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Effect of Light on Nonphotosynthetic Microorganisms. V.[1] Genetic Control of Photoinduced Carotenogenesis in *Mycobacterium smegmatis*

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Genetic control of carotenoid synthesis in *Mycobacterium smegmatis* (*M. smegmatis*), which produces carotenoids both photoinducibly and constitutively, was investigated in several strains of *M. smegmatis* by using a multi-polar conjugation system. The analysis of the recombinants revealed that the amount of carotenoids produced was strain specific, and the photoinduced and constitutive carotenoid syntheses were under the same genetic control. At least 3 genes participate in the genetic control of carotenoid synthesis in *M. smegmatis*. Constitutive carotenoid synthesis of *M. smegmatis* is a result of an incomplete repression of carotenoid gene.

Keywords——photoinduced carotenogenesis; genetic control; carotenoid synthesis; *Mycobacterium smegmatis*; conjugation

Photochromogenicity is a common and widely distributed phenomenon in *Actinomycetes* and related microorganisms. Photochromogenic strains appear to have the ability to synthesize carotenoid inducibly on exposure to light.[1-5]

Previous investigations on various photochromogenic strains indicated that they could be classified into two types. The first type, such as *Brevibacterium sulfureum*[2c] and *Streptomyces canus*,[2e] produces carotenoid only in response to light. The second type, on the other hand, produces carotenoid even if the organisms are incubated in total darkness, but shows enhanced carotenoid synthesis when exposed to light. Most photochromogenic strains tested were grouped into the second type.

It is an interesting question, whether or not the constitutive carotenoid synthesis of photochromogenic strains is under the same genetic control as inducible carotenoid synthesis.

In the previous paper,[1] we reported on the pigmentation of *Mycobacterium smegmatis* (*M. smegmatis*), which had been regarded as a nonphotochromogen, and we showed that *M. smegmatis* produced carotenoid both photoinducibly and constitutively. The mechanism of the photoinduced carotenoid synthesis in *M. smegmatis* was the same as in previously known photochromogenic mycobacteria.[3,6] We proposed two possible genetic controls on inducible and constitutive carotenoid synthesis in *M. smegmatis*. One was that each inducible and constitutive carotenoid synthesis is controlled independently by different carotenoid genes. The other was that both inducible and constitutive syntheses are controlled by the same gene, and constitutive carotenoid synthesis was a result of incomplete repression of that gene.

Mizuguchi *et al.*[7a,b] and Tokunaga *et al.*[7c] described a multi-polar conjugation system among the strains of *M. smegmatis*. To clarify what kind of genetic control is operating in carotenoid synthesis of *M. smegmatis*, a genetic study on carotenoid synthesis of *M. smegmatis* was carried out by means of conjugation. This paper describes the genetic control of inducible and constitutive carotenoid synthesis in *M. smegmatis*.

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TABLE I. Bacterial Strains

Strain[a]	Auxotrophic markers	Phenotype of carotenoid synthesis[b]	Source
L		P^+, S^+	Y. Mizuguchi
L-1	leu^-	P^+, S^+	This work
L-2	arg^-	P^+, S^+	This work
L-4	arg^-	P^-, S^-	This work
L-5	arg^-	S*	This work
N1		P^+, S^+	Y. Mizuguchi
N1-1	his^-	P^-, S^-	This work
N1-2	his^-, leu^-	P^-, S^+	This work
N1-3	his^-, leu^-	P^-, S^-	This work
R		P^+, S^+	Y. Mizuguchi
R-15	arg^-, met^-	P^+, S^+	Y. Mizuguchi

a) L, strain Lacticola; N1, strain Nishi 1; R, strain Rabinowitchi. *b)* P, photoinduced carotenogenesis; S, constitutive carotenogenesis; S*, constitutive mutant of P.

Experimental

Bacterial Strains——*M. smegmatis* strains Lacticola, Nishi 1, Rabinowitchi and their mutants were used. The mutants used in the present paper are listed in Table I. The symbols P and S indicate photoinduced carotenogenesis and constitutive carotenogenesis, respectively.

Media and Cultivation——TB broth base medium (yeast extract, 2 g; polypeptone, 4 g; $Na_2HPO_4 \cdot 12H_2O$, 6.3 g; KH_2PO_4, 1 g; sodium citrate, 1.5 g; $MgSO_4 \cdot 7H_2O$, 0.6 g; glycerol, 10 g; distilled water 1000 ml, pH 7.0) was used as a complete medium. Karlsson's medium (glycerol, 20 g; K_2HPO_4, 4 g; KH_2PO_4, 1.5 g; $(NH_4)_2SO_4$, 1 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; $FeSO_4 \cdot 7H_2O$, 0.02 g; $CaCl_2$, 0.01 g; distilled water 1000 ml, pH 7.0) was used as a minimum medium. If necessary, 2% agar was added to the medium. Incubation was carried out at 37 °C, and bacteria were maintained on TB agar slants.

Determination of Phenotype of Carotenoid Synthesis——The color of colonies incubated on two TB agar plates, one under illumination and the other in the dark, was observed. From the observed color of colonies, the phenotypes of carotenoid synthesis of strains were tentatively designated P^+S^+, P^-S^+, P^+S^- or P^-S^-. With P^+S^+ strain, colonies incubated under illumination showed enhanced pigmentation as compared with those grown in the dark. With P^-S^+ strain, the color of the colonies grown under illumination was identical with that in the dark. With P^+S^- strain, dark-grown colonies were colorless or whitish, but the light-exposed colonies were yellow to yellowish-orange. With P^-S^- strain, neither the light-exposed nor the dark-grown colonies showed coloration. With S* strain, the color of the colonies grown in the dark was identical with that of P^+S^+ strain grown under illumination.

To confirm these designations, organisms were incubated in TB broth under illumination and in the dark for 3 d. Cells were harvested (12000 rpm, 10 min) and transferred to graduated centrifugation tubes to determine the packed volume of the cells. After centrifugation, pigments were extracted as described previously.[1] The extracts were concentrated *in vacuo* to dryness, then the residue was dissolved in methanol (3 times the packed volume) and the absorbance of the solution was measured with a spectrophotometer. The amounts of carotenoids were compared in terms of the absorbance at OD_{450} of the extracts.

Isolation of Mutants——Bacteria incubated in TB broth containing 0.1% Tween 80 were harvested and washed with sterilized saline. Bacteria were suspended in 0.1 M Tris–malate buffer (pH 9.0) containing 0.1% Tween 80 and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) (final concentration, 1 mg/ml). After a 20 min incubation at 37 °C, cells were harvested and washed with saline. Screening for auxotrophic mutants was carried out with cycloserine (100 μg/ml).

Mutants of carotenoid synthesis were isolated as follows. NTG-treated cells were plated on TB agar plates and incubated under illumination. By this procedure, P^-S^+ and P^-S^- mutants were isolated as pale yellow colonies and non-colored colonies, respectively. For the isolation of P^+S^- strain, NTG-treated cells on TB agar plates were incubated in the dark for 3 d, and non-colored or whitish colonies were marked. Then the plates were incubated under illumination for 2 d. The colored colonies among the colonies previously marked were tried to isolate.

Conjugation——Conjugation was carried out as described by Mizuguchi and Tokunaga.[7a] Each donor strain and recipient strain were incubated for 3 d in TB broth. Cells were harvested and homogenized. Equal numbers of cells of donor and recipient strains, adjusted in terms of OD_{660}, were mixed and then plated onto TB agar plates. After a 3-day incubation, the cell mass on the plate was suspended in sterilized saline and homogenized. The

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homogenized cells were diluted appropriately and spread on selective plates, then incubated under illumination for 7 d. The yellow recombinants on each selective plate were transferred to a similar selective plate and incubated. The colonies on the plates were replicated onto two TB agar plates to determine the phenotype of carotenoid synthesis as described above.

Induction of Carotenoid Synthesis by Hydrogen Peroxide——Strains N1 (P^+S^+) and N1-2 (P^-S^+) were incubated in the dark for 3 d. The homogenized cells of strains N1 and N1-2 were inoculated at the final concentration of 1 mg wet cells/ml into a series of 100 ml aliquots of TB broth, and incubated for 36 h in the dark. Cells of each strain were harvested and suspended in phosphate buffer (0.1 M, pH 8.0) containing various concentrations of H_2O_2 (0.01, 0.05, 0.1, 0.2, 0.3 and 0.4 M). Then the cells were incubated at 37 °C for 40 min. Cells, after being washed, were suspended in the supernatant of the culture which had been incubated for 36 h in the dark. After incubation for another 48 h in the dark, pigments produced in the cells were extracted and the amounts of pigments were measured.

Results

Crosses between P^-S^- or P^-S^+ Strain by P^+S^+ Strain

Strain N1-1 [his^-, (P^-, S^-)] was crossed with P^+S^+ strain, L-1 [arg^-, (P^+, S^+)]. His^+ and yellow recombinants were obtained with the frequency of 3.4×10^{-5} (Table II). The phenotype of carotenoid synthesis in these yellow recombinants was tentatively determined as described in the text.

All of the yellow recombinants tested produced carotenoid both constitutively (S^+) and inducibly (P^+). To confirm this, 70 recombinants were selected at random, and incubated under illumination and in the dark in TB broth. Pigments produced in the dark-grown and the light-exposed cells were extracted. Figure 1 shows the absorption spectra of the extracts from light-exposed and dark-grown cells of the recombinants, LNN-2 and LNL-1. From their

Table II. Recombinational Frequencies for Carotenoid Synthesis of Auxotrophically Marked Mutants of *M. smegmatis*

Donor	Recipient	Selected markers		Frequency
L-1	N1-1			
[leu^-, (P^+,S^+)]	[his^-, (P^-,S^-)]	his^+		6.5×10^{-4}
			his^+, (P^+,S^+)	3.4×10^{-5}
L-2	N1-2			
[arg^-, (P^+,S^+)]	[his^-, leu^-, (P^-,S^+)]	his^-, leu^+		2.7×10^{-3}
			his^-, leu^+, (P^+,S^+)	2.9×10^{-5}
		his^+, leu^-		7.1×10^{-4}
			his^+, leu^-, (P^+,S^+)	2.4×10^{-5}
		his^+, leu^+		8.2×10^{-5}
			his^+, leu^+, (P^+,S^+)	4.2×10^{-6}
L-2	N1-3			
[arg^-, (P^+,S^+)]	[his^-, leu^-, (P^-,S^-)]	his^-, leu^+		1.6×10^{-4}
			his^-, leu^+, (P^+,S^+)	2.0×10^{-6}
		his^+, leu^-		2.2×10^{-5}
			his^+, leu^-, (P^+,S^+)	2.0×10^{-7}
		his^+, leu^+		2.4×10^{-6}
			his^+, leu^+, (P^+,S^+)	3.8×10^{-8}
R-15	N1-2			
[arg^-, met^-, (P^+,S^+)]	[his^-, leu^-, (P^-,S^+)]	his^-, leu^+		2.6×10^{-4}
			his^-, leu^+, (P^+,S^+)	6.5×10^{-6}
		his^+, leu^-		1.0×10^{-5}
			his^+, leu^-, (P^+,S^+)	6.8×10^{-7}
		his^+, leu^+		1.2×10^{-6}
			his^+, leu^+, (P^+,S^+)	1.8×10^{-7}

Reversion frequencies of $P^- < 10^{-6}$, each auxotrophic marker $< 10^{-8}$.

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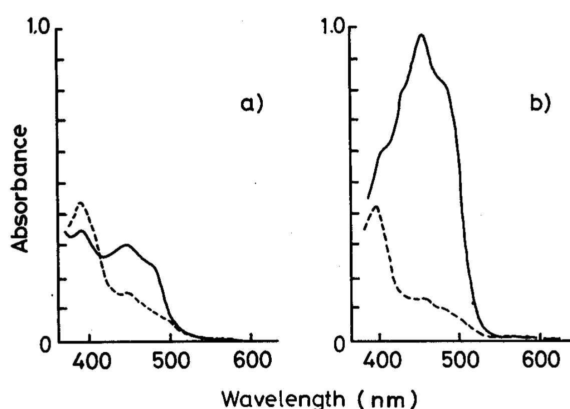

Fig. 1. Visible Light Absorption Spectra of the Extracts from Light-Exposed and Dark-Grown Cells of P^+S^+ Recombinants Obtained from the Crosses between N1-1 (P^-S^-) and L-1 (P^+S^+)

a) Strain LNN-1 showing N type carotenoid synthesis. b) Strain LNL-2 showing L type carotenoid synthesis. (——), extracts from light-exposed cells; (------), extracts from dark-grown cells.

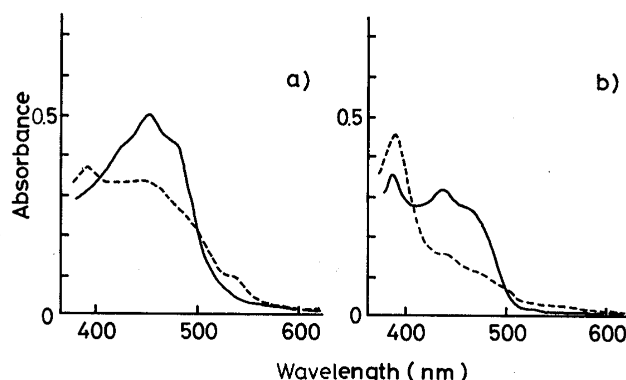

Fig. 2. Visible Light Absorption Spectra of the Extracts from Light-Exposed and Dark-Grown Cells of P^+S^+ Recombinants Obtained from the Crosses between N1-2 (P^-S^+) and R-15 (P^+S^+)

a) Strain RNR-1 showing R type carotenoid synthesis. b) Strain RNN-1 showing N type carotenoid synthesis. (——), extracts from light-exposed cells; (------), extracts from dark-grown cells.

absorption spectra, the phenotype of carotenoid synthesis in all the yellow recombinants tested was confirmed to be P^+S^+. Moreover, the confirmed P^+S^+ recombinants were classified into two groups in terms of the amount of carotenoid produced in light-exposed and dark-grown cells. In one group, the amounts of carotenoids in the light-exposed and dark-grown cells of 12 P^+S^+ recombinants were about 1.0 and 0.15 at OD_{450}, respectively. The other was that the amount of carotenoids produced in the light-exposed and dark-grown cells of the remaining 58 P^+S^+ recombinants were about 0.3 and 0.15 at OD_{450}, respectively. The amount of carotenoids produced in the cells of the former recombinants was the same as that of strain Lacticola, the parent strain of L-1, and this type was designated as L type (Fig. 1b). Similarly, the latter recombinants were designated as N type (Fig. 1a).

While strain N1-1 [his^-, (P^-, S^-)] was isolated directly from P^+S^+ strain by NTG treatment, strain Nl-3 [leu^-, his^-, (P^-, S^-)] was prepared from P^+S^+ strain *via* P^-S^+ strain by two steps of mutation. Crosses between L-2 [arg^-, (P^+, S^+)] and Nl-3 gave P^+S^+ recombinants with frequencies of 2.0×10^{-6} (his^+, leu^-), 2.0×10^{-7} (his^-, leu^+) and 3.8×10^{-7} (his^+, leu^+), as shown in Table II. When N1-2 (leu^-, his^-, P^-S^+) was used as a recipient, P^+S^+ conjugants were obtained with 10 to 100 times higher frequency than when N1-3 was used as a recipient.

The P^+S^+ conjugants obtained from the each cross were also grouped into L type and N type in a ratio of 1 : 6.5 based on the amount of carotenoid produced in both the light-exposed and the dark-grown cells of the recombinants.

Similarly, strain R-15 [arg^-, met^-, (P^+, S^+)] was used as a donor strain and crossed with Nl-2 [leu^-, his^-, (P^-, S^+)]. P^+S^+ recombinants were obtained with frequencies of 6.5×10^{-6} (his^-, leu^+), 6.8×10^{-7} (his^+, leu^-) and 1.8×10^{-7} (his^+, leu^+) (Table II). As shown in Fig. 2, P^+S^+ recombinants were also classified into two groups in terms of the amount of carotenoid produced in the light-exposed and dark-grown cells. The amounts of carotenoid produced in the light-exposed and dark-grown cells of one group of the recombinants designated as R type were about 0.5 and 0.35 at OD_{450}, respectively, almost the same as those produced in the light-exposed and dark-grown cells of strain Rabinowitchi, the parent strain of R-15 (Fig. 2a). The rest were of N type (Fig. 2b). The ratio of the P^+S^+ recombinants showing R type carotenoid

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TABLE III. Recombinational Frequencies for Carotenoid Synthesis of Auxotrophically Marked Mutants of *M. smegmatis*

Donor	Recipient	Selected marker		Frequency
L-4 [arg^-, (P^-,S^-)]	N1-2 [his^-, leu^-, (P^-,S^+)]	his^-, leu^+		8.5×10^{-4}
			his^-, leu^+, (P^+,S^+)	2.3×10^{-5}
		his^+, leu^-		1.7×10^{-4}
			his^+, leu^-, (P^+,S^+)	1.1×10^{-5}
		his^+, leu^+		1.9×10^{-5}
			his^+, leu^+, (P^+,S^+)	1.9×10^{-6}
L-5 [arg^-, (S*)]	N1-3 [his^-, leu^-, (P^-,S^-)]	his^-, leu^+		4.0×10^{-3}
			his^-, leu^+, (P^+,S^+)/(S*)	7.5×10^{-5}
		his^+, leu^-		4.1×10^{-4}
			his^+, leu^-, (P^+,S^+)/(S*)	1.3×10^{-5}
		his^+, leu^+		6.2×10^{-5}
			his^+, leu^+, (P^+,S^+)/(S*)	4.2×10^{-6}

Reversion frequencies of $P^- < 10^{-6}$, each auxotrophic marker $< 10^{-8}$

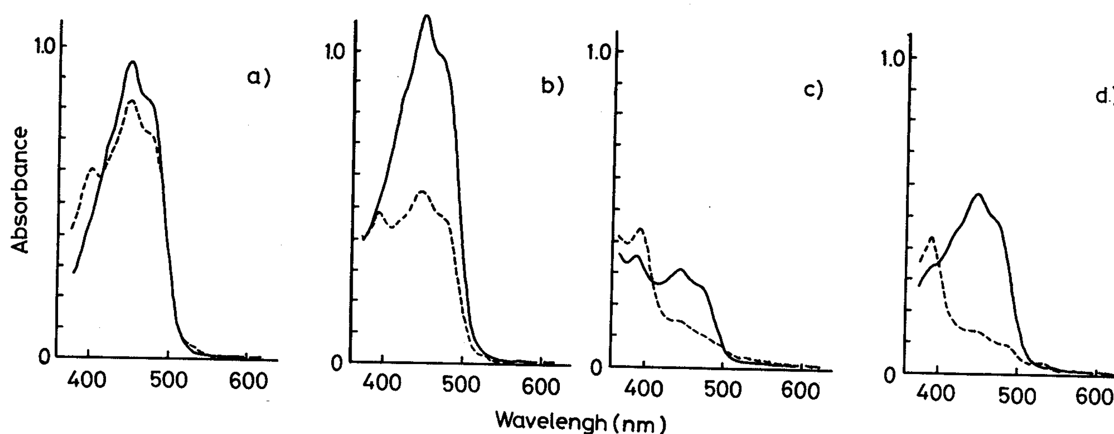

Fig. 3. Visible Light Absorption Spectra of the Extracts from Light-Exposed and Dark-Grown Cells of the Yellow Recombinants Obtained from the Crosses between N1-3 (P^-S^-) and L-5 (S*)

a) Strain LNS-3 showing S* type carotenoid synthesis. b) Strain LNeN-2 showing enN type carotenoid synthesis. c) Strain LNN-11 showing N type carotenoid synthesis. d) Strain LNL-12 showing L type carotenoid synthesis.
(——), extracts from light-exposed cells; (– – – –), extracts from dark-grown cells.

synthesis and N type carotenoid synthesis was 1 : 6.

Crosses between P^-S^+ Strain and P^-S^- Strain

Strain L-4 [arg^-, (P^-, S^-)] derived from strain L-2 [arg^-, (P^+, S^+)] by one step of mutation was crossed with N1-2 [leu^-, his^-, (P^-, S^+)]. The phenotype of carotenoid synthesis in the yellow recombinants obtained from the cross was P^+S^+ as far as tested. P^+S^+ recombinants were obtained with frequencies of 2.3×10^{-5}, (his^-, leu^+), 1.1×10^{-5} (his^+, leu^-) and 1.9×10^{-6} (his^+, leu^+) (Table III). The frequency of the P^+S^+ recombinants at each selection did not significantly differ from that of recombinants obtained from the crosses between strains L-2 [arg^-, (P^+, S^+)] and N1-2 [leu^-, his^-, (P^-, S^+)], as shown in Table II. From the amount of carotenoids produced in the light-exposed and dark-grown cells of the P^+S^+ recombinants, they were also grouped into two types, L type and N type, in a ratio of 1 : 7.

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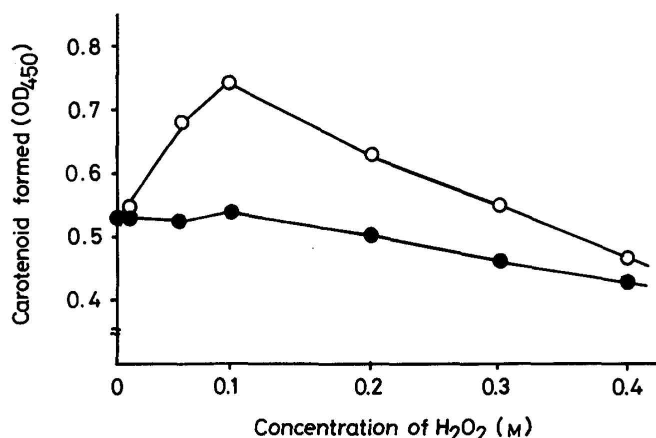

Fig. 4. Induction of Carotenoid Synthesis of *M. smegmatis* Strain N1 (P^+S^+) by Hydrogen Peroxide

The organisms incubated in the dark at 37 °C for 36 h were harvested and suspended in various conc. of H_2O_2 in phosphate buffer. After a 40 min incubation at 37 °C, cells were washed and suspended in the supernatant of the culture incubated for 36 h in the dark. After a further 48 h incubation in the dark at 37 °C, the amounts of carotenoids formed were measured in terms of OD_{450}. (○), strain N1 (P^+, S^+); (●), strain N1-2 (P^-, S^+).

Crosses between P^-S^- Strain and S* Strain

Mutants of carotenoid synthesis symbolized as S* produce almost the same amount of carotenoid in the dark as that produced by the wild strain in the light. Strain L-5 [arg^-, (S*)] derived from L-2 [arg^-, (P^+, S^+)] was used as a donor strain. As a recipient strain, N1-3 [leu^-, his^-, (P^-, S^-)] derived from N1-2 [leu^-, his^-, (P^-, S^+)] was used. Yellow recombinants were obtained with frequencies of 7.5×10^{-5} (his^-, leu^+), 1.3×10^{-5} (his^+, leu^-) and 4.2×10^{-6} (his^+, leu^+) (Table III).

The amounts of carotenoid produced in the light-exposed and the dark-grown cells of 25 yellow recombinants were examined. Yellow recombinants were grouped into 4 groups, shown in Fig. 3, in terms of the amount of carotenoid produced in the light-exposed and dark-grown cells. Two of 25 recombinants were of L type (Fig. 3d). Eleven yellow recombinants were of N type (Fig. 3c). Ten yellow recombinants produced carotenoid with the same ratio of carotenoid produced in the light-exposed cells to that in the dark-grown cells as strain N1, but the amount of carotenoid produced in these yellow recombinants was larger than that of N1; these were designated as enN type (Fig. 3b). The phenotype of carotenoid synthesis of the remaining two yellow recombinants (S* type) was not P^+S^+, and the recombinants produced almost the same amount of carotenoid in the dark as that produced in the light-exposed cells of strain Lacticola (Fig. 3a).

Induction of Carotenoid Synthesis by Hydrogen Peroxide

Whether the carotenoid synthesis of *M. smegmatis* was induced by H_2O_2 or not was investigated. Hydrogen peroxide induced carotenoid synthesis as well as light exposure in the strain N1 (P^+S^+), as shown in Fig. 4. The amount of carotenoid produced by H_2O_2 treatment increased linearly with the concentration of H_2O_2. When H_2O_2 concentration was increased over 0.1 M, the amount of carotenoid produced decreased. However, strain N1-2 (P^-S^+) was not induced by H_2O_2 treatment.

Discussion

The characteristic feature of pigmentation in *M. smegmatis* is that the organism produces carotenoid both inducibly and constitutively.[1] Genetic control of carotenoid synthesis in *M. smegmatis*, used as a representative strain which produces carotenoid both inducibly and constitutively, was investigated. We previously proposed two possible genetic control mechanism of carotenoid synthesis in *M. smegmatis*. One is that the inducible and constitutive carotenoid syntheses of *M. smegmatis* are controlled by different carotenoid genes, and the other is that both carotenoid syntheses are controlled by the same gene, and incomplete repression of the gene results in constitutive carotenoid synthesis.

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From the results described below, we concluded that the inducible and constitutive carotenoid syntheses in *M. smegmatis* are coded on the same carotenoid gene (*car* B); 1) P^+S^- mutant was not obtained by NTG treatment, 2) P^+S^- and P^-S^+ recombinants were not obtained by crosses between P^+S^+ and P^-S^- strain, 3) the crosses between L-4 (P^-S^-) and N1-2 (P^-S^+) gave P^+S^+ recombinants at frequencies similar to those provided by the crosses between L-2 (P^+S^+) and N1-2 (P^-S^+), 4) only 17% of P^+S^+ recombinants obtained from the crosses between L-2 (P^+S^+) and N1-1 (P^-S^+) showed L type carotenoid synthesis, and the remaining P^+S^+ recombinants showed N type carotenoid synthesis. Therefore, the constitutive carotenoid synthesis in *M. smegmatis* is considered to be a result of incomplete repression of the gene.

Consequently, we assumed that the *phc* gene, whose gene product might be a substrate of the photochemical reaction, the reaction product of which would act as an inducer of the carotenoid gene, is involved in the genetic control of carotenoid synthesis in *M. smegmatis*. Rau[8)] and Howes *et al.*[9)] showed that oxygen is essential in photochemical reaction steps of photoinduced carotenoid synthesis. These results indicate that photochemical reaction steps in photoinduced carotenoid synthesis involve oxidative reactions. We also found that *Actinoplanes philippinensis* was induced to synthesize carotenoid by treatment with H_2O_2.[2d)] In P^+S^+ strain of *M. smegmatis*, carotenoid synthesis was also induced by H_2O_2, but P^-S^+ strain treated with hydrogen peroxide was not induced to synthesize carotenoid (Fig. 4). This suggested that the *phc* gene may code a metabolite which serves as the substrate of photochemical oxidation in the photochemical reaction steps of photoinduced carotenogenesis, and the phenotype P^-S^+ is the result of mutation on the *phc* gene. Thus the participation of *phc* and *car* B in carotenoid synthesis of *M. smegmatis* is likely.

However, the results obtained from crosses between L-5 (S*) and N1-3 (P^-S^-), which gave P^+S^+ recombinants showing enN type carotenoid synthesis, are not amenable to interpretation in terms of these two genes. Johnson *et al.*[6e)] reported that in the photochromogenic strain *Mycobacterium* sp., synthesis of geranylgeranyl pyrophosphate synthetase was induced by light but a small amount of the enzyme was synthesized constitutively even in the dark-grown cells. Thus, we assumed the existence of a gene (*car* A) coding synthesis of precursors of carotenoid. Thus, the genetic control of carotenoid synthesis in *M. smegmatis* may be explained as follows. The *phc* gene product would be oxidized by photochemical reaction, and the reaction products would derepress *car* A. However, it remains an open question whether or not derepression of *car* B is caused by the photochemical reaction product.

P^+S^+ recombinants obtained from crosses between P^+S^+ strain and P^-S^- or P^-S^+ strain were classified into two groups, one of which produced the same amount of carotenoid as that produced in the donor strain (P^+S^+), and the other of which produced the same amount of carotenoid as that produced in the wild strain of the recipient strain. From these results, we supposed that activity of promotor or operator of *car* A differs in the various strains of *M. smegmatis*, and that the level of activity determines the amount of carotenoid to be synthesized in each strain, because if the activity of *car* B is strain-specific, P^+S^+ recombinants obtained from the crosses between L-1 (P^+S^+) and N1-1 (P^-S^-), which was obtained by one step of mutation and is supposed to be amutant of *car* B, should show L type carotenogenesis, whereas the obtained P^+S^+ recombinants showed L type and N type carotenogenesis in the ratio of 1:4.8. The crosses between L-5 (S*) and N1-3 (P^-S^-) gave four kinds of yellow recombinants. These results were interpreted as follows: mutation on *car* B gave S^- strain, and *car* B gene of the strain was not strain-specific. S* was thought to be a mutant of the regulator gene (i_A) of *car* A, so that *car* A was free from repression. The regulator genes of *car* A and *car* B were not strain-specific because the cross between L-5 (S*) and N1-3 (P^-S^-) gave the recombinants of enN type and L type. Therefore, the fact that the

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amount of carotenoid produced in each strain is strain-specific can be attributed to the strain-specific activity of promotor or operator of *car* A.

The mechanism of photoinduced carotenogenesis in various photochromogenic bacteria so far investigated appears to be fundamentally the same, which suggests that carotenoid synthesis of other photochromogenic bacteria may be under the same machanism of genetic control as described in the present paper.

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