JOURNAL OF BACTERIOLOGY, Nov. 1992, p. 7352–7359
0021-9193/92/227352-08$02.00/0

Vol. 174, No. 22

Isolation and Characterization of a Light-Sensitive Mutant of *Escherichia coli* K-12 with a Mutation in a Gene That Is Required for the Biosynthesis of Ubiquinone

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Received 13 April 1992/Accepted 16 September 1992

Cells with a novel mutation that is lethal when the cells are exposed to visible light were isolated from *Escherichia coli* K-12. The mutation was mapped at 63 min on the linkage map of the *E. coli* chromosome, and the gene, designated *visB*, was cloned and sequenced. From its map position and the evidence that the gene product VisB exhibits homology with flavin monooxygenase of *Pseudomonas fluorescens*, the *visB* gene was deduced to be identical to the *ubiH* gene, which is a gene required for the biosynthesis of ubiquinone and is thought to be similar to the gene for flavin monooxygenase. The photosensitive phenotype appears to be due to the accumulation of the substrate for the reaction catalyzed by the *visB* (*ubiH*) gene product because other mutations that block earlier steps in the biosynthesis of ubiquinone can reverse the photosensitivity. The accumulated intermediates may produce active species of oxygen in the mutant bacteria upon illumination by visible light, and these active oxygen species may cause the death of the cells by a mechanism similar to that associated with mutations in *visA* (*hemH*).

We previously isolated and characterized several light-sensitive mutants of *Escherichia coli* (14, 15). The mutant bacteria are killed by illumination with visible light at about 460 nm. It has been shown that the gene, designated *visA*, in which almost all the mutations have been mapped, is the same gene as *hemH*. The *visA* (*hemH*) gene encodes ferrochelatase, the enzyme that catalyzes the final step in the biosynthesis of heme. The photosensitivity of the *visA* mutants is probably caused by the harmful effects of active oxygen species generated by the photochemical reactions that involve protoporphyrin IX, a substrate for ferrochelatase.

In this study, to search for such mutations in genes other than *visA*, we attempted to isolate photosensitive mutants from a *visA*$^+$/*visA*$^+$ partial diploid strain. Here we report the isolation and characterization of a new photosensitive mutant from *E. coli* K-12.

MATERIALS AND METHODS

Bacterial strains, phages, and plasmids. All strains of *E. coli* K-12 used in this study are shown in Table 1. By use of phage P1, VS011 and VS551 were constructed by transferring the Φ(*sodA'-'lacZ*) Cm^r marker from QC772 to CA274 and VS550, respectively. VS560 and VS561 were constructed by transferring the Δ*visB*:Km^r marker from VS005 to CA274 and AW385, respectively. VS600 was constructed by transferring the Δ*visC*:Km^r marker from VS006 to CA274. VS005 and VS006 were constructed as described below. The *ubi* mutants, AN172 and AN385, were kindly supplied by I. G. Young (Department of Biochemistry, John Curtin School of Medical Research, Australian National University). *E. coli* genomic libraries were constructed by using λgt-λC (21) and Charon 28 (16) as vectors. λvisB1 is described in Results. Phages from an *E. coli* linking library were described by Kohara et al. (11). Wild-type λ phage was used as helper for lysogeny by a λ phage that cannot lysogenize cells by itself. Plasmids pVPs and pVKs were constructed by subcloning the *Pst*I or *Kpn*I fragments from the insert DNA of λvisB1 into pUC118 (24). The Φ(*nfo'-'lacZ*) fusion plasmid pHI20 was kindly supplied by S. Yonei (Department of Zoology, Faculty of Science, Kyoto University).

Media and growth. The basic media used were LB medium and M9 minimal medium (19). When appropriate, media were supplemented with 0.2% glucose, 0.6% sodium succinate, and 20 μg of each amino acid per ml unless otherwise noted. For the illumination, plates were exposed to the light from two 40-W fluorescent lamps (Hitachi, Sunlight FL40SSD/37-G) at a distance of 10 cm (approximately 7,500 lx) unless otherwise noted. Controls (in the dark) were wrapped in aluminum foil and placed in the same location. Incubations were performed at 37°C.

Mutagenesis. Isolation of the photosensitive mutants after treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) was performed as described by Miyamoto et al. (14). Disruption of the genes was performed by using the *recBC sbcB* strain JC7623. The gene to be disrupted was cloned on a plasmid vector, and a part of the cloned gene was replaced with a kanamycin resistance cassette from pUC4-KAPA (purchased from Pharmacia LKB Biotechnology, Uppsala, Sweden). The inserted fragment was then purified and used to transform JC7623. Genomic DNA of the Km^r transformants was isolated and amplified by the polymerase chain reaction to confirm the insertion of the Km^r cassette at the desired position within the genome. Subsequently, the gene was transferred to other strains by phage P1 (13). To make Δ*visB*:Km^r and Δ*visC*:Km^r, the *Kpn*I-*Sal*I region (662 bp) in the *visB* gene and the *Eco*RV-*Sty*I region (662 bp) in the *visC* gene were replaced by the Km^r cassette.

Primer extension. The 5'-^{32}P-labelled oligonucleotide primer was allowed to hybridize with 50 μg of total RNA

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TABLE 1. Bacterial strains

Strain	Relevant genotype or phenotype	Reference
CA274	*lacZ*(Am) *trp*(Am)	18
VS101	*visA1*	14
VS011	*sodA'-'lacZ* Cm^r	This work
VS550	*visB103*	This work
VS551	*visB103 sodA'-'lacZ* Cm^r	This work
VS560	Δ*visB*:Km^r	This work
VS561	Δ*visB*:Km^r *ubiA*	This work
VS600	Δ*visC*:Km^r	This work
VS005	Δ*visB*:Km^r	This work
VS006	Δ*visC*:Km^r	This work
AN172	*ubiH434*	28
AN385	*ubiA420* Sm^r	1
QC772	*sodA'-'lacZ* Cm^r	4
JC7623	*recB21 recC22 sbcB15 sbcC201*	12

from CA274 and elongated by avian myeloblastosis virus reverse transcriptase as described by Sambrook et al. (19). The elongated DNA was analyzed on 6% acrylamide-urea gels.

Paraquat gradient plates and reagents. Agar plates containing linear concentration gradients of paraquat were prepared by the method of Cunningham et al. (5). To 25 ml of molten LB agar, paraquat (final concentration, 10 μM; Sigma Chemical Co., St. Louis, Mo.) and 25 μl of an Antifoam B emulsion (Sigma) were added. The mixture was poured into a 15-cm^2 petri dish (Falcon no. 1012) and solidified with the petri dish on an incline of about 5°. The plate was placed on the level and overlaid with another 25 ml of molten LB agar. Each plate was uncovered and dried at 37°C for 1 h. Twenty milliliters of molten soft agar was put on a microscope slide, and then 20 μl of a fresh overnight bacterial culture was added to the soft agar on the microscope slide. The edge of a second slide was touched against the surface of the soft agar and stamped onto the gradient plate parallel to the gradient. The plates were incubated for 24 h at 37°C and photographed.

Bovine superoxide dismutase was purchased from Sigma (product no. 2515). Radioactive compounds were purchased from Amersham Japan (Tokyo).

Data base search. A search was made in the GenBank release 64.0 and Swiss-prot release 14.0 data bases for DNA and protein homologies, respectively, by using the Genetyx-CD bio-data base program supplied by Software Development Co. Ltd., Tokyo, Japan.

Nucleotide sequence accession number. The nucleotide sequence described in this report has been assigned the DDBJ/EMBL and GenBank accession number D90281.

RESULTS

Isolation and phenotype of a new mutant that is affected by light. λ phage carrying the *visA* gene (14) was used to lysogenize *E. coli* CA274, to give the *visA*$^+$/*visA*$^+$ diploid strain. This strain was mutagenized with MNNG, and cells were screened for photosensitive mutants. One mutant exhibited clear photosensitivity and was analyzed as follows. The mutant bacteria were cured of the lysogenized λ phage, and the resulting strain was designated VS550. The photosensitivity of VS550 is shown in Fig. 1. Transformation with a plasmid that carried the wild-type *visA*$^+$ gene did not cure the photosensitivity of VS550. The lethal effect of illumination was not due to the UV light because it was not affected

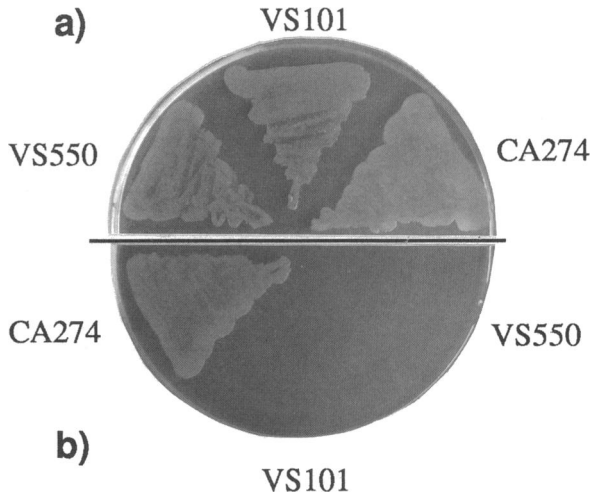

FIG. 1. Photosensitive phenotype of the *vis* mutants. Suspensions of cells were spread on LB plates and incubated overnight under each condition. (a) Dark; (b) light.

by insertion of a glass plate (3-mm thick) between the light source and the plates. When ascorbic acid (2 mM optimal) was added to the medium, VS550 did not exhibit such photosensitivity. This phenomenon was not observed with the *visA* mutants (Fig. 2). Thus, we hypothesized that the photosensitive phenotype of VS550 was not caused by a mutation in the *visA* gene, and we designated the putative gene *visB*.

Cloning of the *visB* gene. Genomic DNA from *E. coli* CA274 was digested with *Eco*RI or *Hin*dIII and cloned into λ phage vector λgt-λC or Charon 28, respectively. VS550 cells were infected separately by the two genomic libraries, and the infected bacteria were incubated in the light. Several light-resistant colonies appeared upon infection with the

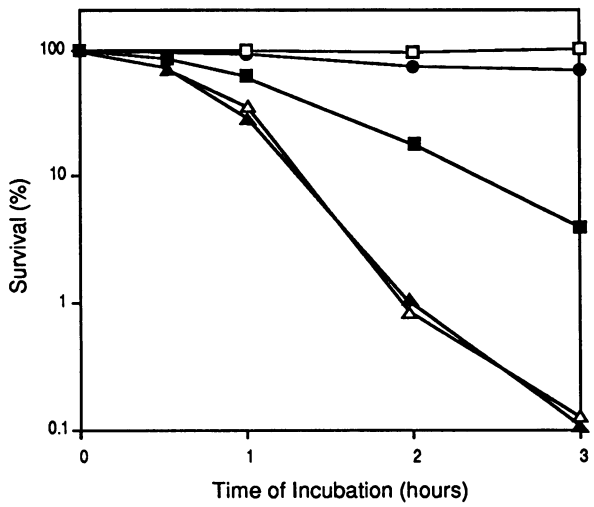

FIG. 2. Suspensions of cells were diluted appropriately and spread on LB plates with or without ascorbate. After incubation in the light, plates were incubated overnight for formation of colonies. Percent survival was calculated by reference to dark controls. Symbols: ●, CA274; ■, VS550; ▲, VS101; □, VS550 (plates were supplemented with 2 mM ascorbate); △, VS101 (with 2 mM ascorbate).

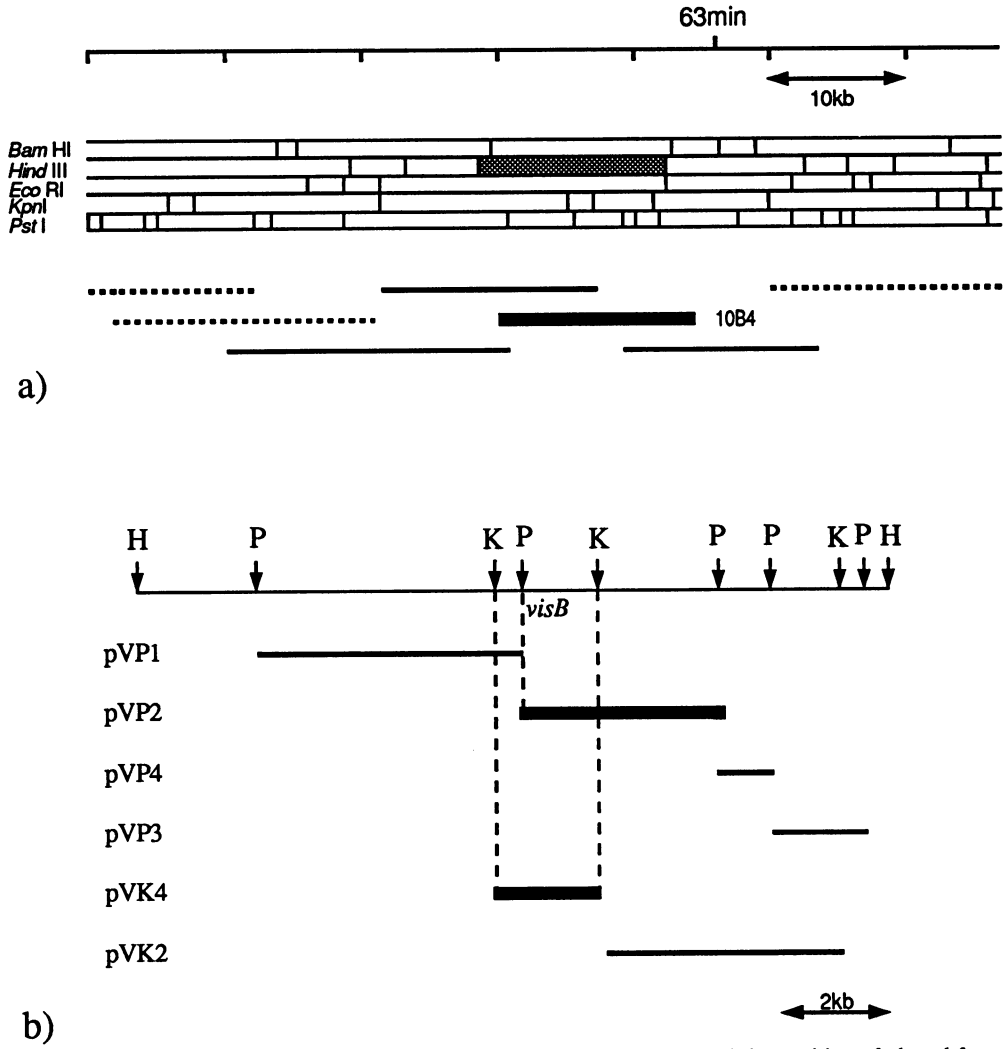

FIG. 3. (a) Restriction map at approximately 62 to 63 min of the *E. coli* chromosome and the position of cloned fragments in Kohara's phages (modified from Kohara et al. [11]). Of the clones, those indicated by regular lines hybridized with the fragment from λvisB1 and the one indicated by a thick line (clone 10B4) complemented the photosensitivity of VS550. The hatched box indicates the *Hin*dIII fragment in λvisB1. (b) Restriction sites in the 12-kb *Hin*dIII fragment are indicated above the plasmids, and the insert DNAs in the plasmids are indicated below these sites. pVP2 and pVK4, indicated by thick lines, complemented the photosensitivity of VS550. Abbreviations: H, *Hin*dIII; P, *Pst*I; K, *Kpn*I.

phage library constructed with Charon 28. From the light-resistant colonies, phages were induced and analyzed further. We obtained one phage clone that was able to complement the photosensitive phenotype of cells which carried a mutation in *visB*. This phage clone was designated λvisB1.

Mapping of the *visB* gene. A 12-kb fragment derived from λvisB1 was hybridized with each member of the *E. coli* linking library prepared by Kohara et al. (11). Four clones from the library at around 63 min (shown in Fig. 3a) hybridized to this probe. These clones were used to lysogenize VS550, and the lysogens were incubated in the light. Only one lysogen, carrying clone 10B4, was able to grow in the light. To define more exactly the position of the *visB* gene, the inserted fragment from λvisB1 was cut into several pieces by *Kpn*I or *Pst*I and recloned in the plasmid vector pUC118. VS550 was transformed by the various plasmids, and the photosensitivity of the resulting strains was examined. As shown in Fig. 3b, the 2-kb *Kpn*I fragment and the 4-kb *Pst*I fragment were able to reverse the photosensitive phenotype of VS550.

Structures of the *visB* gene and the genes around it. We determined the nucleotide sequence of the inserts from the positive clones with lengths of more than about 5 kb. As shown in Fig. 4, we found four open reading frames (ORFs) on the same strand. The ORFs, presumably forming an operon, were 194, 441, 392, and 400 amino acids long (from the 5′ to the 3′ direction). From a comparison with the complementation analysis, the third ORF was the most probable candidate for the *visB* gene. We constructed a plasmid that contained only the third ORF and examined its effect on the photosensitivity of VS550. In fact, the transformants with a plasmid that carried the third ORF did not exhibit a photosensitive phenotype (data not shown). We postulated that the third ORF (i.e., pVK4) could be transcribed from the *lac* promoter of the vector. Thus, we designated the third ORF as the *visB* gene. Furthermore, we

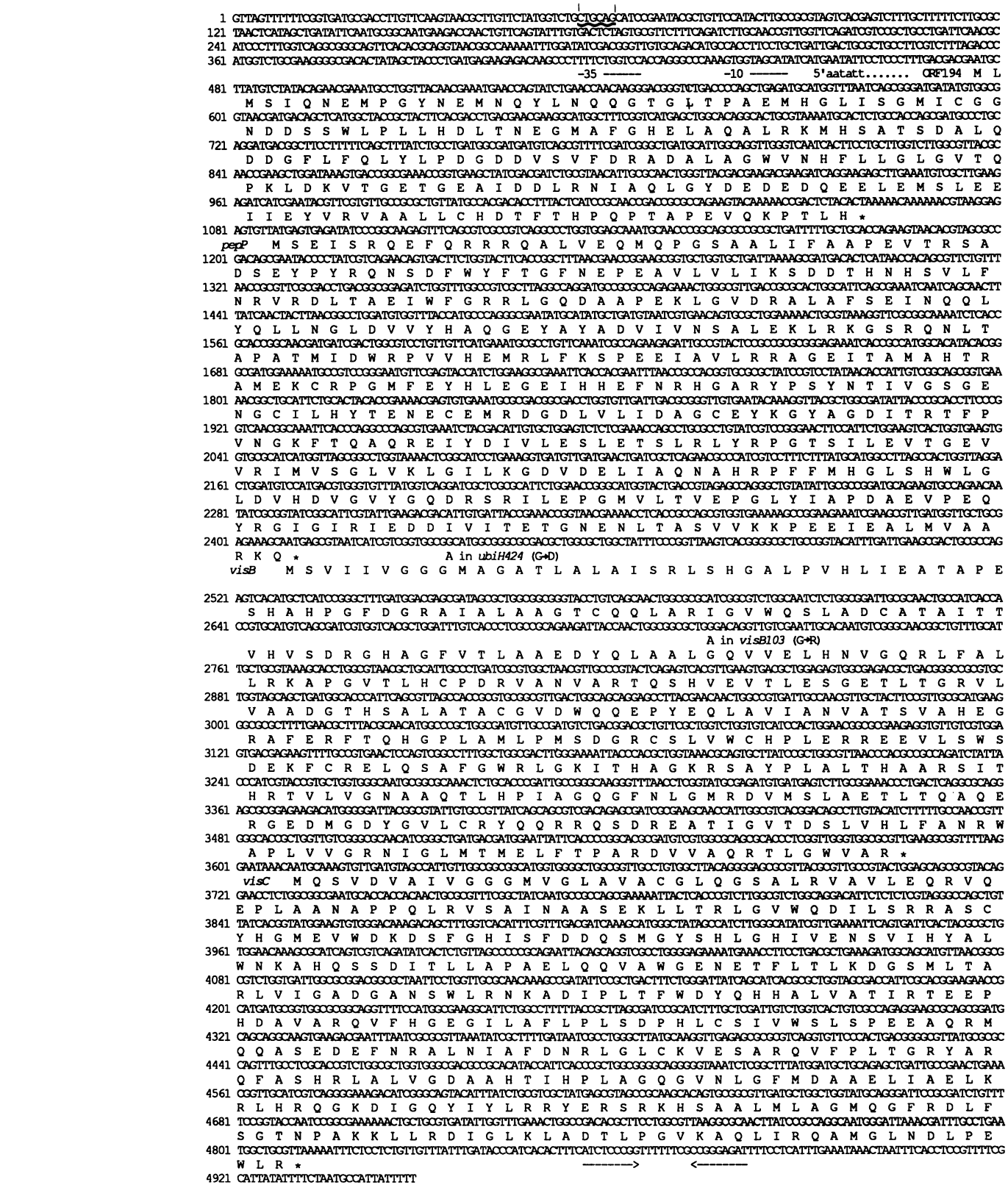

FIG. 4. The nucleotide sequence around the *visB* gene. Deduced amino acid sequences, promoter consensus sequences, and sites of initiation of transcription are shown below the DNA sequence. Names of genes are given before the N terminus of each translated product. Altered bases in *visB103* and the *ubiH424* are shown under the wild-type sequence, and the deduced changes in amino acids are shown in parentheses. The *Pst*I site described in the text is indicated by a wavy underline.

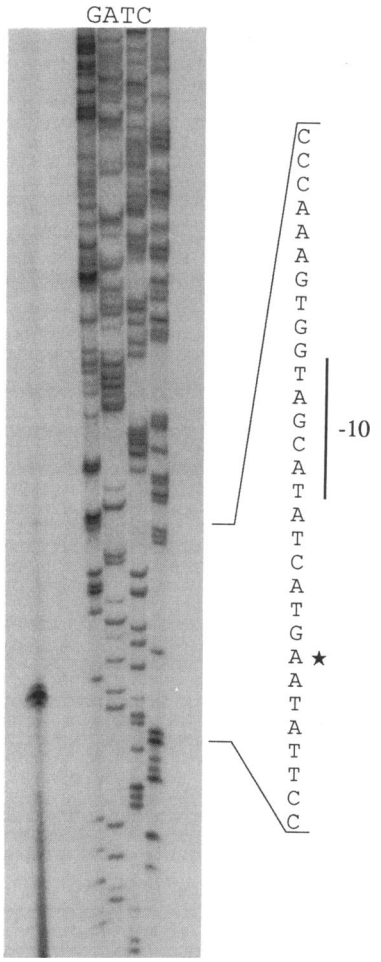

FIG. 5. Primer extension assay upstream of ORF194. A 20-mer oligonucleotide, TCATTTCGTTGTAACCAGGC, complementary to the 5′ end of ORF194, was used for primer extension with total RNA from CA274. DNA sequencing was performed with the same primer and is shown on the same gel. The sequence around the 5′ end of the RNA is shown. The A at the 5′ end and the deduced −10 promoter sequence are indicated. The A band corresponding to +2 is faint in the fractionation of the sequencing reaction.

determined the nucleotide sequence of the mutant *visB* gene from VS550. The mutant gene, designated *visB103*, had a single base change from G to A at the 103rd codon, which causes an amino acid substitution from glycine to arginine.

From a search of a protein data base, we found that the deduced product of the fourth ORF exhibited about 30% homology to the VisB protein. Thus, we designated this ORF *visC*. Furthermore, we found that the amino acid sequence encoded by the second ORF matched perfectly that of aminopeptidase P from *E. coli* HB101 (27). We confirmed that the genes matched at the nucleotide level and concluded that the second ORF in the operon was the *pepP* gene that encodes aminopeptidase P.

A primer extension assay, with oligonucleotides complementary to the 5′ end of the first ORF, gave a band that corresponded to the A residue 23 bases upstream from the ATG codon (Fig. 5). No band was observed with the oligonucleotide complementary to the *pepP* or *visB* gene. A search of a DNA data base revealed that the sequence upstream from the first *Pst*I site matched the sequence of the 1.3-kb *Pst*I fragment that encodes the *ssr* gene for stable 6S RNA (9).

Identification of the function of VisB. We searched for amino acid sequences in the data base that might be homologous to the sequences of the predicted VisB and VisC proteins, and we found that the latter protein, VisC, belongs to a class of proteins that have a βαβ unit or a nucleotide-binding fold (17). Within this class of proteins, 4-hydroxybenzoate hydroxylase (HBHase) from *Pseudomonas fluorescens* (25) exhibits a limited degree of homology, about 20%, to both VisB and VisC (Fig. 6). HBHase is a well-studied enzyme that catalyzes the monooxygenation of 4-hydroxybenzoate to 3,4-dihydroxybenzoate and contains one flavin adenine dinucleotide moiety per molecule (8, 26). In *P. fluorescens*, this enzyme is induced when the bacteria utilize external 4-hydroxybenzoate as their carbon source. However, in *E. coli*, HBHase may not have this function because *E. coli* cannot use this compound as a carbon source. Consequently, we presume that the *visB* and *visC* genes may encode enzymes similar to HBHase.

Among the genes mapped at about 62 to 63 min on the *E. coli* genome, the site of the *visB* and *visC* genes, only the *ubiH* gene encodes a monooxygenase that is thought to contain flavin adenine dinucleotide (10). UbiH protein catalyzes the conversion of 2-octaprenyl-6-methoxyphenol to 2-octaprenyl-6-methoxy-1,4-benzoquinone in the biosynthetic pathway to ubiquinone (28). This reaction appears to be similar to that catalyzed by HBHase. If the function of UbiH is completely lost, the bacterium should be deficient in ubiquinone and consequently should be unable to use nonfermentable sources of carbon (3). The *visB103* strain was able to grow on plates with succinate as the only carbon source. However, there remains the possibility that the function of the VisB protein is not completely lost when the *visB103* allele is present. To examine this possibility, part of the *visB* or *visC* gene on the chromosome of CA274 was replaced by the Km^r gene cassette to generate the Δ*visB* or Δ*visC* strain, VS560 or VS600. We confirmed that the Δ*visB* strain was photosensitive (Fig. 7), while the Δ*visC* strain was not. These strains were also tested for their ability to grow with a nonfermentable source of carbon, and we found that the Δ*visC* strain could use such a carbon source while the Δ*visB* strain could not. This result suggests that *visB*, but not *visC*, might be the same gene as *ubiH*. To confirm this possibility, plasmids with only the *visB* gene fragment or only the *visC* gene fragment were used to transform an authentic *ubiH* mutant, AN172. As expected, only the bacteria transformed by the *visB*-carrying plasmid were able to form colonies on M9 minimal plates with succinate as the sole carbon source.

We isolated a DNA fragment that included the *visB* gene from AN172 by polymerase chain reaction, and the sequence of the mutant *visB* gene was determined. There was a single nucleotide substitution, from G to A, at position 26 from the initiation codon, which corresponds to the replacement of glycine by aspartic acid at the ninth codon.

A *ubiA visB* double mutant is not photosensitive. The photosensitivity of the *visA* (*hemH*) mutants could be reversed by a second mutation in other *hem* genes that lie upstream within the biosynthetic pathway in which the product of the *visA* (*hemH*) gene is involved (15). We postulated that the same scheme might be applicable to the *visB* mutant, i.e., another mutation in a gene required for biosynthesis of ubiquinone might reverse the photosensitive phenotype. To test this hypothesis, Δ*visB* was introduced to the *ubiA* strain, which is unable to catalyze the prenylation

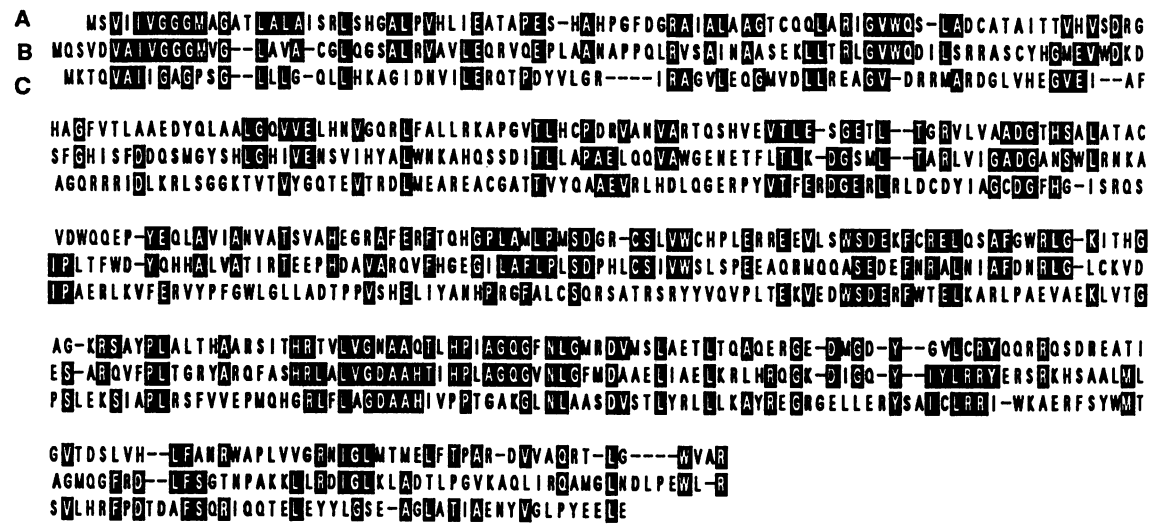

FIG. 6. Comparison of VisB and VisC with 4-hydroxybenzoate 3-monooxygenase from *P. fluorescens*. Amino acid sequences of the polypeptides have been aligned by introducing gaps (hyphens) to maximize identity. Identical residues are printed in white on black. (A) VisB; (B) VisC; (C) 4-hydroxybenzoate 3-monooxygenase from *P. fluorescens*.

of 4-hydroxybenzoate at an early stage in the biosynthesis of ubiquinone (3). In fact, as shown in Fig. 7, the *ubiA* Δ*visB* double mutant did not show photosensitivity, unlike the Δ*visB* single mutant.

Relationships between the *visB* mutation and sensitivity to superoxide. To examine the sensitivity of the photosensitive strains to a redox-cycling agent, we employed the gradient plate method. Suspensions of cells were applied in uniform lines on an agar plate that contained a linear gradient of paraquat. As shown in Fig. 8, it was clear that both *visB103* and Δ*visB* are more sensitive than the wild-type strain.

Mn-containing superoxide dismutase (Mn-SOD) plays the major role in protecting cells against the superoxide produced by redox-cycling agents (20, 22, 23). Extracts of the *vis*$^+$, *visA*, and *visB* cells were separated on a nondenaturing acrylamide gel, and the gel was stained for SOD activity. The results are presented in Fig. 9. Although the activity of Fe-containing SOD (Fe-SOD) in each sample was the same, the activity of Mn-SOD in the extract from *visB103* cells was apparently weaker than in that from other cells. This result was confirmed as follows. The *sodA* gene for Mn-SOD in each strain was replaced by a fusion gene of *sodA* and *lacZ* so that we could monitor the expression of *sodA* by monitoring the activity of β-galactosidase. At the same time, we also examined the expression of the *nfo* gene by use of a *nfo'-'lacZ* fusion gene on plasmid pHI201. As shown in

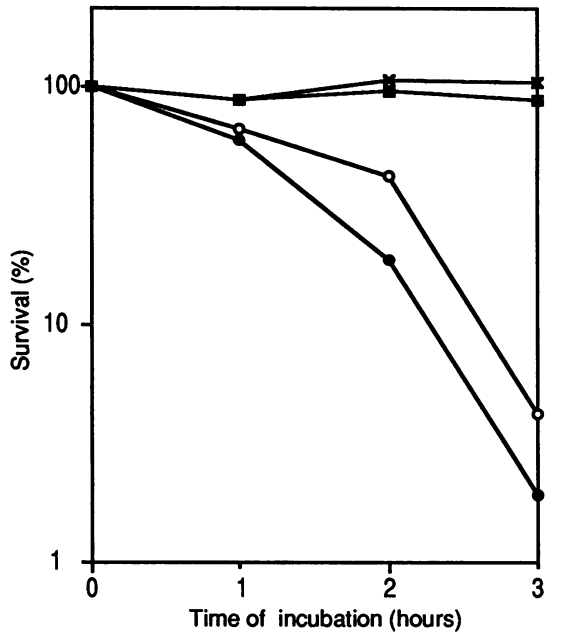

FIG. 7. Photosensitivity of Δ*visB* strains. Experiments were performed as described in the legend to Fig. 2. Symbols: ×, CA274 (wild type); ○, VS550 (*visB103*); ●, VS560 (Δ*visB*); ■, VS561 (Δ*visB ubiA*).

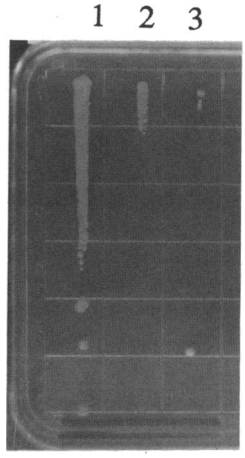

FIG. 8. Sensitivity of photosensitive strains to a redox-cycling agent. The assay for sensitivity to paraquat was performed as described in Materials and Methods. The concentration of paraquat was 0.4 mg/ml. Lanes: 1, CA274 (*visB*$^+$); 2, VS550 (*visB103*); 3, VS560 (Δ*visB*).

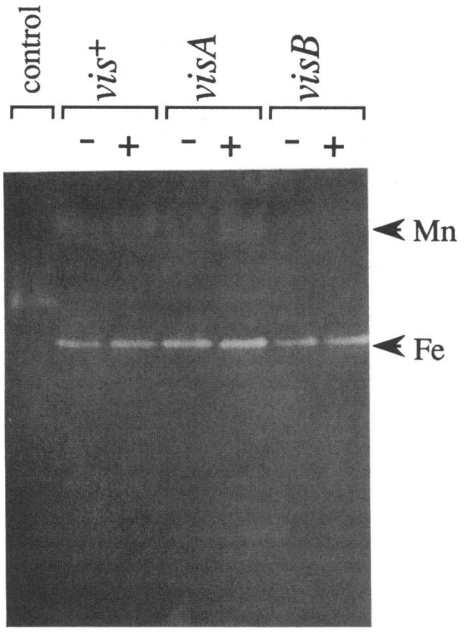

FIG. 9. SOD activity in the *visB* mutant. Cells were grown to 5 × 10^8 cells per ml for preparation of crude extracts. Crude extracts (50 μg of protein) were loaded on a nondenaturing 7% polyacrylamide gel. Bovine SOD (0.01 μg) was included as a positive control. After electrophoresis, the gel was stained for SOD activity as described elsewhere (6). CA274 (wild type), VS101 (*visA*), and VS550 (*visB*) were used for preparation of cell extracts. Symbols: −, cells were not treated with paraquat; +, paraquat (final concentration, 10 μM) was added to the medium 45 min before harvest. Abbreviations: Mn, Mn-SOD; Fe, Fe-SOD.

Table 2, the β-galactosidase activity expressed from Φ(*sodA'-'lacZ*) in the *visB103* strain was lower than that in the *visB*$^+$ strain with or without induction by paraquat. In contrast, the expression from Φ(*nfo'-'lacZ*) remained normal or even increased slightly.

TABLE 2. Activity of the fusion genes

Genotype	β-Galactosidase activity[a]			
	Φ(*sodA'-'lacZ*)		Φ(*nfo'-'lacZ*)	
	−	+	−	+
Wild type[b]	314	801	545	1095
visB103[c]	106	192	761	2229

[a] For the β-galactosidase assay, 0.001 volume of an overnight culture was inoculated into LB broth and incubated for 4 h in the dark. Paraquat was then added to the cultures (+; 10 μM) or not (−), and the cultures were incubated for 45 min. The amount of β-galactosidase was determined as described by Miller (13).

[b] VS011 was used for Φ(*sodA'-'lacZ*), and CA274/pHI201 was used for Φ(*nfo'-'lacZ*).

[c] VS551 was used for Φ(*sodA'-'lacZ*), and VS550/pHI201 was used for Φ(*nfo'-'lacZ*).

DISCUSSION

***visB* and *ubiH* genes.** Since the *ubiH424* mutation could be complemented by the plasmid that carried the wild-type *visB* gene, the *ubiH* gene and the *visB* gene appear to be the same gene. Knoell (10) predicted that the UbiH protein, which encodes 2-octaprenyl-6-methoxyphenol monooxygenase, is a flavin oxygenase. In fact, the amino acid sequence of the VisB protein deduced from the nucleotide sequence is rather similar to those of other flavin monooxygenases, such as 4-hydroxybenzoate-hydroxylase from *P. fluorescens*. The site of the mutation in *ubiH424*, in the ninth codon, is located in a highly conserved region of the nucleotide-binding domain. This mutation should be sufficient to cause the *ubiH* mutant phenotype. However, the *visB103* mutant bacteria retained its respiratory ability and, when the plasmid carrying the *visB103* mutant gene was used to transform the *ubiH424* strain, the transformants had the *ubi*$^+$ phenotype, although they grew slightly more slowly than the *ubiH424* strain which was transformed with the wild-type *visB* gene (unpublished results). From these pieces of evidence, it appears that the *visB103* mutation affects the enzymatic activity only slightly.

Sensitivity to paraquat. Both *visB103* and Δ*visB* mutants displayed high sensitivity to paraquat. This sensitivity is probably due to the low-level expression of Mn-SOD observed in these mutants. Mn-SOD is encoded by the *sodA* gene and protects cells from superoxide generated by paraquat. *sodA* and *nfo* are part of the same regulon and are induced simultaneously under the control of SoxRS when cells are exposed to superoxide stress (2, 7). However, in the case of the *visB* mutant, only the expression of *sodA* was reduced. This result may be due to the situation with respect to the fusion genes, namely, the *sodA'* fusion had been replaced by the original gene whereas the *nfo'* fusion was present as extra copies on a plasmid. Alternatively, there is a possibility that some other regulatory mechanism specific to *sodA* exists. We have not yet excluded this possibility. Because this system is rather complicated, we have to perform more experiments to determine conclusively whether the effects are direct or indirect.

Photosensitivity of VS550. VS550 cells not only failed to grow under illumination but died during the illumination. We have not determined the wavelength of the light that has this toxic effect, but it is not within the UV spectrum because insertion of a glass plate between the light source and the cells did not prevent cell death. This phenotype is similar to tnat of *visA* mutants (14, 15). The salient difference between VS550 and *visA* mutants is reflected in the effect of ascorbic acid on the photosensitivity.

Mechanism of the photosensitivity. The ability of ascorbate to reverse the photosensitivity gives some clues as to the direct origin of the toxic effect of light. Ascorbate is known as a good antioxidant and can quench many forms of active oxygen. If the cause of the toxic effect that is associated with the *visB* mutation is a species of active oxygen, it is not unexpected that the photosensitivity should be cured by ascorbate. It is possible that the addition of ascorbate to aerobic cells could generate an appropriate reducing environment for induction of synthesis or activation of function of the VisB (UbiH) proteins. Alternatively, it is also possible that a mutation in the *visB* gene affects the regulation of expression of *sodA*, which protects cells from active oxygen.

In preliminary experiments, we have found that the *visB103* and the Δ*visB* strains are not photosensitive under anaerobic conditions. This photosensitive phenotype under anaerobic conditions was also observed in the case of the *visA* mutants. The fact that the *ubiA* mutation could suppress the photosensitivity suggests that an accumulation of some intermediates in the biosynthesis of ubiquinone triggers the photosensitive phenotype. However, the evidence is not as clear as in the case of *visA* mutants, in which the

immediate substrate of the reaction catalyzed by the VisA product causes the photosensitivity.

In contrast to *visB103* and Δ*visB*, the *ubiH424* mutant did not show photosensitivity (data not shown). We have not yet eliminated the possibility that the *ubiH424* mutant is simply more leaky than the *visB103* mutant. However, given that it was difficult to isolate the photosensitive *visB* mutant, it seems that a simple loss-of-function mutation in the *visB* (*ubiH*) gene does not cause photosensitivity. It is possible that some alteration in some other system, such as the mechanism of regulation of the biosynthesis of ubiquinone, causes the photosensitivity.

Function of VisC. VisC exhibits striking homology to VisB and other flavin monooxygenases, and it should have a similar activity. Furthermore, the location of the *visC* gene immediately downstream of *visB* suggests that VisB and VisC may have related functions. However, VisC is not necessary for the production of ubiquinone, because deletion of the *visC* gene from the *E. coli* chromosome had no effect on photosensitivity and aerobic respiration. The function of the *visC* gene remains obscure.

ACKNOWLEDGMENTS

We thank S. Yonei for strains, plasmids, and valuable discussions and I. G. Young for strains.

This research was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

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