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Expression of *Erwinia uredovora* Phytoene Desaturase in *Synechococcus* PCC7942 Leading to Resistance against a Bleaching Herbicide[1]

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The gene coding for phytoene desaturase of the bacterium *Erwinia uredovora* (*crtI*) was inserted into the chromosome of the cyanobacterium *Synechococcus* PCC7942 strain R2-PIM8. For expression of *crtI* in the heterologous host, two constructs with different promoters were introduced into *Synechococcus*. In the first, *crtI* was fused to the 5′ region of the *psbA* gene of the xanthophycean microalga *Bumilleriopsis filiformis*. The second construct carried *crtI* inserted downstream of the neomycin phosphotransferase II gene (*nptII*) from the transposon Tn*5*. Expression of *crtI* under the control of the respective promoter was shown by immunodetection of the gene product. The functionality of the heterologously expressed phytoene desaturase CRTI in the transformants was demonstrated by enzymic assays. The transformants acquired very strong resistance toward the bleaching herbicide norflurazon.

Carotenoids are synthesized by all photosynthetic organisms as well as by several nonphotosynthetic bacteria and fungi. In phototrophs these pigments serve a dual function: protection against photosensitized destruction of the light-harvesting Chl's and light harvesting in the blue-green spectral region (Krinsky, 1971; Britton, 1988).

Carotenoid biosynthesis in cyanobacteria is essentially similar to that of higher plants and algae. The desaturation of phytoene, catalyzed by phytoene desaturase, is assumed to be the rate-limiting reaction in carotenogenesis (Bramley, 1985; Linden et al., 1990). Based on amino acid sequence homology, two different groups of phytoene desaturases have been identified (Chamovitz et al., 1991). The known phytoene desaturases differ in the number of desaturation steps they catalyze and in sensitivity toward bleaching herbicides (Linden et al., 1991). In plants, algae, and cyanobacteria, phytoene desaturase has been found to be a target of various commercial bleaching herbicides such as norflurazon, fluridone, and flurtamone (Sandmann and Böger, 1989). The inhibition of phytoene desaturation by these herbicides results in an accumulation of phytoene and concurrent bleaching of the organism. For norflurazon it was shown that the herbicide binds to the phytoene desaturase and inhibits its catalytic activity in a noncompetitive manner (Sandmann et al., 1989; Sandmann, 1993). The phytoene desaturase of the bacterium *Erwinia uredovora*, on the other hand, differs in amino acid composition and the end product of the desaturation sequence, and was shown to be insensitive to norflurazon (Sandmann and Fraser, 1993).

Due to its similar physiology and its oxigenic photosynthesis, the cyanobacterium *Synechococcus* PCC7942 can be used as a functional model for higher plant chloroplasts. Because of its prokaryotic nature and its inherent competence for the uptake of DNA, this bacterium is easily amenable to genetic manipulation (Porter, 1985). Accordingly, *Synechococcus* provides an excellent system for cloning genes that confer resistance toward a bleaching herbicide and for analyzing the effect of their expression in relation to oxigenic photosynthesis.

A large cluster of genes coding for carotenogenic enzymes was previously identified in *E. uredovora* (Misawa et al., 1990). In the present study we introduced the gene coding for phytoene desaturase (*crtI*), which belongs to this cluster, into *Synechococcus* PCC7942 strain R2-PIM8 (Van der Plas et al., 1990). The effect of *crtI* expression was analyzed by investigating the influence of norflurazon on the carotenoid content in the respective transformants.

MATERIALS AND METHODS

Microorganisms and Growth Conditions

Cultures of *Synechococcus* PCC7942, strain R2-PIM8 (Van der Plas et al., 1990), a derivative of the small-plasmid-cured strain R2-SPc (Kuhlemeier et al., 1983), were maintained on BG11 medium (Rippka et al., 1979) buffered with 5 mM Tes (pH 8.0). Liquid batch cultures were grown either in Erlenmeyer flasks with constant shaking or in culture tubes in a thermostat (Edward-Kniese, Marburg, Germany). The cultures were illuminated with continuous fluorescent white light (60 μE m^{-2} s^{-1}) and supplied with an air-CO_2 mixture (98:2 [v/v], 60 mL min^{-1}) at 30°C. Taking into account the Met auxotrophy of strain R2-PIM8, the medium was supple-

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Abbreviations: CRTI, phytoene desaturase of *Erwinia uredovora*; *crtI*, gene coding for phytoene desaturase of *E. uredovora*; I_{50}, molar concentration for 50% inhibition; *nptII*, gene coding for neomycin phosphotransferase II, which confers kanamycin resistance; pcv, packed cell volume; *pds*, gene coding for phytoene desaturase of *Synechococcus* PCC7942; *psbA*, gene coding for the D1 protein of PSII.

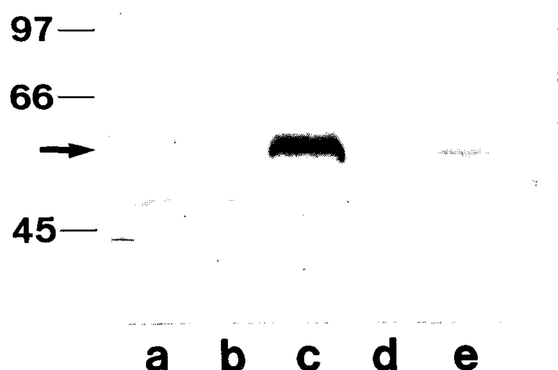

Figure 4. Immunodetection of the phytoene desaturase of *E. uredovora* in *Synechococcus* with an antibody raised against CRTI. Cell extracts of the following strains were separated by 10% SDS-PAGE: (lane a) *Synechococcus* R2-PIM8, (lane b) *Synechococcus* R2-PIM8-BGO, (lane c) *Synechococcus* R2-PIM8-BG1, (lane d) *Synechococcus* R2-PIM8-BGOP, and (lane e) *Synechococcus* R2-PIM8-BG2. The arrow points to the 54-kD bands, which correspond to the calculated size of CRTI. The faint signals at 48 kD are due to a nonspecific immunoreaction. Numbers refer to apparent molecular mass (in kD).

For strains R2-PIM8-BG1 and R2-PIM8-BG2 the conversion of phytoene to β-carotene was higher compared with the control, as indicated by the conversion rate. The contribution of CRTI activity to the overall activity of the endogenous and newly introduced phytoene desaturase can be estimated by comparing the activity with and without norflurazon. The conversion rate for phytoene desaturation in R2-PIM8-BG1 was decreased by norflurazon from 36 to 12%, indicating that about one-third of the activity can be attributed to the foreign enzyme CRTI. In case of R2-PIM8-BG2, the residual rate with norflurazon was 2.6%, which accounted for about one-tenth of contribution of CRTI to total activity.

Analyses of Herbicide Resistance of Transformants

The content of colored carotenoids was determined in the *Synechococcus* PCC7942 control transformant R2-PIM8-BG0 as well as in the transformants R2-PIM8-BG1 and R2-PIM8-BG2 after growth with various concentrations of norflurazon (Table II). Cultures grown without herbicide were taken as references. Comparison of the carotenoid content of untreated cultures of the three transformants showed that the overall content of colored carotenoids was not influenced by the heterologously expressed phytoene desaturase. The analyzed strains produced about 1.2 mg of colored carotenoids per mL of pcv, which is consistent with the values given in the literature for *Synechococcus* PCC7942 wild type (Linden et al., 1990).

The phenotypic pigmentation of the *Synechococcus* cultures exhibited no visible differences between R2-PIM8-BG1 cells grown with or without norflurazon up to a concentration of 10^{-6} M, and only a slight bleaching of the culture was observed at a concentration of 10^{-5} M. The control strain R2-PIM8-BG0, on the other hand, was already slightly bleached at a concentration of 10^{-7} M and totally bleached at a concentration of 3×10^{-7} M. In the latter transformant, growth with about 3×10^{-7} M norflurazon resulted in a decrease of two-thirds of the colored carotenoids (Table II). In contrast, the same concentration applied to R2-PIM8-BG1 had no bleaching effect, and an increase of norflurazon to 10^{-5} M inhibited the formation of colored carotenoids by only 29%. About the same degree of inhibition was caused in transformant R2-PIM8-BG2 with 10^{-6} M norflurazon, whereas this transformant still contained more colored carotenoids at a concentration of 10^{-7} M norflurazon than the control strain R2-PIM8-BG0.

DISCUSSION

In this investigation *crtI* (Misawa et al., 1990) was introduced successfully and expressed under the control of two different promoters in the cyanobacterium *Synechococcus* PCC7942. The immunoreactive bands in Figure 4 demonstrated that the levels of the heterologously synthesized phytoene desaturase CRTI in the respective transformants were different. In strain R2-PIM8-BG2 only a small amount of CRTI was produced under the control of the *psbA* gene promoter compared with the high production in strain R2-PIM8-BG1. In the latter the expected co-transcription of *npt*II and *crtI*, which depended on *npt*II promoter activity, was obviously very effective. This difference points to a differential efficiency of the promoter used to provide expression and demonstrates the usefulness of the *npt*II gene promoter, which is active in many gram-negative bacteria, to express foreign genes in *Synechococcus.* Gene constructs in which *crtI* was inserted in the opposite direction to the respective promoter did not result in any detectable formation of CRTI (data not shown), indicating that there are no sequences upstream of *crtI* that are recognized as a promoter in *Synechococcus.*

Table I. *In vitro phytoene desaturase activity of Synechococcus transformants*

[^{14}C]Phytoene was generated in vitro from [^{14}C]mevalonate using extracts from *Phycomyces* mutant C5. Incubation time was 2 h. One micromolar norflurazon (NFZ) was added as indicated to inhibit the endogenous enzyme.

Strain and Additions	Radioactivity		Conversion Rate[a]
	Phytoene	β-Carotene	
	dpm		%
R2-PIM8-BG0	16,738	4602	21.6
R2-PIM8-BG0 + NFZ	19,320	112	0.6
R2-PIM8-BG1	13,497	7477	35.7
R2-PIM8-BG1 + NFZ	17,637	2360	11.8
R2-PIM8-BG2	14,957	4775	24.2
R2-PIM8-BG2 + NFZ	18,424	486	2.6

[a] Conversion rate, 100 × (dpm in β-carotene/[dpm in phytoene + dpm in β-carotene]).

suspended in 200 μL of BG11 medium (G. Schmetterer, personal communication). The cells were plated on a BG11-agar plate supplemented with Met and streptomycin. After about 3 d of incubation the light-green cyanobacterial lawn was washed away with BG11 medium supplemented with Met. To 100 μL of the obtained cell suspension, plasmid DNA was added to a final concentration of about 100 ng mL^{-1}. This mixture was incubated for 4 h in the light before plating on BG11 medium supplemented with ampicillin and kanamycin. Antibiotic resistance of the transformant colonies obtained after 5 to 7 d was checked again by transferring them to fresh, selective medium.

DNA/DNA hybridization was performed according to Southern (1975) with a digoxigenin-labeled probe following the instructions accompanying the Digoxigenin Nonradioactive DNA Labeling and Detection Kit from Boehringer (Mannheim, Germany).

SDS-PAGE and Immunodetection of CRTI

Extracts of *Synechococcus* cells were produced following a modified procedure of Omata and Murata (1983). Cyanobacterial cells of 20-mL aliquots were harvested from a liquid culture (OD_{730} = 3) by centrifugation (10 min, 4000*g* at room temperature). After resuspension in 2 mL of lysis buffer (2 mg mL^{-1} lysozyme in 10 mM Tes/NaOH, pH 7.0, 600 mM Suc, and 2 mM EDTA) and incubation for 2 h at 30°C, the cells were broken by passing them through a French press at 140 MPa. Unbroken cells were removed by centrifugation at 4000*g* and room temperature.

Aliquots of the resulting supernatant were subjected to SDS-PAGE on a 10% gel according to Laemmli (1970). Separated proteins were electrophoretically transferred to nitrocellulose (Towbin et al., 1979). An immunoreaction was carried out with antiserum raised against purified phytoene desaturase of *E. uredovora* heterologously expressed in *E. coli* (Fraser et al., 1992). Antigenic polypeptides were visualized by incubation with anti-rabbit IgG antibody conjugated to a horseradish peroxidase, followed by enzymic color development through incubation with peroxide and chloronaphthol. Lanes containing molecular mass markers were cut from the filters and stained with amido black.

Carotenoid Analysis

Cyanobacterial cultures were grown until an OD_{730} of about 2 was attained. Cells of 20-mL aliquots were harvested by centrifugation. Carotenoids were extracted from the pelleted cells with hot methanol (20 min, 65°C) containing 6% (w/v) KOH and partitioned against 10% (v/v) diethylether in petrol (b.p. 35–80°C). The content of colored carotenoids was determined by measuring the absorption of the diethylether/petrol phase at 445 nm. An overall specific extinction coefficient ($E^{1\%}_{1cm}$ (i.e. extinction of 1 g of carotenoid in 100 mL of solution in a 1-cm light-path spectrophotometer cuvette) of 2500 was used for calculation (Davies, 1976). The content was referred to the pcv of the culture.

In Vitro Phytoene Desaturase Assay

Activity of phytoene desaturase was determined in *Synechococcus* transformants. Membranes active in phytoene desaturation were obtained by French press treatment (50 MPa). [^{14}C]Phytoene was generated in vitro from [^{14}C]mevalonate using extracts from the *Phycomyces* mutant C5. Incubation time was 2 h. Details of this procedure including the assay conditions have been published (Linden et al., 1990; Sandmann, 1993).

RESULTS

Construction of *Synechococcus* Strains Carrying the *crtI* Gene

Synechococcus strain R2-PIM8 (Van der Plas et al., 1990) was used as recipient for the various constructs carrying the *crtI* gene. This strain is a descendent of strain R2-SPc (Kuhlemeier et al., 1983), which lacks the endogenous plasmid pUH24 of the wild type. R2-PIM8 carries a so-called "integration platform" in its chromosome, which consists of a streptomycin resistance gene (*aad*) flanked by the origin of replication of plasmid pBR322 and a promoterless ampicillin resistance gene (*bla*). The location of the platform is the *metF* gene, which is involved in Met biosynthesis in this organism. Provided that the medium is supplemented with Met, *metF* can be inactivated without affecting the viability of the strain. Due to the effective recombination system of *Synechococcus* PCC7942 (Golden et al., 1987), any fragment cloned into a pBR-derived vector can integrate into this platform as the result of a "double cross-over" event. Homologous recombination between the transformed DNA molecule and the integration platform both in the *bla* and the *ori* sequences is expected to result in restoration of a functional *bla* gene and in concomitant replacement of the *aad* gene of the PIM8 platform by the cloned insert of the donor plasmid.

The source of the *crtI* gene was plasmid pCRT-I (Fraser et al., 1992), a subclone of plasmid pCAR25, which carries the whole *crt* gene cluster of *E. uredovora* (Misawa et al., 1990). We intended to compare the expression of *crtI* under the control of two different promoters. In plasmid pBG1 the *npt*II gene of transposon Tn5, including the promoter residing on the left insertion element IS50L (Beck et al., 1982), was inserted upstream of *crtI* in plasmid pCRT-1 as outlined in Figure 1.

The *npt*II gene confers kanamycin resistance in a wide variety of gram-negative bacteria (Berg and Berg, 1983). Since there is no transcriptional stop signal downstream of *npt*II (Putnoky et al., 1983), the latter construct was expected to provide co-transcription of *npt*II and *crtI* under the control of the *npt*II promoter. In plasmid pBG2 *crtI* was fused to the 5′ region of the *psbA* gene, which codes for the D1 protein of the microalga *B. filiformis* (Scherer et al., 1991). This region contains sequences that resemble prokaryotic promoter elements; therefore, it was expected that the *psbA* promoter would function in *Synechococcus.* After amplification in *E. coli* and verification by restriction analysis, plasmids pBG1, pBG2, and, as controls, pBG0 and pBG0P were transferred into *Synechococcus* PCC7942 strain R2-PIM8, resulting in strains

R2-PIM8-BG1, R2-PIM8-BG2, R2-PIM8-BG0, and R2-PIM8-BG0P, respectively.

Transformants with insertions of the various plasmids in the "integration platform" were selected by plating the bacteria on medium supplemented with ampicillin and kanamycin. The correct insertion of the respective plasmids in the transformants was checked by DNA/DNA hybridization experiments. Plasmid pBG2 was chosen as hybridization probe with the intention of mapping the insertions in the transformant DNA and the original platform of R2-PIM8 in one blot. This is possible because this plasmid contains all information necessary to recognize the integrated foreign DNA and provides homology to the pBR322 sequences that are present in the original platform. The DNA to be analyzed was digested with restriction endonucleases *Eco*RV and *Bam*HI. *Eco*RV cleaves only the flanking *metF* sequences and not the DNA interrupting the *metF* gene (Van der Plas et al., 1990). As shown in Figure 1, *Bam*HI sites are located in the multiple cloning site of pUC18 and in the *crtI* gene. The theoretical sizes of hybrid fragments were calculated from the published map of the platform (Van der Plas et al., 1990) and from the restriction maps of the plasmids that were used for transformation (Fig. 1). A schematic map of the integrations is presented in Figure 2. The band pattern of the Southern blot shown in Figure 3 illustrates the correct integration of the transformed constructs because the sizes of hybrid fragments obtained match the calculated ones.

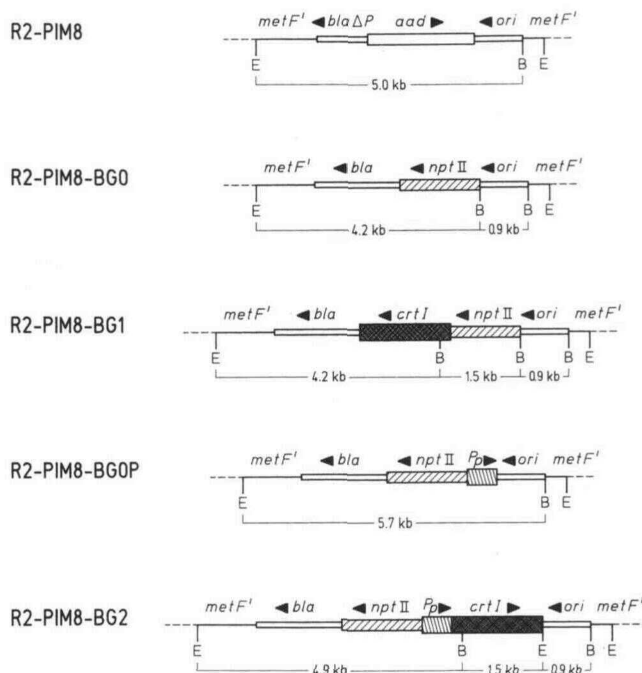

Figure 2. Schematic presentation of the platform region of *Synechococcus* strain R2-PIM8 before and after insertion of the constructs shown in Figure 1. The sizes of fragments that hybridize to plasmid pBG2 in the Southern blot analysis (Fig. 3) are given. Restriction sites: E, *Eco*RV; B, *Bam*HI. Abbreviations are as in Figure 1, with the addition of *bla*Δ*P*, a promoterless *bla* gene, and *metF*, a gene involved in Met biosynthesis of *Synechococcus* PCC7942.

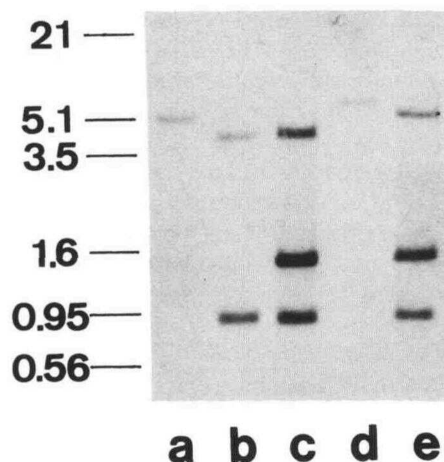

Figure 3. Southern blot analysis of *Synechococcus* strain R2-PIM8 transformants carrying various constructs in the "integration platform." Total DNA from strains (lane a) *Synechococcus* R2-PIM8, (lane b) *Synechococcus* R2-PIM8-BG0, (lane c) *Synechococcus* R2-PIM8-BG1, (lane d) *Synechococcus* R2-PIM8-BG0P, and (lane e) *Synechococcus* R2-PIM8-BG2 was double-digested with *Eco*RV and *Bam*HI and hybridized to digoxygenin-labeled plasmid pBG2 as a probe. Numbers indicate the sizes of DNA molecular mass markers in kb.

Heterologous Expression of *crtI*

Expression of the integrated *crtI* gene in the transformants was shown by western blot analysis with cell extracts of *Synechococcus* PCC7942 strains R2-PIM8, R2-PIM8-BG0, R2-PIM8-BG0P, R2PIM8-BG1, and R2-PIM8-BG2. A polyclonal antiserum raised against CRTI, the product of the cloned *E. uredovora* phytoene desaturase gene (Fraser et al., 1992), was used. As shown in Figure 4, only the extracts of the transformants R2-PIM8-BG1 and R2-PIM8-BG2, which carried *crtI* co-linear to the *npt*II gene or *psbA* promoter, respectively, exhibited an immunoreaction corresponding to a protein of about 54 kD apparent molecular mass (Fig. 4, lanes c and e). Judged by the intensity of the immunoreaction of this protein, the content of CRTI in strain R2-PIM8-BG1 is considerably higher than in strain R2-PIM8-BG2. With the extracts of the control strains R2-PIM8, R2-PIM8-BG0, and R2-PIM8-BG0P (Fig. 4, lanes a, b, and d), no specific cross-reacting protein of corresponding size was detected. A faint, nonspecific immunoreaction was observed with a protein of about 48 kD.

In Vitro Phytoene Desaturation

Synechococcus strain R2-PIM8-BG0 with the integrated vector pBG0 alone showed substantial in vitro phytoene desaturation activity (Table I). More than 20% of the radioactivity of the substrate was converted into β-carotene during the incubation period of 2 h. Labeling of intermediate carotenes was negligible. In parallel experiments the same reaction was carried out with 1 μM norflurazon, which is 10-fold higher than the I_{50} value of the endogenous phytoene desaturase (Sandmann et al., 1989). In the strain without the *Erwinia crtI* gene, inhibition was almost complete under these conditions.

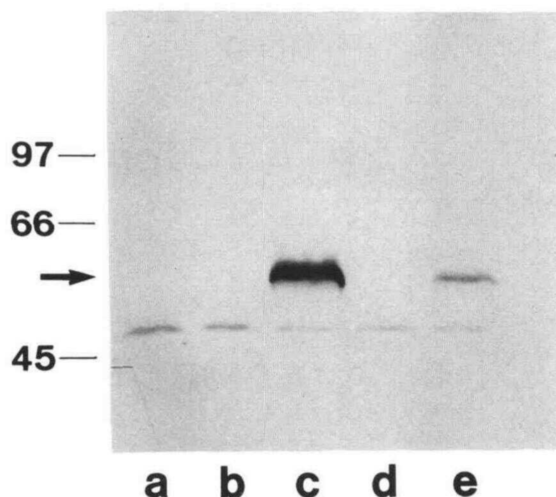

Figure 4. Immunodetection of the phytoene desaturase of *E. uredovora* in *Synechococcus* with an antibody raised against CRTI. Cell extracts of the following strains were separated by 10% SDS-PAGE: (lane a) *Synechococcus* R2-PIM8, (lane b) *Synechococcus* R2-PIM8-BG0, (lane c) *Synechococcus* R2-PIM8-BG1, (lane d) *Synechococcus* R2-PIM8-BG0P, and (lane e) *Synechococcus* R2-PIM8-BG2. The arrow points to the 54-kD bands, which correspond to the calculated size of CRTI. The faint signals at 48 kD are due to a nonspecific immunoreaction. Numbers refer to apparent molecular mass (in kD).

For strains R2-PIM8-BG1 and R2-PIM8-BG2 the conversion of phytoene to β-carotene was higher compared with the control, as indicated by the conversion rate. The contribution of CRTI activity to the overall activity of the endogenous and newly introduced phytoene desaturase can be estimated by comparing the activity with and without norflurazon. The conversion rate for phytoene desaturation in R2-PIM8-BG1 was decreased by norflurazon from 36 to 12%, indicating that about one-third of the activity can be attributed to the foreign enzyme CRTI. In case of R2-PIM8-BG2, the residual rate with norflurazon was 2.6%, which accounted for about one-tenth of contribution of CRTI to total activity.

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DISCUSSION

In this investigation *crtI* (Misawa et al., 1990) was introduced successfully and expressed under the control of two different promoters in the cyanobacterium *Synechococcus* PCC7942. The immunoreactive bands in Figure 4 demonstrated that the levels of the heterologously synthesized phytoene desaturase CRTI in the respective transformants were different. In strain R2-PIM8-BG2 only a small amount of CRTI was produced under the control of the *psbA* gene promoter compared with the high production in strain R2-PIM8-BG1. In the latter the expected co-transcription of *npt*II and *crtI*, which depended on *npt*II promoter activity, was obviously very effective. This difference points to a differential efficiency of the promoter used to provide expression and demonstrates the usefulness of the *npt*II gene promoter, which is active in many gram-negative bacteria, to express foreign genes in *Synechococcus*. Gene constructs in which *crtI* was inserted in the opposite direction to the respective promoter did not result in any detectable formation of CRTI (data not shown), indicating that there are no sequences upstream of *crtI* that are recognized as a promoter in *Synechococcus*.

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R2-PIM8-BG2 + NFZ	18,424	486	2.6

[a] Conversion rate, 100 × (dpm in β-carotene/[dpm in phytoene + dpm in β-carotene]).

Table II. *Content of colored carotenoids in Synechococcus R2-PIM8-BG0, R2-PIM8-BG1, and R2-PIM8-BG2*

Cells were grown in BG11 supplemented with Met, kanamycin, and ampicillin with a light intensity of 60 μE m^{-2} s^{-1} and a temperature of 30°C (see "Materials and Methods").

Strain	Norflurazon	pcv	Colored Carotenoids	Colored Carotenoids
	M	μg/mL culture	μg/mL pcv	%
R2-PIM8-BG0	0	1.7	1285	100
	10^{-7}	1.7	932	73
	3×10^{-7}	1.1	364	28
R2-PIM8-BG1	0	1.4	1190	100
	10^{-7}	1.4	1230	104
	3×10^{-7}	1.3	1200	100
	5×10^{-7}	1.3	1110	93
	10^{-6}	1.3	1090	91
	5×10^{-6}	1.4	860	70
	10^{-5}	1.2	890	71
R2-PIM8-BG2	0	1.7	1375	100
	10^{-7}	1.9	1145	83
	10^{-6}	1.8	1010	73

The western blot experiments support the basic difference between the phytoene desaturases from *E. uredovora* and *Synechococcus*. The antiserum that was raised against the *Erwinia* protein (Fraser et al., 1992) showed no cross-reaction with the endogenous enzyme of *Synechococcus*, which would be shown by the appearance of a band corresponding to a protein of 53 kD (Chamovitz et al., 1991; Fraser et al., 1993).

Two strategies can be followed to genetically engineer herbicide resistance. Other than the introduction of genes coding for detoxifying enzymes, transformation with genes of resistant target enzymes for the corresponding herbicide is a feasible alternative (Comai and Stalker, 1984). This approach has already been used successfully to obtain plants resistant to herbicides whose target enzymes are involved in amino acid biosynthesis (see Mazur and Falco, 1989, for review).

In our case, expression of the foreign gene *crtI* for a phytoene desaturase that is naturally resistant to bleaching herbicides such as norflurazon (Sandmann and Fraser, 1993) resulted in norflurazon-resistant *Synechococcus* transformants. This resistance also demonstrated the functionality of the heterologously expressed enzyme in the foreign host. Norflurazon, a noncompetitive inhibitor of the cyanobacterial phytoene desaturase of *Synechococcus* PCC7942 (Sandmann et al., 1989), does not affect the *Erwinia* enzyme (Sandmann and Fraser, 1993). The major difference in the degree of herbicide resistance between the two *crtI*-containing transformants reflects the different amounts of CRTI produced in the two strains. In contrast to the wild-type strain with an I_{50} value of 10^{-7} M (Linden et al., 1991), the highly expressing strain R2-PIM8-BG1 contained a normal content of colored carotenoids with 10^{-6} M norflurazon present in the medium. Even 10^{-5} M norflurazon lowered the content of carotenoids in this strain by only 29% (Table II). Although strain R2-PIM8-BG2 showed a considerably lower rate of expression of *crtI* compared with R2-PIM8-BG1, it produced more carotenoids than strain R2-PIM8-BG0 exposed to different norflurazon concentrations (Table II), which is indicative of a certain degree of resistance due to expression of the foreign gene. More detailed studies on herbicide resistance of the *Synechococcus* transformants, including cross-resistance to chemically different bleaching herbicides, are in progress.

The mutagenized endogenous phytoene desaturase of *Synechococcus* was less active in norflurazon-resistant strains that synthesized a decreased amount of carotenoids (Linden et al., 1990). In the present study we followed a different approach to produce herbicide-resistant cyanobacteria by inserting a foreign gene, whose product is naturally resistant to the herbicide, into the chromosome of *Synechococcus*. The construction of the CRTI-producing cyanobacterial strains provides a model that can be used to analyze the effect of resistance toward a bleaching herbicide in an organism that combines the plant-type photosynthetic apparatus with the comparatively rapid growth rate of a prokaryote. The results obtained from our investigation can be used as a basis for experiments leading to the construction of higher plants that are resistant to bleaching herbicides.

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