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Isolation of Catalase-Deficient *Escherichia coli* Mutants and Genetic Mapping of *katE*, a Locus That Affects Catalase Activity

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A number of catalase-deficient mutants of *Escherichia coli* which exhibit no assayable catalase activity were isolated. The only physiological difference between the catalase mutants and their parents was a 50- to 60-fold greater sensitivity to killing by hydrogen peroxide. For comparison, mutations in the *xthA* and *recA* genes of the same strains increased the sensitivity of the mutants to hydrogen peroxide by seven- and fivefold, respectively, showing that catalase was the primary defense against hydrogen peroxide. One class of mutants named *katE* was localized between *pfkB* and *xthA* at 37.8 min on the *E. coli* genome. A second class of catalase mutants was found which did not map in this region.

Catalase, which is sometimes referred to as a hydroperoxidase, employs a two-electron transfer in the dismutation of hydrogen peroxide to oxygen and water, thereby protecting the cell from the harmful effects of H_2O_2. It was one of the first bacterial enzymes described and has been the object of research for nearly a century, during which time a considerable body of biochemical information has accumulated. In *Escherichia coli*, two electrophoretically distinct catalase activities with associated peroxidase activities (employing a one-electron transfer to a hydroperoxide acceptor) have been identified and labeled HPI and HPII (5). After its purification, the main activity, HPI, was characterized as a tetramer with a molecular weight of 337,000 and two molecules of protoheme IX per tetramer (5). A third catalase activity without an associated peroxidase activity has also been reported (18) but not characterized.

Some information, albeit contradictory, has also accumulated regarding the regulation of catalase gene expression. For example, a link between the synthesis of components of the respiratory chain and the synthesis of catalase has been suggested (13), and catabolite repression has been implicated in the regulation of catalase synthesis in yeasts (22) and bacteria (12, 13, 26), although evidence to the contrary has been presented (10, 20). The addition of H_2O_2 or ascorbate (11, 13, 26) to cultures of *E. coli* caused a rapid sixfold increase in the basal catalase levels, but the mechanism of this induction by H_2O_2 has remained undefined.

By contrast, very little genetic data concerning bacterial catalase have accumulated. A number of loci involved in catalase synthesis in *Salmonella typhimurium* have been reported, but only one locus was mapped by phage transduction (14). In *E. coli*, the genetic relationship among the three catalase activities remains obscure and there has been no confirmation of the presence of the regulatory genes suggested by H_2O_2 induction. A more complete understanding of catalase gene regulation demands greater knowledge of the genetics of the system. As a first step in this direction, this paper describes the isolation of mutants completely deficient in catalase activity and the genetic mapping of a locus affecting catalase synthesis in *E. coli*.

MATERIALS AND METHODS

Bacterial strains. The strains used in this work and their characteristics are listed in Table 1. All genetic manipulations and strain constructions involving recombination and generalized phage-mediated transduction were carried out as described by Miller (16).

Media. LB medium (16) contained 10 g of tryptone (Difco Laboratories), 5 g of yeast extract (Difco), and 10 g of NaCl per liter. Nutrient broth contained 8 g of nutrient broth (Difco) and 4 g of NaCl per liter. M9 minimal medium (16) contained 6 g of Na_2HPO_4, 3 g of KH_2PO_4, 0.5 g of NaCl, and 1 g of NH_4Cl per liter supplemented after autoclaving with 10 μM $CaCl_2$, 1 mM $MgSO_4$, 3 μM vitamin B1, and 0.16 mM L-amino acids as required and various carbon sources at 0.2% (wt/vol). Solid media were prepared with 1.5% agar. Cultures were incubated with shaking at temperatures dictated by the individual markers present.

Mutagenesis and isolation of catalase-deficient mutants. Bacteria were grown to early exponential phase (10^8 cells per ml) in 10 ml of LB medium, collected by centrifugation, and resuspended in 10 ml of citrate buffer, pH 5.5, containing 100 μg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine per ml. After shaking gently at 37°C for 15 min, the cells were collected by centrifugation, washed one time in 10 ml of unsupplemented M9 medium, and resuspended in 10 ml of appropriately supplemented M9 medium. The culture was grown overnight at 37°C and plated on solid LB medium suitably diluted to result in approximately 50 colonies per plate after growth at 37°C (42°C in the case of temperature-sensitive selections). A drop of 30% H_2O_2 was applied with a syringe to the edge of each colony. Catalase-deficient strains failed to evolve bubbles of oxygen and were immediately streaked on a new LB plate. After growing overnight at 37°C (or 42°C), the apparent catalase deficiency was retested with H_2O_2. Catalase-deficient strains were then grown at 37°C in 10 ml of LB for oxygraph analysis of the catalase activity.

Enzyme assays. Catalase activity was determined by the method of Rørth and Jensen (21) in a Gilson oxygraph equipped with a Clark electrode. One unit of catalase was defined as the amount that decomposes 1 μmol of H_2O_2 in 1 min at 37°C. Exonuclease was assayed by the procedure described by Milcarek and Weiss (15). One unit of exonuclease activity was defined as the amount causing the production of 1 nmol of acid-soluble, ^{32}P-labeled nucleotides in 30 min at 37°C.

Screening of other genetic markers. *aroD* strains were scored by their requirement for a mixture of 25 μg each of tryptophan, tyrosine, and phenylalanine per ml and 1 μM *p*-

TABLE 1. *E. coli* K-12 strains used

Strain	Genotype	Reference or source
AB1157	*thr-1 leuB6 thi-1 argE3 his-4 proA2 lacY1 galK2 ara-14 xyl-5 mtl-1 rpsL tsx-33 supE44*	C.G.S.C.[a]
BW9091	As AB1157 but *xthA1*	B. Weiss (15)
BW9109	As AB1157 but Δ(*pncA-xthA*)	B. Weiss (23)
CSH57A	*leuB6 proC83 purE42 trpE38 his-208 argG77 ilvA681 met-160 thi-1 ara-14 lacY1 galK2 xyl-5 mtl-1 azi-6 rpsL109 tonA23 tsx67 supE44 malA38 xthA*	C.S.H.C.[b] (16)
CSH7	*lacY rpsL thi-1*	C.S.H.C. (16)
CSH74(KL16)	*thi-1* Hfr	C.S.H.C. (16)
MP180	*thi-1* HfrH	M. L. Pearson (17)
MLD2	*pps*	M. L. Duckworth[c]
NP37	*pheS5 relA1 tonA22* $T2^r$ *pit-10 spoT1* Hfr	C.G.S.C.
GMS343	*aroD6 argE3 lacY1 galK2 man-4 mtl-1 rpsL700 tsx-29 supE44*	C.G.S.C.
DF801	*edd-1 galK his pyrD rpsL sup* Δ_{15}(*rha-pfkA*) *pfkB21*::Tn*10*	D. Fraenkel (8)
E5014	F'128 Δ(*gpt-lac*)*5 relA1 thi-1 mal-24 rpsE2112*	C.G.S.C.
X573	F'254 Δ(*lac-lip*)*71 serA12 supE42* $T3^r$	C.G.S.C.
CSH28	F'(lac^+ $proA^+$ B^+) Δ(*lac-pro*) *supF trp pyrF his thi rpsL*	C.S.H.C.
F597/EB54	F'(*proAB lac proC*) *proB trpA trpR his met lacI lacZ azi rpsL nalA recA*	C.G.S.C.
KL723	F'104 *thr-1 leuB6 proA2 his-4 recA13 argE3 thi-1 ara-14 lacY1 galK2 xyl-7 mtl-1 rpsL31 tsx-33 supE44*	C.G.S.C.
NH4104	F'42 *thr-1 leuB6 thi-1 his-4 proA2 uvrA6 lacY1 ara-14 supE44*	C.G.S.C.
KL16-99	Hfr(KL16) *thi-1 recA1 relA1 deoB13*	C.G.S.C.
ZSC114	*ptsM1 glk rpsL*	7
UM1	As CSH7 but *katE1*	See text
UM2	As CSH57a but *katF2*	See text
UM5	As UM1 but *his*	Nitrosoguanidine
UM22	As UM2 but *ptsM*	P1(ZSC114) × UM2→*ptsM*
UM25	As UM5 but *pps*	P1(MLD2) × UM5→*pps*
UM30	As UM2 but *pps*	P1(MLD2) × UM2→*pps*
UM40	As UM2 but *pheS*	P1(NP37) × UM30→pps^+ *phes*
UM41	As UM5 but *aroD*	P1(GMS343) × UM25→pps^+ *aroD*
UM50	As UM2 but *thyA*	UM2 · aminopterin
UM51	As UM2 but his^+	Spontaneous his^+
UM53	As UM2 but *recA*	KL16-99 × UM50→$thyA^+$ *recA*
UM60	As UM5 but *pheS5*	P1(NP37) × UM41→$aroD^+$ *pheS5*
UM94	As UM1 but *thyA*	UM1 · aminopterin
UM96	As UM1 but *recA*	KL16-99 × UM94→$thyA^+$ *recA*
UM104	As UM1 but *pfkB*::Tn*10*	P1(DF801) × UM1→Tet^r *kat*
UM105	As UM2 but *pfkB*::Tn*10*	P1(DF801) × UM2→Tet^r *kat*
UM119	As UM2 but *katE1 pfkB*::Tn*10*	P1(UM104) × UM2→Tet^r *kat*

[a] Coli Genetic Stock Center, B. Bachmann, Curator.
[b] Cold Spring Harbor Collection.
[c] Ph.D. Thesis, University of Manitoba, 1980.

aminobenzoic acid and 1 μM *p*-hydroxybenzoic acid (18). The *pps* strains were scored by an inability to grow on 0.8% sodium pyruvate as carbon source on minimal agar plates (6). The temperature-sensitive *pheS5* marker was scored by a failure to grow at 42°C (6). The very useful *pfkB*::Tn*10* marker was scored by the ability to grow on plates supplemented with 15 μg tetracycline per ml (8). The *xthA* marker was scored by an in vitro assay for exonuclease (15). The *ptsM* marker was scored by the inability to grow on minimal agar plates with mannose as the carbon source (7).

H_2O_2 killing assay. Cultures were grown to early-exponential phase with shaking, and H_2O_2 was added to a final concentration of 1 mM. Samples were removed before and 15 min after H_2O_2 addition and plated with suitable dilutions on LB plates to determine the viable cell concentration.

RESULTS

Effect of growth medium on catalase mutant selection. After nitrosoguanidine mutagenesis, the growth medium affected the number and type of catalase mutants found. The use of LB plates resulted in about 1 in 2,000 colonies being catalase deficient both on plates and in the oxygraph assay. By contrast, nutrient agar plates or LB plates with a glucose supplement resulted in up to 1 in 200 colonies being deficient in catalase on plates. However, like the catalase-deficient mutants of *Salmonella typhimurium* also isolated on nutrient agar plates, most of these latter mutants exhibited some catalase activity in the oxygraph assay. It has been noted in other work (12, 19) that the composition of liquid growth media affected catalase levels, and apparently the composition of solid media can elicit the same effect, with lower levels of catalase appearing on nutrient agar and glucose minimal plates as compared to LB plates. Therefore, to make the initial selections for catalase deficiency as stringent as possible, LB plates were used to select the catalase mutants studied in this report. Specifically, UM1 (*kat-1*), isolated from CSH7, and UM2 (*kat-2*), isolated from CSH57A, are the two mutants characterized in this paper. Subsequently, other catalase-deficient mutants (*kat-3* to *-11*) were isolated by using various media, and their relationship to the *kat-1* and *kat-2* mutants was determined.

Physiology of catalase-deficient strains. Neither UM1 nor UM2 exhibited any assayable catalase activity in either

TABLE 2. Catalase and exonuclease III levels and hydrogen peroxide sensitivities of various *E. coli* strains growing in exponential phase

Strain	Catalase (U/mg of dry cell weight)	Exonuclease III (U/mg of dry cell weight)	Plating efficiency after H_2O_2 treatment
CSH7	3.6	10.7	0.81
UM1	0	12.3	0.013
CSH57A	4.9	0.8	0.11
UM2	0	1.0	0.0022
UM53	0	0.9	0.00047
AB1157	5.0	11.0	0.46
BW9091	2.5	0.7	0.056
BW9109	4.1	0.7	0.10

exponential phase (Table 2) or stationary phase. Despite this complete absence of catalase, both mutants grew normally with no apparent difference in growth rate or extent of growth as compared with the parental strains in LB medium or minimal medium supplemented with glucose, succinate, glycerol, acetate, or Casamino Acids as carbon source. Similarly, there was no difference between parent and daughter strains in the loss of viability during storage at room temperature for periods of up to 2 weeks. The only visible difference between mutant and parental physiologies was in their sensitivities to hydrogen peroxide. Consistent with the role of catalase being to protect the cell from hydrogen peroxide, the catalase mutants were 50- to 60-fold more sensitive to killing by 1 mM hydrogen peroxide (Table 2).

During this analysis, it was observed that strain CSH57A was 10-fold more sensitive to hydrogen peroxide than strain CSH7 (Table 2). Enhanced sensitivity to H_2O_2 has been reported in *recA* strains (1, 4, 25) and in *xthA* or exonuclease III-deficient strains (9). Because strain CSH57A was not more sensitive than strain CSH7 to UV irradiation, eliminating a *recA* genotype, the strains were tested for exonuclease III activity (Table 2). By using the authentic *xthA* mutants BW9091 and BW9109 for comparison, it was found that strain CSH57A and its derivative, UM2, were deficient in exonuclease III, suggesting the presence of the *xthA* lesion. This conclusion was verified by cotransduction mapping studies described below.

Genetic analysis of *kat-1* and *kat-2*. Because of the lack of suitable markers in UM1, the location of *kat-2* in UM2 was investigated first by conjugative crosses with strains MP180 and CSH74, selecting for the appearance of *trp*$^+$ or *his*$^+$ colonies, respectively, and scoring for the appearance of catalase. The apparent time of entry of *kat-2*$^+$ with CSH74 was 6 to 8 min after the *his* marker, suggesting a map location around 36 to 38 minutes. By using MP180 as the donor, *kat*$^+$ entered 12 to 14 min after the entry of *trp*$^+$, suggesting a location around 38 to 40 minutes. To map *kat-1*, a *his* derivative (UM5) was isolated and used in a cross with strain CSH74. As with UM2, catalase activity appeared 6 to 8 min after the *his*$^+$ entry, indicating that *kat-1* and *kat-2* were in the same region of the chomosome.

The gene order between 37 and 40 min on the *E. coli* chromosome includes *aroD*, *pps*, *pheS*, *pfkB*, *xthA*, and *ptsM* (3). These markers were introduced into *kat-1* and *kat-2* strains to be used in three-factor transductional crosses. No linkage of either *kat-1* or *kat-2* was found with *ptsM* at 40 min, but as shown in Table 3, there were various degrees of linkage between *kat-1* and each of *pps*, *pheS*, and *pfkB*::Tn*10*, establishing the order *pps-pheS-pfkB-kat-1*. By using *aroD* and *xthA* markers as well, a similar order, *aroD-pps-pheS-pfkB-kat-2-xthA*, was established for *kat-2* (Table 4), revealing that *kat-1* and *kat-2* lesions were located very close together on the genome. The linkages of *pfkB*::Tn*10* with *kat-1* and *kat-2* were verified by direct transductional crosses introducing *pfkB*::Tn*10* into UM1 and UM2 (Tables 3 and 4).

Phenotypic differences between *kat-1* and *kat-2* mutants. Because of the presence of multiple catalase activities in *E.*

TABLE 3. Mapping of *kat-1* relative to adjacent genes by three-factor transductional crosses

Donor	Recipient	Selected marker	Unselected marker(s)	
			Class	No. (%)
NP37 (*pheS*)	UM25 (*kat pps*)	*pps*$^+$	*pheS kat*$^+$	141 (47)
			pheS kat	86 (29)
			pheS$^+$ *kat*$^+$	12 (4)
			pheS$^+$ *kat*	60 (20)
DF801 (*pfkB*::Tn*10*)	UM25 (*kat pps*)	*pps*$^+$	Tet^r *kat*$^+$	28 (47)
			Tet^r *kat*	8 (13)
			Tet^s *kat*$^+$	1 (2)
			Tet^s *kat*	23 (38)
DF801 (*pfkB*::Tn*10*)	UM25 (*kat pps*)	Tet^r	*pps*$^+$ *kat*$^+$	110 (33)
			pps$^+$ *kat*	52 (16)
			pps kat$^+$	115 (35)
			pps kat	54 (16)
DF801 (*pfkB*::Tn*10*)	UM41 (*aroD kat*)	*aroD*	Tet^r *kat*$^+$	47 (20)
			Tet^r *kat*	62 (27)
			Tet^s *kat*$^+$	0 (0)
			Tet^s *kat*	121 (53)
DF801 (*pfkB*::Tn*10*)	UM41 (*aroD kat*)	Tet^r	*aroD*$^+$ *kat*$^+$	41 (17)
			aroD$^+$ *kat*	70 (29)
			aroD kat$^+$	86 (36)
			aroD kat	43 (18)
DF801 (*pfkB*::Tn*10*)	UM60 (*kat pheS*)	Tet^r	*pheS*$^+$ *kat*$^+$	140 (47)
			pheS$^+$ *kat*	81 (27)
			pheS kat$^+$	53 (17)
			pheS kat	26 (9)
DF801 (*pfkB*::Tn*10*)	UM1 (*kat-1*)	Tet^r	*kat*$^+$	179 (75)

TABLE 4. Mapping of *kat-2* relative to adjacent genes by three-factor transductional crosses

Donor	Recipient	Selected marker	Unselected marker(s)	
			Class	No. (%)
NP37 (*pheS*)	UM30 (*pps kat*)	*pps*$^+$	*pheS kat*$^+$	80 (22)
			pheS kat	49 (14)
			phe$^+$ *kat*$^+$	42 (12)
			phe$^+$ *kat*	189 (52)
DF801 (*pfkB*::Tn*10*)	UM30 (*pps kat*)	*pps*$^+$	Tet^r *kat*$^+$	7 (12)
			Tet^r *kat*	23 (38)
			Tet^s *kat*$^+$	3 (5)
			Tet^s *kat*	27 (45)
DF801 (*pfkB*::Tn*10*)	UM30 (*pps kat*)	Tet^r	*pps*$^+$ *kat*$^+$	77 (26)
			pps$^+$ *kat*	60 (20)
			pps kat$^+$	104 (35)
			pps kat	59 (19)
DF801 (*pfkB*::Tn*10*)	UM40 (*pheS kat*)	Tet^r	*pheS*$^+$ *kat*$^+$	78 (43)
			pheS$^+$ *kat*	53 (29)
			pheS kat$^+$	35 (20)
			pheS kat	14 (8)
DF801 (*pfkB*::Tn*10*)	UM2 (*kat xthA*)	Tet^r	*xth*$^+$ *kat*$^+$	72 (30)
			xth$^+$ *kat*	23 (10)
			xth kat$^+$	66 (27)
			xth kat	79 (33)
DF801 (*pfkB*::Tn*10*)	UM2 (*kat-2*)	Tet^r	*kat*$^+$	120 (59)

coli extracts, it was expected that multiple mutagenic events would be necessary to create a phenotype of complete catalase deficiency. The following observations suggested that the *kat-1* and *kat-2* mutations were superimposed on a background of other mutations affecting catalase expression. (i) An undefined episome in strain CSH28 enhanced catalase synthesis in *kat-2* (UM53) strains but not in *kat-1* (UM96) strains. The known episomes F'128, F'104, F'254, F'42, and F'597, which cover portions of the chromosome surrounding the *lac* marker (2), did not enhance catalase synthesis in either strain. (ii) The phenotype of complete catalase deficiency could not be transduced into parent strain CSH57A with either UM104 (*kat-1 pfkB*::Tn*10*) or UM105 (*kat-2 pfkB*::Tn*10*) as donor, although a partial deficiency was observed in 63% of the tetracycline-resistant colonies when UM104 was the donor. (iii) Of the tetracycline-resistant colonies resulting from the transduction of UM1 (*kat-1*) with UM105 (*kat-2 pfkB*::Tn*10*) as donor, 60% exhibited catalase activity. The reciprocal transductive cross of UM2 (*kat-2*) with UM104 (*kat-1 pfkB*::Tn*10*) as donor did not produce any *kat*$^+$ tetracycline-resistant colonies. Clearly more work is required to fully explain the differences between the *kat-1* and *kat-2* phenotypes.

Naming of the catalase locus. Because four catalase loci have already been named in *S. typhimurium*, *katA* to *-D*, it is proposed that the region around *kat-1* and *kat-2* be named *katE*. The map of the region around 38 min is presented in Fig. 1. Other catalase mutants (*kat-3* to *-11*), including *kat-8* isolated as a temperature-sensitive mutant at 42°C, were partially characterized by cotransduction with the *pfkB*::Tn*10* marker, giving rise to two distinct classes of mutants. One class exhibited 47 to 76% cotransduction with *pfkB*::Tn*10* (*kat5-9* and *-11*) and the second class exhibited no cotransduction (*kat3*, *-4*, and *-10*) with *pfkB*::Tn*10*. This verified the presence of at least one other gene unrelated to *katE* involved in catalase synthesis in *E. coli*.

DISCUSSION

A locus affecting catalase expression in *E. coli* was mapped in three-factor cotransductional crosses and located between *pfkB* and *xthA* at 37.8 min on the chromosome. In naming the locus, the report of four separate loci affecting catalase synthesis in *S. typhimurium* labeled *katA* to *-D* (14) was considered. Of these loci, only *katC* was mapped by phage transduction near *proAB*, and this is the only marker included in the *E. coli* map (3). However, the existing nomenclature is retained, and the *E. coli* locus at 37.8 min is designated *katE*.

The presence in bacterial extracts of three catalase activities, two of which possess a hydroperoxidase activity, implies that there is more than one gene encoding catalase. Consequently, two or more separate mutations should be required to produce a completely catalase-deficient strain, and the phenotypic differences between *kat-1* and *kat-2* could be the result of variations in genetic background. Alternatively, the different phenotypes of *kat-1* and *kat-2* strains coupled with the differences in linkage to *pfkB*::Tn*10*

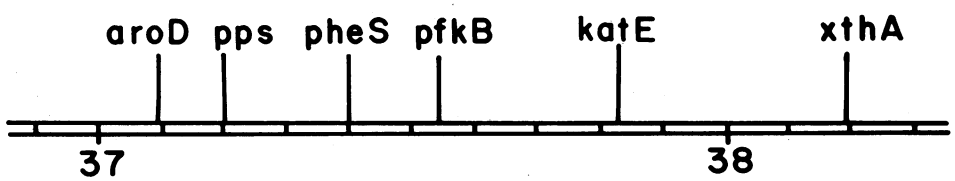

FIG. 1. Genetic map orienting *katE* relative to nearby genes in *E. coli* K-12. Gene locations (in minutes) are based on the 100-min *E. coli* map (3), using *pps* at 37.2 min as a reference point. The map distances were calculated by the equation of Wu (24) from the cotransduction frequencies in Tables 3 and 4.

(75% for UM1 and 59% for UM2) could also be interpreted to mean that the two lesions occur in different but closely linked genes. Other linkage data in Tables 3 and 4 are not consistent with this interpretation, however. Unfortunately, a clear definition of the function of the *katE* gene product is not yet possible but it may encode or enhance the synthesis of the electrophoretically slower catalase found in extracts of recombinants of *kat-2* strains (unpublished data). The ability of these mutants to grow effectively on a wide variety of fermentable and nonfermentable carbon sources, which would necessitate a functional heme group for cytochrome activity, confirmed that neither lesion affected the synthesis of the protoheme required by mature catalase.

Previously, both *recA* (1, 4, 25) and *xthA* (9) mutants have been shown to be 20- and 14-fold more sensitive to H_2O_2 killing, respectively. As expected, the catalase mutants were also sensitive to H_2O_2 killing and proved to be more sensitive than either the *xthA* or *recA* strain. A complete deficiency in catalase resulted in strains that were 50- to 60-fold more sensitive to H_2O_2, confirming the role of catalase as a key protective enzyme against H_2O_2. This contradicted an earlier report (4) that the *recA* product was more important than catalase in this function; the discrepancy may lie in the growth and assay procedures used in the earlier work. Catalase levels are quite sensitive to the growth medium and growth phase (5, 19), and variations in the catalase levels observed by Carlsson and Carpenter (4) may have arisen from their use of overnight cultures for catalase determinations. In addition, actual catalase-deficient strains complementing the *rec*A strains were not isolated in that work for comparison. The sequential introduction of deficiencies in exonuclease III, catalase, and *recA* (CSH57A, UM2, and UM53 in Table 2) resulted in strains that were 7, 370, and 1,700 times more sensitive to H_2O_2, respectively, consistent with each protein having a distinct role in protection against H_2O_2. The *xthA* and *recA* proteins are involved in the repair of DNA lesions caused by H_2O_2, whereas catalase is responsible for the removal of H_2O_2 before it can damage cell components.

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