (Fig. 2F). The concentration of  
174 TDHL in acetone was determined by photospectroscopy [extinction coefficient 5933  
175  $(\text{g}/100\text{ml})^{-1} \text{cm}^{-1}$ , Unrean et al. 2010]. The amount of TDHL in LB cultures of *E. coli* BW-BE  
176 pJF119-*crtI* increased upon IPTG induction (Figs. 1 and 2). The highest TDHL concentration  
177 was after induction with 0.5 mM IPTG ( $253 \pm 33 \mu\text{g g}^{-1} \text{cdw}$ , 59% of all carotenoids).  
178 Induction with 1 mM IPTG gave the highest percentage of TDHL with 80% ( $169 \pm 27 \mu\text{g g}^{-1}$   
179  $\text{cdw}$ ). Extracts of cultures with a high TDHL content as well as the purified TDHL samples  
180 also reveal the existence of two further isomeric forms of TDHL ( $M^+$  532) as indicated in Fig.  
181 2. The formation of TDHL was not observed in any of the other tested lycopene producing  
182 strains, in which *crtI* is expressed from single copy or by the plasmid encoded gene cluster  
183 (pCAS58).

184 For the CrtI-type phytoene-desaturase from *P. ananatis* and other organisms, the general  
185 ability to carry out up to six desaturation reactions on phytoene has been shown (Linden et al.  
186 1991; Sandmann 2009). Additionally, the ratio of substrate and desaturase can further define  
187 the degree of phytoene desaturation (Stickforth & Sandmann 2007). Sandmann and co-  
188 workers used a two plasmid system for the differentiated expression of *crtEB* and *crtI* in *E.*  
189 *coli*, which resulted in TDHL at approx. >10% of all carotenoids. Here, the reported  
190 combination of single-copy (*crtE*, *crtB*) and multi-copy (*crtI*) expression led to a TDHL  
191 portion of up to 80% of the *E. coli* synthesized carotenoids which verifies that the kinetics of  
192 CrtI from *P. ananatis* can determine the degree of desaturation. By the use of directed  
193 evolution methods a CrtI-mutant was generated that led, compared to the wild type enzyme,

194 to a partly conversion of lycopene to TDHL in *E. coli* (Schmidt-Dannert et al. 2000). Due to  
195 the lack of biochemical data it is not yet clarified if the mutein has an increased catalytic  
196 activity or if the amino acid exchanges lead to a higher protein overproduction or stability that  
197 caused the formation of TDHL.

198 This report demonstrates that the expression level of heterologous biosynthesis pathways may  
199 have a significant impact on the product yield as well as on product specificity. The  
200 expression of single chromosomal copies of either *crtE*, *crtB* or *crtI* leads to an about 90%  
201 higher yield of lycopene compared to the plasmid expressed gene cluster. *E. coli* BW-BE  
202 pJF119-crtI produced TDHL as the predominant carotenoid, due to the high expression of  
203 *crtI*, which opens the opportunity for a preparative synthesis of this rare carotenoid.

204 **In conclusion**, this report demonstrates that an efficient heterologous *in vivo* biosynthesis of  
205 carotenoid compounds requires an adapted expression of the heterologous genes to avoid  
206 metabolic burden effects and unspecific side reactions.

207

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210 for providing carotenoid standards. I thank Josef Altenbuchner (Universität Stuttgart) for  
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213

214 **References**

215

216 Albermann C, Trachtmann N, Sprenger G (2010) A simple and reliable method to conduct  
217 and monitor expression cassettes integration into the *Escherichia coli* chromosome.  
218 Biotechnol J 5:32-38

219

220 Albermann C, Ghanegaonkar S, Lemuth et al (2008) Biosynthesis of the vitamin E compound  
221  $\delta$ -tocotrienol in recombinant *Escherichia coli* cells. ChemBioChem 9:2524–2533

222

223 Albrecht M, Klein A, Huguency P, et al (1995) Molecular cloning and functional expression  
224 in *E. coli* of a novel plant enzyme mediating zeta-carotene desaturation. FEBS Lett 372:199-  
225 202

226

227 Cherepanov PP, Wackernagel W (1995) Gene disruption in *Escherichia coli*: Tc<sup>R</sup> and Km<sup>R</sup>  
228 cassettes with the option of Flp-catalyzed excision of the antibiotic-resistance determinant.  
229 Gene 158:9-14

230

231 Das A, Yoon SH, Lee SH, et al (2007) An update on microbial carotenoid production:  
232 application of recent metabolic engineering tools. Appl Microbiol Biotechnol 77:505-12

233

234 Datsenko KA, Wanner BL (2000) One-step inactivation of chromosomal genes in *Escherichia*  
235 *coli* K12 using PCR products. Proc Natl Acad Sci USA 97:6640-6645

236

237 Harada H, Misawa N (2009) Novel approaches and achievements in biosynthesis of  
238 functional isoprenoids in *Escherichia coli*. Appl Microbiol Biotechnol 84:1021-1031  
239

240 Hundle BS, Beyer P, Kleinig H, et al (1991) Carotenoids of *Erwinia herbicola* and an  
241 *Escherichia coli* HB101 strain carrying the *Erwinia herbicola* carotenoid gene cluster.  
242 Photochem Photobiol 54:89-93  
243

244 Linden H, Misawa N, Chamovitz D et al (1991) Functional complementation in *Escherichia*  
245 *coli* of different phytoene desaturase genes and analysis of accumulated carotenes. Z  
246 Naturforsch C 46:1045-1051  
247

248 Misawa N, Nakagawa M, Kobayashi K et al (1990) Elucidation of the *Erwinia uredovora*  
249 carotenoid biosynthetic pathway by functional analysis of gene products expressed in  
250 *Escherichia coli*. J Bacteriol 172:6704-6712  
251

252 Nagao A (2009) Absorption and function of dietary carotenoids. Forum Nutr. 61:55-63  
253

254 Neudert U, Martínez-Férez IM, Fraser PD et al (1998) Expression of an active phytoene  
255 synthase from *Erwinia uredovora* and biochemical properties of the enzyme. Biochim  
256 Biophys Acta 1392:51-58  
257

258 Sandmann G (1994) Carotenoid biosynthesis in microorganisms and plants. *Eur J Biochem*  
259 223:7-24  
260

261 Sandmann G (2009) Evolution of carotene desaturation: the complication of a simple  
262 pathway. *Arch Biochem Biophys* 483:169-74  
263

264 Sandmann G, Misawa N (1992) New functional assignment of the carotenogenic genes *crtB*  
265 and *crtE* with constructs of these genes from *Erwinia* species. *FEMS Microbiol Lett* 69:253-  
266 257  
267

268 Schmidt-Dannert C, Umeno D, Arnold FH (2000) Molecular breeding of carotenoid  
269 biosynthetic pathways. *Nat Biotechnol* 18:750-753  
270

271 Seren S, Lieberman R, Bayraktar UD, et al (2008) Lycopene in cancer prevention and  
272 treatment. *Am J Ther* 15:66-81  
273

274 Stickforth P, Sandmann G (2007) Kinetic variations determine the product pattern of  
275 phytoene desaturase from *Rubrivivax gelatinosus*. *Arch Biochem Biophys* 461:235-241  
276

277 To KY, Lai EM, Lee LY, et al (1994) Analysis of the gene cluster encoding carotenoid  
278 biosynthesis in *Erwinia herbicola* Eho13. *Microbiology* 140:331-339

279

280 Unrean P, Trinh CT, Srienc F (2010) Rational design and construction of an efficient *E. coli*  
281 for production of diapolycopendioic acid. *Metab Eng* 12:112-122

282

283 Vallon T, Ghanegaonkar S, Vielhauer O et al (2008) Quantitative analysis of isoprenoid  
284 diphosphate intermediates in recombinant and wild-type *Escherichia coli* strains. *Appl*  
285 *Microbiol Biotechnol* 81:175-182

286

287 Zhang Y, Buchholz F, Muyrers JP et al (1998) A new logic for DNA engineering using  
288 recombination in *Escherichia coli*. *Nat Genet* 20:123-128

289

290 **Legends to the figures**

291

292 Figure 1: Formation of lycopene and tetrahydrolycopene (TDHL) by recombinant *E. coli*  
293 stains upon different inductor (IPTG) concentration. Green bars: cellular dry weight per  
294 volume (g cdw L<sup>-1</sup>); blue bars: lycopene concentration (mg L<sup>-1</sup>); black bars: lycopene  
295 concentration (mg g<sup>-1</sup> cdw); purple bars: TDHL concentration (mg L<sup>-1</sup>); yellow bars: TDHL  
296 concentration (mg g<sup>-1</sup> cdw). Values represent the average of  $\geq 3$  independent cultivations.

297

298

299 Figure 2: HPLC-analysis of carotenoids in extracts from recombinant *E. coli* strains after  
300 cultivation for 24h in LB medium. (A) *E. coli* BW-Lyco, (B) *E. coli* BW-BE pJF119-crtI w/o  
301 IPTG, (C) *E. coli* BW-BE pJF119-crtI + 0,05 mM IPTG, (D) *E. coli* BW-BE pJF119-crtI +  
302 0,2 mM IPTG, (E) *E. coli* BW-BE pJF119-crtI + 1 mM IPTG. (F) HPLC analysis of TDHL  
303 after purification. UV/Vis spectrum of lycopene (G), of TDHL (H), of TDHL isomer T'(I), of  
304 TDHL isomer T'' (J). L lycopene, T tetrahydrolycopene.

305

306

307 Table 1: Strains and plasmids

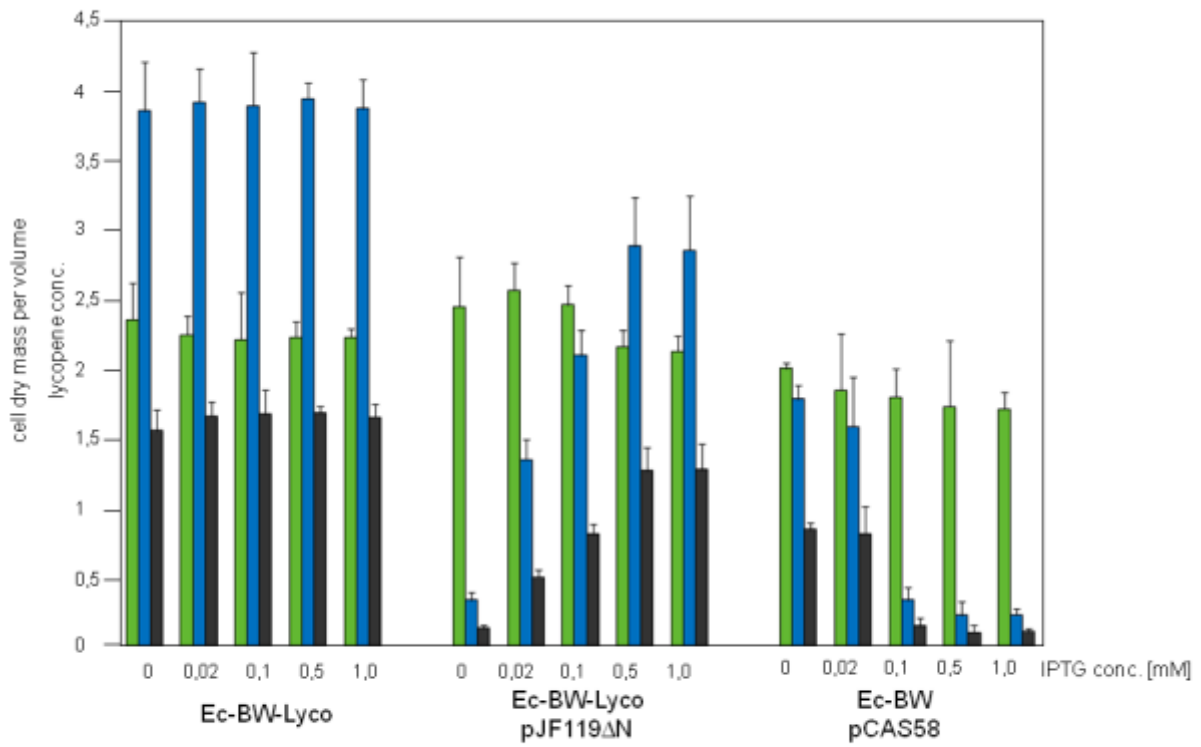
Strains and plasmids	Relevant genotype or sequence	Source
<i>E. coli</i> DH5 $\alpha$	F <sup>-</sup> , $\phi$ 80d, <i>lacZ</i> $\Delta$ M15, <i>endA1</i> , <i>recA1</i> , <i>hsdR17</i> (r <sub>K</sub> <sup>-</sup> m <sub>K</sub> <sup>-</sup> ), <i>supE44</i> , <i>thi-1</i> , <i>gyrA96</i> , <i>relA1</i> , $\Delta$ ( <i>lacZYA-argF</i> )U169	laboratory strain
<i>E. coli</i> BW25113 <i>lac</i> <sup>+</sup>	<i>rrnB</i> <sub>T14</sub> , <i>hsdR514</i> , $\Delta$ <i>araBAD</i> <sub>AH33</sub> , $\Delta$ <i>rhaBAD</i> <sub>LD78</sub>	Albermann et al (2010)
<i>E. coli</i> BW-EI	<i>E. coli</i> BW25113 <i>lac</i> <sup>+</sup> , <i>malEFG::crtE</i> , <i>xylAB::crtI</i>	this study
<i>E. coli</i> BW-BE	<i>E. coli</i> BW25113 <i>lac</i> <sup>+</sup> , <i>malEFG::crtE</i> , <i>fucIK::crtB</i>	Albermann et al (2010)
<i>E. coli</i> BW-BI	<i>E. coli</i> BW25113 <i>lac</i> <sup>+</sup> , <i>xylAB::crtI</i> , <i>fucIK::crtB</i>	this study
<i>E. coli</i> BW-Lyco	<i>E. coli</i> BW25113 <i>lac</i> <sup>+</sup> , <i>malEFG::crtE</i> , <i>fucIK::crtB</i> , <i>xylAB::crtI</i>	this study
pKD46	P <sub>araB</sub> $\gamma$ $\beta$ <i>exo</i> (red recombinase), Amp <sup>R</sup>	Datsenko Wanner (2000)
pCP20	FLP <sup>+</sup> , $\lambda$ cI857 <sup>+</sup> , $\lambda$ p <sub>R</sub> Rep <sup>ts</sup> , Amp <sup>R</sup> , Cm <sup>R</sup>	Cherepanov Wackernagel (1995)
pCAR25	pUC19, <i>P. ananatis crtEXYBZ</i> gene cluster	Misawa et al (1990)
pJOE5559	pJeM1, P <sub>RhaBAD</sub> - <i>dxs-idi-ispA</i> , Km <sup>R</sup>	J. Altenbuchner, Universität Stuttgart (unpublished)
pJF119 $\Delta$ N	cloning vector, RBS, P <sub>tac</sub> , Amp <sup>R</sup>	Albermann et al (2008)
pCAS58	pJF119 $\Delta$ N, <i>P. ananatis crtEBI</i> genes, Amp <sup>R</sup>	this study
pCAS30	pJF119 $\Delta$ N, <i>P. ananatis crtE</i> gene, Amp <sup>R</sup>	Vallon et al (2010)
pCAS30-FRT- <i>cat</i> -FRT	pJF119 $\Delta$ N, <i>P. ananatis crtE</i> gene, FRT-sites, Amp <sup>R</sup> , Cm <sup>R</sup>	Vallon et al (2010)
pJF119- <i>crtB</i>	pJF119 $\Delta$ N, <i>P. ananatis crtB</i> gene, Amp <sup>R</sup>	Albermann et al (2010)
pJF119- <i>crtB</i> -FRT- <i>kan</i> -FRT	pJF119 $\Delta$ N, <i>P. ananatis crtB</i> gene, FRT-sites, Amp <sup>R</sup> , Km <sup>R</sup>	Albermann et al (2010)
pJF119- <i>crtI</i>	pJF119 $\Delta$ N, <i>P. ananatis crtI</i> gene, Amp <sup>R</sup>	Albermann et al (2010)
pJF119- <i>crtI</i> -FRT- <i>cat</i> -FRT	pJF119 $\Delta$ N, <i>P. ananatis crtI</i> gene, FRT-sites, Amp <sup>R</sup> , Cm <sup>R</sup>	Albermann et al (2010)

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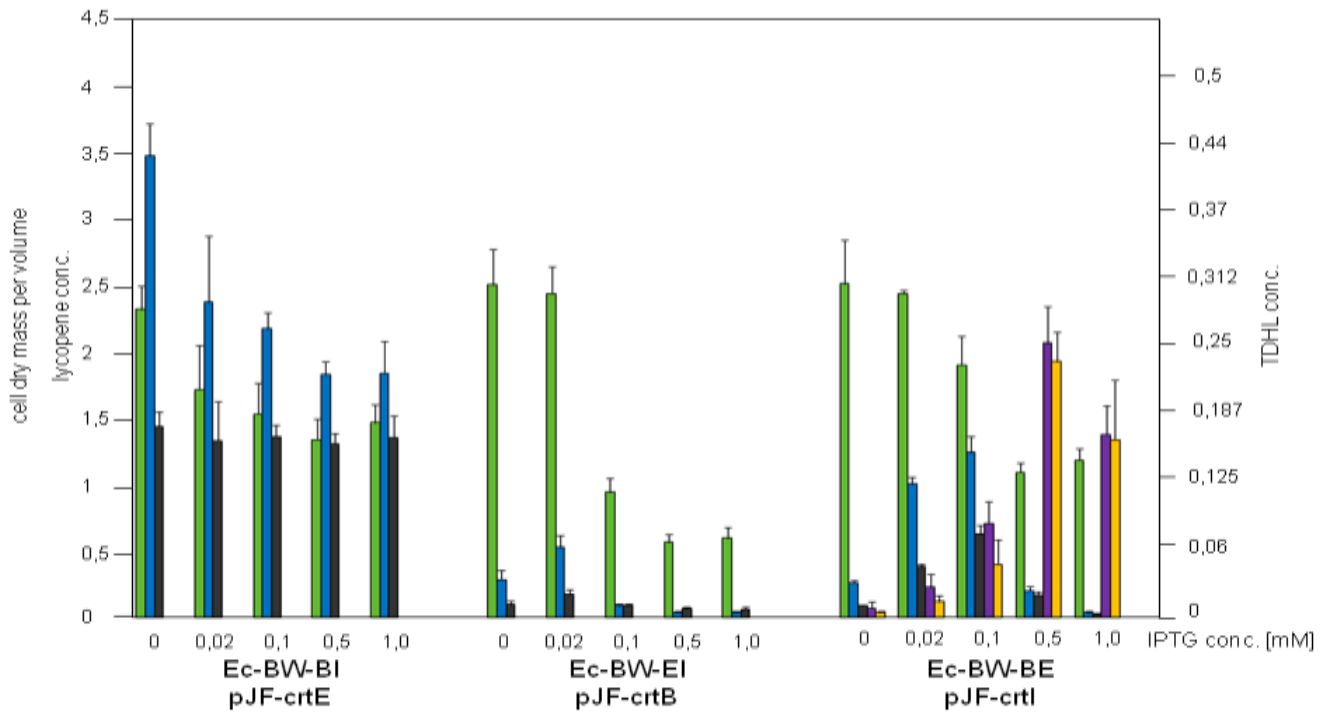
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310 Figure 1



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