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Isolation and Characterization of *Escherichia coli* Strains Containing New Gene Fusions (*soi*::*lacZ*) Inducible by Superoxide Radicals

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Gene fusions in *Escherichia coli* that showed increased β-galactosidase expression in response to treatment with a superoxide radical (O_2^-) generator, methyl viologen (MV), were obtained. These fusions were constructed by using a Mud(Ap *lac*) phage to insert the lactose structural genes randomly into the *E. coli* chromosome. Ampicillin-resistant colonies were screened for increased expression of β-galactosidase on X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) plates containing MV at 1.25 μg/ml. Other O_2^- generators, menadione and plumbagin, also induced β-galactosidase activity in these fusion strains. The induction by these drugs occurred only under aerobic conditions. Hyperoxygenation also elicited an induction of the fusions. On the other hand, no significant induction was observed with hydrogen peroxide and cumene hydroperoxide. The induction of these fusions by MV was not dependent on the peroxide stress control mediated by the *oxyR* gene or on the *recA*-dependent SOS system. These fusions were named *soi* (superoxide inducible)::*lacZ*. The induction of β-galactosidase was significantly reduced by introducing a *soxS*::Tn*10* locus into the fusion strains, indicating that the *soi* genes are members of the *soxRS* regulon. Five of the fusions were located in 6 to 26 min of the *E. coli* genetic map, while three fusions were located in 26 to 36 min, indicating that these fusions are not related to genes already known to be inducible by O_2^- under the control of *soxRS*. At least five mutants containing the *soi*::*lacZ* fusion were more sensitive to MV and menadione than the wild-type strain, suggesting that the products of these *soi* genes play an important role in protection against oxidative stress.

Active oxygens such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical naturally occur during normal metabolism in aerobically growing cells as a result of incomplete reduction of molecular oxygen and also arise from a variety of endogenous and environmental sources such as ionizing radiation and redox-cycling agents (11, 13, 17, 25, 37, 42). Cells maintain a strong defense against the oxidative threat. Aerobically growing *Escherichia coli* cells, for example, have two superoxide dismutases (SODs), Mn-SOD and Fe-SOD, which dismutate O_2^- to H_2O_2 (15, 33, 35, 37). H_2O_2 is disposed of by two catalase-hydroperoxidases, HPI and HPII, to yield H_2O and O_2 (22, 37). Under certain circumstances, however, the concentration of active oxygen increases to a level over the basal level of the cell's defense capacity, giving rise to an oxidative stress. *E. coli* cells respond to increased flux of active oxygen by activating a set of coregulated genes (5, 9, 11, 30). In response to increased flux of H_2O_2 and other organic peroxides such as cumene hydroperoxide, the expression of at least 30 proteins resolved by two-dimensional polyacrylamide gel electrophoresis becomes elevated over the basal levels (5). The peroxide stress proteins include eight proteins in *E. coli* that are positively regulated by the locus, *oxyR* (5, 6, 29, 31, 32). On the other hand, upon exposure to elevated levels of O_2^-, *E. coli* cells respond to superoxide stress by invoking different proteins (11, 39). Treatment of cells with methyl viologen (MV), for example, elicits an induction of more than 30 proteins (14, 39). At least nine of these proteins are produced by a regulon controlled by two regulatory genes, *soxR* and *soxS* (14, 36, 40, 41), which are positioned head to head with an intervening sequence of 85 bp (40). Gene products already identified to be controlled by the *soxRS* include Mn-SOD, glucose-6-phosphate dehydrogenase, paraquat diaphorase, a modified ribosomal protein, an antisense RNA for *ompF* mRNA, and a DNA repair enzyme, endonuclease IV (4, 9, 11, 14, 26, 39, 40). Only a few of the proteins induced by O_2^- generators have been identified with enzymatic activities and genes. The present experiments were done in order to clarify the nature, regulation, and function of genes induced by superoxide stress in *E. coli*.

Several approaches to identify proteins and genes induced in oxidative stress conditions have been taken. An important approach is to screen random operon fusions to a reporter gene (e.g., *lacZ*) for inducibility by oxidative stress. Mud(Ap *lac*) phage which randomly inserts into the *E. coli* chromosome (3, 18) is often used to create operon fusions (11, 18, 31, 34, 40). With this approach, Kogoma et al. (19) have isolated two new O_2^--inducible gene fusions, *soi-28* and *soi-17*/*soi-19*, that are different from genes already known to be induced by O_2^-. In this article, we report several more O_2^--inducible gene fusions in *E. coli* isolated by using the Mud(Ap *lac*) phage.

MATERIALS AND METHODS

Bacterial strains and growth media. All bacterial strains used during this study are derivatives of *E. coli* K-12 and listed in Table 1. Bacterial cells were routinely grown in Luria-Bertani medium (23) unless otherwise stated. Preparation of Mud(Ap *lac*) lysates and transduction with the phage were done as described by Silhavy et al. (27). The

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TABLE 1. *E. coli* strains used

Strain	Description	Source or reference
GC4468	F⁻ *lac4169 rpsL*	2
QC774	GC4468 with Δ(*sodA-lacZ*)*49* Δ(*sodB-kan*)*1*-Δ_2 Cm^r Kan^r	2
MAL103	Δ(*gpt-lac*) Mud(Ap *lac*)	27
JC10284	*srlR*::Tn*10* Δ(*srlR-recA*)*306*	20
CAG8396	*argE86*::Tn*10*	28
TA4112	*oxy*Δ*3*	5
KS290	TA4112 with *argE86*::Tn*10*	P1(CAG8396) × TA4112
BW840	*soxS3*::Tn*10*	36
KS260	GC4468 with *soi-5*::*lacZ*	This study
KS262	GC4468 with *soi-7*::*lacZ*	This study
KS263	GC4468 with *soi-8*::*lacZ*	This study
KS264	GC4468 with *soi-9*::*lacZ*	This study
KS265	GC4468 with *soi-10*::*lacZ*	This study
KS288	GC4468 with *soi-11*::*lacZ*	This study
KS266	GC4468 with *soi-12*::*lacZ*	This study
KS267	GC4468 with *soi-13*::*lacZ*	This study
KS301	KS260 with *oxy*Δ*3 argE86*::Tn*10*	P1(KS290) × KS260
KS302	KS262 with *oxy*Δ*3 argE86*::Tn*10*	P1(KS290) × KS262
KS303	KS263 with *oxy*Δ*3 argE86*::Tn*10*	P1(KS290) × KS263
KS304	KS264 with *oxy*Δ*3 argE86*::Tn*10*	P1(KS290) × KS264
KS305	KS265 with *oxy*Δ*3 argE86*::Tn*10*	P1(KS290) × KS265
KS306	KS288 with *oxy*Δ*3 argE86*::Tn*10*	P1(KS290) × KS288
KS307	KS266 with *oxy*Δ*3 argE86*::Tn*10*	P1(KS290) × KS266
KS308	KS267 with *oxy*Δ*3 argE86*::Tn*10*	P1(KS290) × KS267
KS309	KS260 with Δ(*srlR-recA*)	P1(JC10284) × KS260
KS310	KS262 with Δ(*srlR-recA*)	P1(JC10284) × KS262
KS311	KS263 with Δ(*srlR-recA*)	P1(JC10284) × KS263
KS312	KS264 with Δ(*srlR-recA*)	P1(JC10284) × KS264
KS313	KS265 with Δ(*srlR-recA*)	P1(JC10284) × KS265
KS314	KS288 with Δ(*srlR-recA*)	P1(JC10284) × KS288
KS315	KS266 with Δ(*srlR-recA*)	P1(JC10284) × KS266
KS316	KS267 with Δ(*srlR-recA*)	P1(JC10284) × KS267
KS317	KS260 with *soxS3*::Tn*10*	P1(BW840) × KS260
KS318	KS262 with *soxS3*::Tn*10*	P1(BW840) × KS262
KS319	KS263 with *soxS3*::Tn*10*	P1(BW840) × KS263
KS320	KS264 with *soxS3*::Tn*10*	P1(BW840) × KS264
KS321	KS265 with *soxS3*::Tn*10*	P1(BW840) × KS265
KS322	KS288 with *soxS3*::Tn*10*	P1(BW840) × KS288
KS323	KS266 with *soxS3*::Tn*10*	P1(BW840) × KS266
KS324	KS267 with *soxS3*::Tn*10*	P1(BW840) × KS267
HfrH	*valS*(97)←*uxuAB*(98)	28
KL96	*his*(44)→*mgl*(45)	28
BW372	*nth-1*::*kan*	8
GW2100	*umuC*::*kan*	20
CAG18515	*proAB3096*::*kan*	28
CAG1681	*proAB81*::Tn*10*	28
CAG18497	*fadR13*::Tn*10*	28
CAG12016	*zcg-3060*::Tn*10*	28
CAG12169	*zch-506*::Tn*10*	28
CAG1655	*zci-233*::Tn*10*	28
CAG12081	*zcj-3061*::Tn*10*	28
CAG12026	*trg-2*::Tn*10*	28
CAG18461	*zdc-235*::Tn*10*	28
CAG18459	*zde-234*::Tn*10*	28
CAG18462	*zdg-603*::Tn*10*	28
KS500	KL96 with *zdg-603*::Tn*10 umuC*::*kan*	This study
KS501	KL96 with *zcg-3060*::Tn*10 nth-1*::*kan*	This study
KS502	HfrH with *zcg-3060*::Tn*10 proAB3096*::*kan*	This study
KS503	HfrH with *proAB81*::Tn*10 umuC*::*kan*	This study

transduction experiments with P1 phage were performed according to Miller (23). The concentrations of antibiotics used were (per milliliter) 50 μg of ampicillin, 25 μg of tetracycline, 50 μg of kanamycin, and 50 μg of streptomycin sulfate. X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) was added to give a concentration of 40 μg/ml in Luria-Bertani agar.

Construction of gene fusions. Colonies of *E. coli* GC4468 containing random insertions of Mud(Ap *lac*) were prepared as described by Silhavy et al. (27). Ampicillin-resistant (Ap^r)

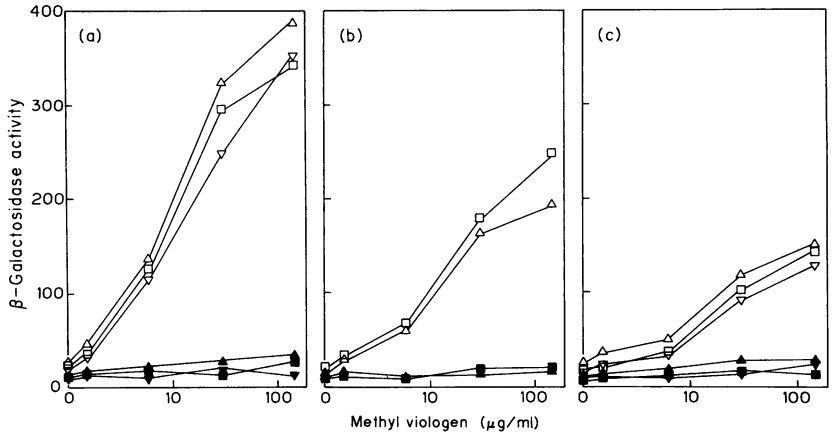

FIG. 1. Induction of β-galactosidase by MV in *soi*::*lacZ* fusion strains of *E. coli*. Exponentially growing *E. coli* cells containing the *soi*::*lacZ* fusion were treated with MV at various concentrations at 30°C for 1 h under aerobic (open symbols) or anaerobic (closed symbols) conditions, and then β-galactosidase activity was assayed. (a) KS262 (△ and ▲), KS264 (□ and ■), and KS265 (▽ and ▼); (b) KS288 (□ and ■) and KS266 (△ and ▲); (c) KS260 (△ and ▲), KS263 (□ and ■), and KS267 (▽ and ▼).

colonies were replica plated on X-Gal–ampicillin plates in the absence or presence of MV at 1.25 μg/ml and then incubated at 30°C for about 18 h under aerobic conditions. Colonies that appeared to produce more β-galactosidase in the presence of MV than in the absence of the agent were picked and further used to test for O_2^- inducibility.

Treatment with various oxidizing agents. Overnight cultures of *E. coli* with fusions were diluted 100-fold in prewarmed Luria-Bertani medium and grown at 30°C with aeration. When the concentration reached about 2×10^8 cells per ml, cell suspensions were mixed with drugs at various concentrations and incubated at 30°C for 1 h, and then β-galactosidase activity was assayed. Anaerobic and hyperoxic conditions were achieved by bubbling nitrogen gas and pure oxygen gas, respectively, through a medium during incubation with or without drugs.

Measurement of β-galactosidase activity. β-Galactosidase activity in whole cells was determined as described by Miller (23). Values represent the means of at least three independent assays.

Tests for MV and menadione sensitivities. *E. coli* cells were grown overnight at 30°C in M9 medium (23) supplemented with 0.25% Casamino Acids (EM9 medium) and diluted 10^3-fold with phosphate-buffered saline. Sterilized papers (0.4 by 12 cm) were put in the cell suspensions, kept at room temperature for 5 min, and then placed on EM9 plates containing MV or menadione with a linear gradient of concentration (0 to 50 μg/ml). These plates were incubated at 30°C overnight before the zone of growth inhibition was determined.

Mapping of fusions. Mapping of fusions on the *E. coli* genetic map was carried out by interrupted mating with Hfr strains (23) and by a Tn*10* marker P1 mapping kit. The procedures followed were described by Miller (23) and Singer et al. (28). Four subsets of Hfr strains carrying Tet^r and Kan^r markers were constructed by P1 transduction. These Hfr strains and recipient *soi*::*lacZ* fusion strains were mixed and incubated at 30°C for up to 2 h.

Chemicals. MV and cumene hydroperoxide were obtained from Nakarai chemicals. Ampicillin, tetracycline hydrochloride, kanamycin, streptomycin sulfate, hydrogen peroxide (30%), and 2-nitrophenyl-β-D-galactopyranoside were purchased from Wako Pure Chemicals. Menadione and plumbagin were obtained from Sigma Chemicals. X-Gal was obtained from Takara Shuzo. All other reagents used were of highest purity commercially available.

RESULTS

Construction of O_2^--inducible gene fusions. The insertion of Mud(Ap *lac*) phage into *E. coli* chromosomal DNA is random (3). If the insertion creates an operon fusion to a gene, *lacZ* will be transcribed by the gene promoter (3, 18, 34) and the resulting colonies become blue on an X-Gal plate. About 20,000 Ap^r colonies were picked and tested for O_2^- inducibility. These colonies were replica plated on X-Gal–ampicillin plates in the absence or presence of MV at 1.25 μg/ml. MV is a typical redox-cycling agent which generates O_2^- within cells in the presence of oxygen (13, 16, 17). About 30 colonies were strongly blue in the presence of MV but only pale in the absence of the agent. Among these, 12 strains were proved, on further in vitro measurement of β-galactosidase activity, to contain gene fusions which became induced (or derepressed) during incubation with MV. As described below, the fusions were most strongly induced by O_2^--generating agents. Therefore, these fusions were called *soi* (for superoxide-inducible)::*lacZ* fusions. In this study, eight strains, KS260 (*soi-5*), KS262 (*soi-7*), KS263 (*soi-8*), KS264 (*soi-9*), KS265 (*soi-10*), KS288 (*soi-11*), KS266 (*soi-12*), and KS267 (*soi-13*), which showed the highest induction by MV and had normal levels of SOD and catalase activities were selected for detailed characterization.

Induction of the *soi*::*lacZ* fusions with O_2^--generating agents. β-Galactosidase activity in *soi*::*lacZ* strains treated with MV was measured by using freshly prepared extracts. Figure 1 shows that, in these cells, the specific activity of β-galactosidase increased with increasing dose of MV under aerobic conditions. On the other hand, no significant induction of β-galactosidase activity under anaerobic conditions was observed. The other superoxide generators, plumbagin and menadione, also induced these *soi*::*lacZ* fusions (Fig. 2). Both drugs also required the presence of oxygen to induce the *soi*::*lacZ* fusions. The induction of β-galactosidase activity in all fusion strains occurred extensively during con-

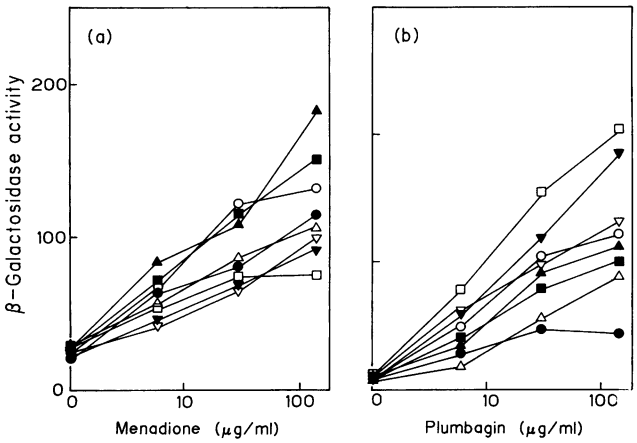

FIG. 2. Induction of β-galactosidase by menadione and plumbagin in *soi*::*lacZ* fusion strains of *E. coli*. Exponentially growing *E. coli* cells were treated with menadione (a) or plumbagin (b) at various concentrations under aerobic conditions. The activity of β-galactosidase was determined according to the method of Miller (23). ○, KS260; ●, KS262; □, KS263; ■, KS264; △, KS265; ▲, KS288; ▽, KS266; ▼, KS267.

tinuous bubbling of pure oxygen gas through a culture (data not shown).

Induction of the *soi*::*lacZ* fusions with H_2O_2. Incubation of *E. coli* cells with low concentrations of H_2O_2 causes induction of about 30 proteins, including HPI (5, 9, 11, 30), which is encoded by the *katG* gene (22). Treatment with H_2O_2 causes increased expression of the *katG*::*lacZ* fusion (5, 6, 31), whereas it does not induce *sodA*::*lacZ* fusion (34). Kogoma et al. (19) have reported that treatment of three *soi*::*lacZ* fusion strains with H_2O_2 at 50 μM resulted in only a slight increase in β-galactosidase activity whereas significant levels of induction required a concentration of above 400 μM. In this study, induction of the *soi*::*lacZ* fusions was tested with 50 and 500 μM H_2O_2. No significant induction was observed with H_2O_2 at concentrations employed (data not shown). It was also demonstrated that cumene hydroperoxide at 500 μM did not cause the induction of β-galactosidase activity (data not shown).

Effects of *recA*, *oxyR*, and *soxS* mutations on the induction of the *soi*::*lacZ* fusions. Expression of eight of the peroxide-inducible proteins is under the control of a positive regulator, *oxyR*, and is not induced in the *oxyR* mutants (5, 9, 11, 30). The *oxyR* deletion mutation was introduced into *soi*::*lacZ* fusion strains by use of P1 phage to examine the effect of the mutation on the expression of *soi*::*lacZ* fusions. These *oxyR* derivatives were treated with MV at 30°C for 1 h, and then in vitro β-galactosidase activity was measured. The *oxyR* mutation did not affect the rate of induction of β-galactosidase activity (Table 2). It was also demonstrated that the induction of *soi*::*lacZ* fusions was independent of the *recA*-mediated SOS response (38) (Table 2).

Superoxide stress induces more than 30 proteins in *E. coli*, resolved by two-dimensional polyacrylamide gel electrophoresis, which are different from the peroxide-induced proteins (11, 39). The expression of Mn-SOD, endonuclease IV, glucose-6-phosphate dehydrogenase, and an antisense RNA for *ompF* (1, 24) is positively regulated by two regulatory loci, *soxR* and *soxS* (4, 9, 11, 14, 26, 39, 40). It was of interest to know whether the *soi* genes are members of the *soxRS* regulon. The effect of the *soxS*::Tn*10* mutation on the induction of β-galactosidase by MV in the *soi*::*lacZ* fusion strains was examined (Fig. 3). The induction of *soi*::*lacZ* fusions by MV was strongly reduced in the fusion strains containing *soxS*::Tn*10*.

TABLE 2. MV-induced expression of the *soi*::*lacZ* fusions in *E. coli oxyR* and *recA* strains

Fusion	Fold increase in β-galactosidase[a]		
	Wild type	*oxyR*	*recA*
soi-5::*lacZ*	2.3	2.7	2.3
soi-7::*lacZ*	6.9	6.9	6.2
soi-8::*lacZ*	5.0	7.3	6.3
soi-9::*lacZ*	6.4	5.5	6.7
soi-10::*lacZ*	5.5	5.4	6.3
soi-11::*lacZ*	5.1	5.6	5.3
soi-12::*lacZ*	4.8	4.9	4.9
soi-13::*lacZ*	2.6	3.4	2.9

[a] Assayed by the method of Miller (23) after *E. coli* cells were treated with MV at 30 μg/ml for 1 h.

Sensitivities of the *soi*::*lacZ* fusion strains to superoxide-generating drugs. The insertion of Mud(Ap *lac*) phage into the *E. coli* chromosome is a mutational event. Construction of the fusion presumably brings about the disruption of the functions of *soi* genes that might play an important role in protection against superoxide stress. Therefore, the sensitivities of the *soi*::*lacZ* fusion strains to the lethal effects of superoxide-generating drugs were examined (Table 3). At least five strains bearing the *soi*::*lacZ* fusion, KS262, KS263, KS264, KS265, and KS266, showed increased sensitivities to MV and menadione compared with those of the wild-type GC4468 strain. The sensitivity of QC774 lacking active SODs (2) was greater than that of any *soi*::*lacZ* fusion strain.

Genetic mapping of *soi*::*lacZ* fusions. We used Hfr strains to determine the approximate positions of *soi*::*lacZ* fusions on the *E. coli* genetic map. KS500 and KS501 are derivatives of Hfr KL96 and transfer the chromosome counterclockwise from 44 min, while KS502 and KS503, derivatives of HfrH, transfer the chromosome clockwise from 98 min (28). A cross of each fusion strain with four Hfr strains allowed selection for Sm^r and Tet^r, and then conjugants were scored for Ap^s and Kan^r. In a cross of KS265, carrying the *soi-10*::*lacZ* fusion, with KS500 and KS501, for example, the Ap^s marker appeared around 60 min between the Tet^r (around 45 min) and Kan^r (around 75 min) markers during conjugation at 30°C. On the other hand, in a cross of KS262, carrying *soi-7*::*lacZ*, with KS502 and KS503, the Ap^s marker could be detected between the Tet^r and Kan^r markers (data not shown). These results demonstrated that the *soi-7*::*lacZ* and *soi-10*::*lacZ* fusions are located at 6 to 26 min and at 26 to 36 min, respectively, on the *E. coli* genetic map. By the same methods, it was also revealed that four fusions (*soi-5*, *soi-8*, *soi-11*, and *soi-13*) were located at 6 to 26 min while another two fusions (*soi-9* and *soi-12*) were located at 26 to 36 min (data not shown). P1 linkage experiments showed that both *soi-10*::*lacZ* and *soi-12*::*lacZ* were cotransduced with the *zcj* locus at 29.50 min (Table 4).

DISCUSSION

In order to clarify the nature, regulation, and functions of the superoxide-inducible proteins, we first attempted to isolate and characterize O_2^--inducible genes in *E. coli* by using the same methodology as that described by Kogoma et al. (19). Mud(Ap *lac*) phage was used to construct fusions of

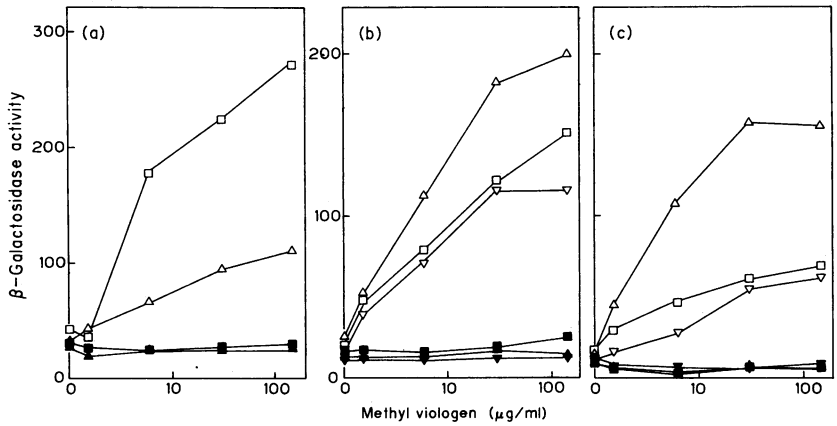

FIG. 3. The *soxS* gene-dependent induction of *soi*::*lacZ* fusions upon treatment with MV in *E. coli*. *soxS*::Tn*10* was transferred to *soi*::*lacZ* fusion strains by P1 transduction (23). (a) △, KS260; □, KS288; ▲, KS317; ■, KS322. (b) △, KS262; ▽, KS264; □, KS267; ▲, KS318; ▼, KS320; ■, KS324. (c) △, KS263; ▽, KS265; □, KS266; ▲, KS319; ▼, KS321; ■, KS323.

O_2^--inducible genes with *lac* genes. Eight clones for which β-galactosidase activity was elevated over the basal level upon treatment with low concentrations of MV were isolated (Fig. 1). The other O_2^--generating drugs, menadione and plumbagin, also induced the fusions (Fig. 2), while hydrogen peroxide and cumene hydroperoxide did not. The induction with MV, menadione, and plumbagin occurred only under aerobic conditions. Hyperoxygenation also induced the fusions. In addition, *E. coli* QC774, lacking active SODs (2), is highly sensitive to the drugs and hyperoxygenation, indicating that such agents generate O_2^- within *E. coli* cells (10, 33). Therefore, it is concluded that the fusions are induced by O_2^- produced by the agents. Thus, we also named these gene fusions *soi* (for superoxide-inducible)::*lacZ* fusions, as did Kogoma et al. (19).

The induction of these fusions was not affected by mutations in *oxyR* and *recA* genes (Table 2). This indicates that *soi* genes are not one of the regulons of the *oxyR* and *recA* genes. In contrast, the induction of these *soi*::*lacZ* fusions was markedly reduced by the mutation in the *soxS* regulator gene (Fig. 3). Therefore, the *soi* genes are members of the *soxRS* regulon. In *E. coli*, at least four genes have been identified to be members of the *soxRS* regulon and mapped on the *E. coli* chromosome (9, 11, 14, 40). These are *sodA*, *nfo*, *zwf*, and *micF*, which encode Mn-SOD, endonuclease IV, glucose-6-phosphate dehydrogenase, and an antisense RNA for *ompF* translation, respectively (4, 9, 11, 14, 26, 30, 40). These genes are found to be located at 88, 47, 41, and 48 min on the *E. coli* genetic map, respectively (1, 7, 11, 14, 24, 26, 33). In addition, two *soi* genes isolated by Kogoma et al., *soi-28* and *soi-17*/*soi-19*, are located at 47 and 45 to 61 min, respectively (19). The expression of the two *soi* genes is also shown to be regulated by *soxRS* (11). Five of the *soi*::*lacZ* fusions obtained in this study were located at 6 to 26 min on the *E. coli* genetic map, while three fusions were located at 26 to 36 min. Therefore, none of the *soi* genes are related to the six *soxRS*-regulated genes described above. The *soxS* gene itself is inducible by O_2^- under the control of the *soxR* gene (41). However, none of the *soi* genes are identical with the *soxS* gene, because the induction of the *soi*::*lacZ* fusions strongly depends on the function of the *soxS* gene (Fig. 3). Recently, Liochev and Fridovich (21) found that fumarase C

TABLE 3. Sensitivities of *soi*::*lacZ* fusion strains to MV and menadione[a]

Strain	Zone of growth inhibition (cm)[b]	
	MV	Menadione
Wild type	1.2	1.0
sodA sodB	8.2	9.0
soi-5::*lacZ*	1.8	1.2
soi-7::*lacZ*	5.0	3.5
soi-8::*lacZ*	2.8	5.2
soi-9::*lacZ*	3.5	3.5
soi-10::*lacZ*	5.6	5.2
soi-11::*lacZ*	2.0	3.0
soi-12::*lacZ*	5.8	5.5
soi-13::*lacZ*	1.8	1.6

[a] EM9 plates contained MV or menadione with a linear gradient of concentration of 0 to 50 μg/ml.
[b] Values are means of at least three independent assays.

TABLE 4. Mapping of the *soi-10*::*lacZ* and *soi-12*::*lacZ* fusions on the *E. coli* genetic map by P1 transduction[a]

Fusion	Tn*10* (min)	No. of transductants		% Ap[s] transductants
		Tet[r]	Tet[r] Ap[s]	
soi-10::*lacZ*	26.75	140	0	0
	27.25	98	0	0
	28.50	84	0	0
	29.50	222	92	41.4
	31.00	404	34	8.4
	32.00	282	0	0
	33.50	79	0	0
	35.75	117	0	0
soi-12::*lacZ*	26.75	222	0	0
	27.25	77	0	0
	28.50	60	0	0
	29.50	157	57	36.3
	31.00	187	16	8.6
	32.00	84	0	0
	33.50	66	0	0

[a] The Tn*10* marker was transferred from Tn*10* mapping strains (28) to each *soi*::*lacZ* fusion strain.

is also expressed under the control of the *soxRS* genes. The *fumC* gene encoding fumarase C is located at 35.5 min (21). Thus, it is concluded that the *soi* genes described in this paper are newly isolated genes. However, it is likely that some *soi*::*lacZ* fusions occur in one locus. Identification of the *soi* genes needs further detailed studies.

A goal of the present study was to identify the products and their functions involved in protection against superoxide stress. Cloning and characterization of the *soi* genes are under investigation in our laboratory. Functions of the *soi* genes are yet unknown, but five strains containing the *soi*::*lacZ* fusion were more sensitive to the lethal effect of MV and menadione than the parental strain (Table 3). This interesting property of the *soi* mutants allows us to conclude that the products of these *soi* genes play an important role in protection against superoxide stress. Farr et al. (11, 12) have found that treatment of *E. coli* cells with low doses of plumbagin results in an induction of the DNA repair enzyme. It is of interest to examine whether some *soi* mutants are deficient in such an inducible DNA repair pathway. The clarification of the functions of the Soi proteins is a critical step for understanding the mechanisms of protection against oxidative stress in *E. coli*.

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