Markerless Gene Deletion with Cytosine Deaminase in Thermus thermophilus Strain HB27

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We developed a counterselectable deletion system for Thermus thermophilus HB27 based on cytosine deaminase (encoded by codA) from Thermoaerobacter marianensis DSM 12885 and the sensitivity of T. thermophilus HB27 to the antimetabolite 5-fluorouracil (5-FU). The deletion vector comprises the pUC18 origin of replication, a thermostable kanamycin resistance marker functional in T. thermophilus HB27, and codA under the control of a constitutive putative trehalose promoter from T. thermophilus HB27. The functionality of the system was demonstrated by deletion of the bgLT gene, encoding a β-glicosidase, and three carotenoid biosynthesis genes, CYP175A1, crtY, and crtI, from the genome of T. thermophilus HB27.

Thermus thermophilus HB27 is a Gram-negative, yellow-pigmented, aerobic bacterium growing at temperatures up to 85°C (1). It belongs to the phylum Deinococcus-Thermus and has a GC content of 69%. Due to its specific amenable characteristics, like high growth rates, cell yields, constitutive natural competence, and, not least, the availability of its genome sequence comprising the 1.90-Mb chromosome and a 0.23-Mb megaplasmid (2). T. thermophilus emerged as a laboratory model for studying the molecular basis of thermophila (3, 4). The small genome of T. thermophilus contains few functional paralogues, and consequently, studying knockout mutants is one appropriate approach to elucidate specific gene functions in the organism (3).

A number of genetic tools have been developed for genetic manipulation of T. thermophilus HB27, including various T. thermophilus-Escherichia coli shuttle vectors and different deletion or integration systems (5–9). For plasmid maintenance or identification of desired mutants, only a few engineered thermostable antibiotic resistance markers, namely, kanamycin nucleotidyltransferase (10, 11), bleomycin binding protein (12), and hygromycin B phosphotransferase (13), are available. An alternative positive-selection strategy is complementation of gene defects of auxotrophic host strains by supplying gene functions, such as tryptophan synthetase (trpB) (14) or malate dehydrogenase (mdh) (15), in trans.

The limited number of selection markers and the demand for mutants whose construction does not irreversibly consume these rare markers pushed the development of alternative, counterselectable systems for markerless genome manipulation in T. thermophilus, including negative-selection systems based on pyrE (6, 16), the rpsL1 allele (17), bgLT-lacZ (18), or the pheS allele (5). These systems allow clearance of any positive-selection marker, thus enabling its reuse in the next step of sequential strain construction or maintenance of expression plasmids. The general principle of a counterselection strategy follows two steps: The first step includes the targeted chromosomal integration of a suicide plasmid carrying the desired allele to be exchanged, an antibiotic resistance marker, and a counterselectable marker by homologous recombination. Clones with integrated plasmids are identified by their antibiotic resistance. The second step employs the counterselectable trait, allowing excision of the plasmid, together with the selection markers, via homologous recombination, thereby leaving the allele to be exchanged in the chromosome.

A reliable counterselection strategy is inhibition of thymidylate synthetase by the uracil analog 5-fluourouracil (5-FU). The most commonly used marker of this kind is uracil phosphoribosyltransferase (upp) (UPRTase, Upp) (EC 2.4.2.9), which converts uracil to UMP (see Fig. S1 in the supplemental material). Transformation of the uracil analog 5-FU to 5-fluorouridine monophosphate (5-FUMP) by Upp and further conversion of 5-FUMP results in irreversible inhibition of thymidylate synthetase (19, 20). An alternative counterselectable marker is cytosine deaminase (codA) (EC 3.5.4.1), which has been applied for various bacteria (21–23). CodA (EC 3.5.4.1) catalyzes the deamination of cytosine and its analog, 5-fluorocytosine (5-FC), to uracil and 5-FU, respectively, which are subsequently converted to UMP and 5-FUMP by Upp (24). A deletion system developed for T. thermophilus employs pyrE, encoding orotic acid phosphoribosyltransferase (EC 2.4.2.10), as a negative-selection marker (6). In UMP de novo synthesis, PyrE catalyzes the synthesis of orotidine monophosphate (OMP) from orotic acid, which is then converted to UMP by PyrE. Conversion of 5-fluoroorotic acid (5-FO) finally also results in synthesis of 5-FUMP (see Fig. S1 in the supplemental material).

In T. thermophilus HB27, a pyrE gene (TT_C1380) and an upp gene (TT_C0946), but no codA gene or orthologs, have been identified. In contrast to pyrE and upp, application of heterologous codA as a counterselectable marker for T. thermophilus HB27 has the great advantage that the wild type can be used directly because prior construction of a codA deletion strain is not required. We constructed a new markerless deletion system for T. thermophilus HB27 using codA (Tmar_1477) from Thermaerobacter marianensis DSM 12885. T. marianensis DSM 12885 has a GC content of...
was principally derived from two methods described previously (4, 33, 34), by GATC Biotech (Constance, Germany). Eurofins MWG (Ebersberg, Germany). DNA sequencing was performed using the protocol “pretreatment for Gram-positive bacteria.” Oligonucleotides were obtained with the DNeasy blood and tissue kit (Qiagen, Hilden, Germany) from the putative trehalose operon (TT_C0611 to TT_C0615) (2) and used for the construction of the deletion strain. The plasmids, bacterial strains, and growth conditions. MATERIALS AND METHODS Plasmids, bacterial strains, and growth conditions. The bacterial strains and plasmids used in this study are listed in Table S1 in the supplemental material. E. coli DH5α was grown at 37°C with shaking in LB medium (10 g liter⁻¹ tryptone, 5 g liter⁻¹ yeast extract, 5 g liter⁻¹ NaCl) supplemented with 50 μg ml⁻¹ kanamycin (Km), as appropriate. T. thermophilus strains were cultured in Thermus broth (TB) medium (8 g liter⁻¹ tryptone, 4 g liter⁻¹ yeast extract, 3 g liter⁻¹ NaCl, pH 7.5) (29) at 70°C with shaking. For selection, the medium was supplemented with 25 μg ml⁻¹ kanamycin, as appropriate. T. thermophilus strains were also grown in minimal medium 162 (M162) (30) with a minor modification [100 μg liter⁻¹ nitrilotriacetic acid, 0.4 mg liter⁻¹ Bacto nutrient broth, 0.005 mM iron(III) citrate, 40 mg liter⁻¹ CaSO₄·2H₂O, 0.2 g liter⁻¹ MgCl₂·6H₂O, 0.1 g liter⁻¹ (NH₄)₂SO₄, 15 mM Na₂HPO₄, 5 mM KH₂PO₄, pH 7.5], supplemented with 0.1 liter⁻¹ broth, 1 mg liter⁻¹ thiamine, and 2.0 g liter⁻¹ d-glucose. Negative selection was carried out on M162 agar plates containing 1.5% (wt/vol) agar with 15 μg ml⁻¹ 5-FC (Sigma-Aldrich, Steinheim, Germany).

Transformation procedures. The electroporation method (31) was used to transform competent E. coli DH5α (32) with plasmids. The transformation method used for naturally competent T. thermophilus HB27 was principally derived from two methods described previously (4, 33, 34). The cells were first grown in TB medium at 70°C overnight. The culture was diluted 1:100 with fresh TB medium and incubated at 70°C until the optical density at 550 nm (OD₅₅₀) reached a value of 0.8. Next, 500 μl of cell culture was mixed with 50 to 100 ng of plasmid DNA and incubated further at 70°C. After 2 h of incubation, 150 μl of the cultures was plated on TB agar medium supplemented with 25 μg ml⁻¹ kanamycin. The plates were incubated at 65°C overnight.

Plasmid construction. Standard protocols were used for recombinant DNA techniques (31). Plasmid DNA was prepared with the innuPrep Plasmid minikit (Analytik Jena, Jena, Germany). To isolate genomic DNA of T. thermophilus strains, 4 × 10⁷ cells were harvested from an overnight culture by centrifugation (5 min; 4,500 × g) and resuspended in 180 μl lysis buffer (25 mM Tris-HCl, pH 8.0, 25 mM EDTA, 10% [wt/vol] sucrose, 0.5 mM glycine, 20 mg ml⁻¹ lysozyme). Genomic DNA was extracted with the DNeasy blood and tissue kit (Qiagen, Hilden, Germany) using the protocol “pretreatment for Gram-positive bacteria.” Oligonucleotides (see Table S2 in the supplemental material) were synthesized by Eurofins MWG (Ebersberg, Germany). DNA sequencing was performed by GATC Biotech (Constance, Germany).

The basic integrative vector pLEI269.1 (Fig. 1) is based on pUC18 (35) and the Thermus vector pMY1 (33, 36). It was constructed by ligation of several PCR fragments carrying a different restriction site on each end. The pUC18 origin of replication was amplified with primers s9088 and s9436 from pJOE5751.1 (37) as the template and positioned downstream of codA using HindIII and NheI restriction sites. For plasmid maintenance in both E. coli and T. thermophilus HB27, pLEI269.1 carries a thermostable kanamycin resistance marker (38) under the control of the S-layer protein A promoter (PslpA) (39). PslpA, together with the thermostable kanamycin resistance (Km') gene, was amplified with s9289 and s9290 (NheI and PstI) from pMY1. The Ndel restriction site between PslpA and the Km’ gene was deleted by PCR using primers s9289 and s9400 (NheI and Asel).

For the construction of the different plasmids carrying deletion cassettes, flanking sequences (each about 800 bp) of the genes to be deleted were amplified by PCR and integrated into pLEI269.1 using its MCS. The up- and downstream regions of the carotenoid biosynthesis genes CYP175A1, crtY, and ctt were amplified with the primers listed in Table S2 in the supplemental material and genomic DNA of T. thermophilus HB27 as the template. The PCR fragments were fused with their PsI (CYP175A1 and cttY) and Sall (cttY) restriction sites and inserted into pLEI269.1 via Munl and SplI restriction sites, thereby creating pLEI270.5, pLEI271.7 (Fig. 2), and pLEI273.2, respectively. The flanking regions of bgLT were amplified with primers s9978, s10236, s9980, and s9981, fused by their PsI sites, and integrated into the basic vector by Munl and KpnI restriction sites to create pLEI257.1. All the plasmids were verified by DNA sequencing.

Deletion strain construction. T. thermophilus HB27 was transformed with the plasmids carrying the constructed deletion cassettes as described above. Due to their inability to autonomously replicate in T. thermophilus HB27, the plasmids can be maintained only by integration into the megaplasmid pPT27 via homologous recombination (Fig. 2). Cells with integrated plasmids were selected on TB agar with kanamycin at 65°C over-
night. Several clones were isolated and streaked out three times on TB agar plates supplemented with kanamycin. From the last plate, kanamycin-resistant clones were checked for 5-FC sensitivity on M162 agar plates at 65°C for 72 h. The presence of \( \textit{codA} \) causes sensitivity to 5-FC. Next, a kanamycin-resistant and 5-FC-sensitive clone was incubated in TB medium without antibiotics at 70°C overnight. The overnight culture was diluted 1:10\(^4\) with TB medium containing 15 \( \mu \text{g} \) 5-FC and incubated further at 70°C for 6 h. Meanwhile, the integrated plasmid should be excised via a second homologous recombination causing either deletion of the desired marker or reconstitution of the wild type (Fig. 2). In both cases, loss of the plasmid causes resistance to 5-FC and sensitivity to kanamycin. The cell loses the integrated plasmid by a second homologous recombination. Depending on where the recombination occurs, \( \textit{crtY} \) is cut out, together with the plasmid, or the wild-type situation is restored. The \( \Delta \textit{crtY} \) strain, as well as the reconstituted wild type, is resistant to 5-FC and sensitive to Km.

**Identification of deletion mutants by PCR.** The deletion mutants grown on TB plates were scraped off and treated with the DNeasy blood and tissue kit as described above. The prepared genomic DNAs were applied as templates for PCRs with primers s10068/s10069, s10363/s10364, s10365/s10366, and s10369/s10370 (see Table S2 in the supplemental material) for verification of \( \textit{bglT} \), \( \textit{CYP175A1} \), \( \textit{crtY} \), and \( \textit{crtI} \) deletion, respectively. The primers were designed with AT-rich sequences of the flanking regions in order to minimize nonspecific binding with the GC-rich genomic DNA of \( \textit{T. thermophilus} \) HB27. Genomic DNA of the \( \textit{T. thermophilus} \) HB27 wild type and the plasmid DNAs used for deletion strain construction served as controls.

**\( \beta \)-Glycosidase assay.** The \( \textit{bglT} \) gene from \( \textit{T. thermophilus} \) HB27 encodes a \( \beta \)-glycosidase with a broad substrate specificity for the \( \beta \)-anomeric linkage and catalyzes the hydrolysis of \( \beta \)-galacto-, \( \beta \)-gluco-, and \( \beta \)-fucopyranosides (40). We used \( p \)-nitrophenyl \( \beta \)-galactopyranoside (pNPGal) as the substrate to measure its enzyme activity. Cell cultures grown in TB medium at 70°C for 6 h were used for the assay. The cells were harvested by centrifugation (5 min; 4,500 \( \times \) g), washed with 0.1 M potassium phosphate buffer (pH 6.5), and then resuspended in the same buffer. An ultrasonic homogenizer (Sonopuls HD2070; Bandelin, Berlin, Germany) was applied for cell disruption. The supernatant containing soluble proteins was assayed after centrifugation (15 min; 16,100 \( \times \) g). The reaction mixture, containing 25 \( \mu \text{l} \) of the supernatant and 450 \( \mu \text{g} \) of 0.1 M potassium phosphate buffer (pH 6.5), was...
preincubated at 70°C for 5 min. Then, 25 μl pNPGal (4 mg ml⁻¹; Sigma-Aldrich, Steinheim, Germany) was added to the mixture. After 2 to 5 min incubation at 70°C, the reaction was stopped by adding 1 ml 400 mM sodium borate buffer (pH 9.4). The release of p-nitrophenol (pNP) was measured at 405 nm. One unit of enzyme activity was defined as the amount of enzyme catalyzing the liberation of 1 μmol of pNP per minute, using an extinction coefficient (ε₄₀₅/pH 10) of 18.5 × 10³ M⁻¹ cm⁻¹ for pNP. The amount of total soluble protein was determined by the Bradford method (41). Specific activity was expressed as units per milligram of protein.

**TLC of carotenoid extracts from T. thermophilus strains.** T. thermophilus HB27 wild type and mutants were cultivated overnight in TB medium at 70°C. The next day, 2.6 × 10¹⁰ cells were harvested by centrifugation (5 min; 4,500 × g), washed with 1 ml H₂O₂, and stored at -20°C until use. For carotenoid extraction, the cells were resuspended in 1 ml potassium phosphate buffer (0.1 M; pH 6.5) with 10 mg ml⁻¹ lysozyme and incubated at 37°C for 1 h. The suspensions were centrifuged (5 min; 16,100 × g), and the cells were washed with 1 ml H₂O and extracted with 600 μl acetone. The acetone extracts were mixed with 200 μl H₂O and 200 μl hexane for reextraction. The samples were mixed and centrifuged (5 min; 16,100 × g), and the upper hexane phase was isolated. Reextraction with 200-μl portions of hexane was repeated until the hexane phase remained colorless. The hexane extracts were pooled and dried in a vacuum centrifuge. The extracts were dissolved in 20 μl hexane and applied for thin-layer chromatography (TLC) (silicagel 60 F₂₅₄; Merck, Darmstadt, Germany) with petroleum-diethyl ether-acetone (40:10:10) as the mobile phase. β-Carotene (Sigma-Aldrich, Steinheim, Germany) and lycopene (extracted from tomato puree) were used as authentic standards.

**RESULTS**

**The integrative deletion vector.** The basis of the presented codA counterselection system for T. thermophilus HB27 is an integrative plasmid derived from pUC18 and the Thermus vector pMY1 (33). Only a few constitutive and even fewer regulated promoters are available for gene expression in T. thermophilus HB27. We have observed that the promoter region of the putative trehalose operon (P₆₆₁) shows a constitutive promoter activity together with bglT as a reporter gene in T. thermophilus HB27 (data not shown). Thus, P₆₆₁ was applied for codA expression on the integrative deletion vector. Expression of codA from P₆₆₁ in T. thermophilus HB27 was verified on M162 agar supplemented with 5-FC. The presence of the genomically integrated vector caused sensitivity to 5-FC when a concentration of 15 μg ml⁻¹ was applied.

**Deletion of selected genes from the T. thermophilus HB27 genome.** In order to demonstrate the functionality of the new codA counterselection system, we chose bglT as the first target for deletion. BglT is a widely used reporter protein for studying promoter activities in T. thermophilus HB27 (16, 42). The additional copy of bglT on the megaplasmid pTT27 of T. thermophilus HB27 interferes with these reporter assays. Therefore, a bglT-deficient strain is a desirable host for promoter studies using bglT as a reporter gene. We deleted bglT with pLEI257.1 carrying the homologous up- and downstream regions of bglT following the procedure described in Materials and Methods. In order to control the substeps of the deletion procedure, analytical PCRs were performed using primers s10068/s10069 and genomic DNA of T. thermophilus HB27 (wild type), T. thermophilus LW14 (with pLEI257.1 integrated into the genome after the first recombination), T. thermophilus LW19 (ΔbglT), or pLEI257.1 (deletion plasmid) as the template (Fig. 3). The primers used bind within the up- and downstream regions of bglT. The wild type should show a PCR fragment (1,723 bp) that contains bglT between these flanking sequences. The PCR fragment of the bglT deletion strain should have a size reduced by 1,308 bp, based on the size of bglT. The strain with the integrated plasmid should show both PCR fragments and an additional fragment corresponding to the whole plasmid (Fig. 2).

As expected, the PCR with T. thermophilus HB27 wild-type DNA resulted in a fragment of 1,723 bp, and the PCR with pLEI257.1 yielded a fragment of 415 bp (Fig. 3). The PCR with T. thermophilus LW14 DNA showed both fragments. The large fragment (6,943 bp) corresponding to the whole integrated plasmid could not be detected under the PCR conditions used. PCR with DNA of the deletion strain T. thermophilus LW19 exhibited only the small fragment of 415 bp, suggesting the deficiency of bglT.

The β-glycosidase assay with crude extracts of T. thermophilus HB27 and LW19 with pNPGal as the substrate revealed reduced β-glycosidase activity of the ΔbglT strain (3 mU mg⁻¹ of total soluble proteins) compared to the wild type (8 mU mg⁻¹). This result is in agreement with the data published previously (42) and further substantiates the loss of bglT.

T. thermophilus HB27 produces carotenoids (43), especially thermosteazanxin (44, 45). The carotenoid biosynthesis genes are located on the megaplasmid pTT27. Deletion of genes of the carotenoid-biosynthetic pathway is a very colorful way to demonstrate the applicability of the codA counterselection system, because removal of a gene involved in the multistep process of carotenoid biosynthesis results in change of color of the mutant strain.

Similar to the bglT deletion, the three genes CYP175A1, crtY, and crtf participating in the carotenoid synthesis pathway were
deleted with the three plasmids pLEI270.5, pLEI271.7, and pLEI273.2 carrying the appropriate deletion cassettes. The ΔCYP175A1, ΔcrtY, and ΔcrtI strains are referred to as T. thermophilus LW35, LW37, and LW40, respectively. For carotenoid analyses, the strains were incubated in TB medium at 70°C for 24 h. Cells were collected by centrifugation and suspended in potassium phosphate buffer. All of the cell suspensions had the same cell concentration (see Fig. S3 in the supplemental material). T. thermophilus HB27 showed the typical yellow color, whereas T. thermophilus LW35 (ΔCYP175A1) lacking β-carotene hydroxylase presented an orange color due to the enrichment of β-carotene in the cell membrane. The deletion of crtY, coding for lycopene β-cyclase, led to an accumulation of red lycopene (T. thermophilus LW37), which could be observed as a light-rose color of the cell suspension. The phytoene desaturase Crtl converts colorless phytoene to lycopene. Therefore, the cells of T. thermophilus LW40 (ΔcrtI) appeared white. The development of cell colors caused by the gene deletions corresponds to those of the accumulated intermediates and is summarized in Fig. S2 in the supplemental material. The carotenoids of T. thermophilus HB27, LW35, and LW37 were extracted as described in Materials and Methods and analyzed by thin-layer chromatography, together with authentic standards of β-carotene and lycopene (see Fig. S4 in the supplemental material). T. thermophilus LW37 (ΔcrtY) accumulated lycopene, as expected. β-Carotene and smaller amounts of lycopene could be detected in the extract of T. thermophilus LW35 (ΔCYP175A1). The obtained Rf values of the samples were 0 (T. thermophilus HB27), 0.97 (β-carotene, authentic standard), 0.97 (T. thermophilus LW35 ΔCYP175A1), 0.91 (lycopene, authentic standard), and 0.91 (T. thermophilus LW37 ΔcrtY).

DISCUSSION

Commonly used reporter genes of expression vectors for T. thermophilus encode thermostable α-galactosidase and β-galactosidase (42, 46, 47), β-glycosidase (bglT) (16), or phytoene synthase (crtB) (9, 48). All the named reporter genes originate from T. thermophilus HB27 or a closely related species whose native enzyme activities interfere with reporter assays. Hence, mutant strains deficient in these interfering enzymes would facilitate promoter studies employing glucosidases or galactosidases as reporter enzymes. In addition, clarifying specific gene functions of T. thermophilus is another issue of great interest at this time, as it is an indispensable part of uncovering the molecular mechanisms underlying thermophila.

Deletion systems allowing markerless genome manipulation provide powerful tools for genetic manipulation, enabling the construction of mutant strains for biotechnology or the study of specific gene functions with knockout mutants. Compared to other reported deletion methods for T. thermophilus, the codA-based deletion system described here has some advantages. (i) As a markerless and negative deletion system, the antibiotic resistance gene used for selection is excised from the genome. This allows the generation of multiple gene deletions/mutations in the parental strain to produce a final strain unmarked by an antibiotic resistance gene. (ii) The previously reported deletion systems based on pyrE (6), bgl (18), or upp need a pyrE-, bgl-, or upp-deficient host strain. In contrast, no codA ortholog exists in T. thermophilus HB27, and the wild type can be used directly as a parental strain in the reported deletion system. An additional disadvantage of the pyrE deletion system is the uracil auxotrophic feature of the pyrE-deficient host strain. (iii) Furthermore, the codA sequence used in the integrative plasmid should not cause unexpected homologous recombination with the genome sequence. However, pheS (5), rpsL1 (17), or bgl-lacZ (18), as well as the two promoters Pgal and Pnara, that are also part of the integrative vector, originate from T. thermophilus and could potentially recombine with the native genomic genes, leading to false-positive clones after the first recombination step. Using pheS as a counter-selectable marker, a spontaneous large-scale deletion, including the carotenoid synthesis genes and the β-glycosidase gene in the megaplasmid, was observed, apparently mediated by insertion sequence (IS) elements (5). However, unexpected recombination or large-scale deletions have not been observed so far with the codA deletion system.

The ΔbglT strain T. thermophilus LW19 shows considerable β-galactosidase activity despite the lack of bglT. This is in agreement with the ΔbglT strain of T. thermophilus HB27 (T. thermophi- losis PKKU) constructed by Park and Kilbane (42), with a measured reduction of 50% of the β-galactosidase level compared to the wild type. Furthermore, inactivation of the identical bglT gene in the T. thermophilus strain TH125 led to 55% reduction of the pNP-β-galactoside-hydrolyzing activity (49). This background activity was probably due to the existence of two putative β-galactosidases genes (TT_P0220 and TT_P0222) (2). Despite the reduced β-galactosidase activity of T. thermophilus LW19, the deletion strain should allow the use of the β-galactosidase gene as a reporter gene. The purified BglT protein exhibits the highest catalytic efficiency for the substrate β-NP-glucoyranoside (40). Additionally, we used this substrate to measure the BglT activity and observed loss of activity in the deletion strain T. thermophilus LW19, but still with some background level, which is in agreement with the data published by Ohta et al. (16).

Until now, the genes crtY (TT_P0060) and crtI (TT_P0066) of T. thermophilus have been identified exclusively by genome sequence analysis. TLC analysis of carotenoid extracts revealed that lycopene is the main carotenoid produced by the ΔcrtY strain. This result supports the sequence annotation of TT_P0060 as crtY (encoding lycopene β-cyclase). The colorless ΔcrtI strain strongly suggests an interruption of the carotenoid synthesis pathway at a step before lycopene synthesis. In accordance with the sequence analysis, it is likely caused by a deficiency of phytoene desaturase. In this respect, crtI codes for phytoene desaturase of T. thermophilus HB27. The accumulation of β-carotene in the T. thermophilus ΔCYP175A1 (TT_P0059) strain reflects the results obtained with enzyme assays with the Thermus β-carotene hydroxylase described previously (26). Interestingly, the carotenoid extract of the ΔCYP175A1 strain revealed considerable amounts of lycopene, in addition to β-carotene, in TLC (see Fig. S4 in the supplemental material). This is most likely the result of an incomplete conversion of lycopene by Crty.

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