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Genetic Mapping of *katA*, a Locus That Affects Catalase 1 Levels in *Bacillus subtilis*

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Several mutants of *Bacillus subtilis* deficient in catalase synthesis generated by nitrosoguanidine mutagenesis have been used to map a locus affecting catalase activity. Two- and three-factor bacteriophage PBS1 transductional crosses were used to locate the locus, named *katA*, between *recH* and *thiA* with 98% linkage to *thiA* at 70° on the *B. subtilis* genome. The synthesis of catalase 1, found only in vegetative cells, was affected by *katA*.

Catalase (EC 1.11.1.6) uses a two-electron transfer in the dismutation of hydrogen peroxide to oxygen and water. Despite an extensive body of literature pertaining to the enzyme (5), little genetic data have accumulated except for *Salmonella typhimurium* (1, 8) and *Escherichia coli* (9, 11–13, 20), for which three loci have been identified, precisely mapped, and cloned. In *Bacillus subtilis*, the presence of at least two distinct catalases has been confirmed by a combination of biochemical and genetic techniques (10). Catalase 1, found in vegetative cells, was the predominant species, and its synthesis could be induced by H_2O_2. Catalase 2, present in smaller amounts, was shown to be the only catalase species present in spores. The purification and characterization of the enzymes has recently been completed. Mutants deficient in one or both of the catalases were isolated as part of the demonstration of two distinct species. This paper discusses the uses of those catalase-deficient strains and describes the genetic mapping of one locus that affects the synthesis of catalase 1.

The genotypes and sources of the strains used in this work and their characteristics are listed in Table 1. Cultures were grown in Penassay broth prepared with antibiotic medium 3 as directed (Difco Laboratories), on tryptose blood-base agar plates prepared as directed (GIBCO/BRL Life Technologies), in modified Schaeffer medium (7) and the minimal medium of Spizizen (18) containing specific amino acids at 20 μl/ml, and thymine at 50 μg/ml (4) as required by specific strains. The isolation of catalase-deficient mutants UM1001 and UM1005 has been described previously (10). Other mutants used in this work were isolated by a similar procedure. The procedures used for transduction were as described by Sun and Takahashi (19). Transposon Tn*917*-containing transductants were selected in a two-stage plating protocol selecting for resistance to macrolides-lincosamides-streptogramin B antibiotics (MLS^r) on plates containing erythromycin and lincomycin (23). Catalase activity in colonies was scored by applying a drop of 30% H_2O_2 to the edge of the colony with a syringe. The evolution of bubbles signified catalase activity, and differences in rates of oxygen evolution could be used to distinguish among various levels of activity. Catalase 1 was clearly the predominant activity in colonies, making it possible to screen for catalase 1 in the presence of catalase 2 but preventing the reciprocal selection. Catalase activity was visualized after electrophoretic separation on an 8.5% polyacrylamide gel run as described by Davis (3), except in Tris hydrochloride (pH 8.1) and on slab gels (15 by 15 cm), by the method of Clare et al. (2) but using 20 mM H_2O_2 for better contrast. Catalase activity was quantitated by the method of Rorth and Jensen (17), whereas protein was determined after the method of Layne (6).

The approximate location of the locus affecting catalase 1 activity in mutant UM1001 was determined in a series of transductions with the nine strains of Dedonder and coworkers (4). These strains contain markers suitably distributed around the chromosome so as to ensure some degree of linkage with all parts of the chromosome during bacteriophage PBS1 transduction. A PBS1 lysate of UM1001 was prepared and used to transduce each of the Dedonder strains by selecting for prototrophy in the various markers. Selected colonies were then scored for catalase by the H_2O_2 drop test. All of the Dedonder strains exhibited normal vigorous oxygen evolution indicative of the wild-type phenotype, making introduction of the catalase 1-deficient phenotype, which substantially reduced oxygen evolution, clearly evident. In the initial screening, the only marker which appeared to have a linkage with the locus affecting catalase 1 was *glyB* in strain QB934 (kit 3). Upon repetition, there was found to be a 7.4% linkage between *kat-1* and *glyB* (Table 2). The *spo* mutation in UM1001 and its derivatives arose from the nitrosoguanidine mutagenesis (14) but did not affect catalase 1 synthesis. To confirm that catalase 1 was the catalase activity being affected by the introduction of the catalase-deficient phenotype, a number of the catalase-deficient transductants, including UM1008, were chosen and extracts were prepared for visualization of the catalase activities after separation by electrophoresis on native polyacrylamide gels. As shown in Fig. 1, the transductant UM1008 exhibited essentially the same pattern of catalase 2 bands as was present in the wild-type strain but lacked any evidence of catalase 1. Similar patterns were observed for the other catalase-deficient strains, indicating that *kat-1* affected catalase 1 levels independently of catalase 2.

The transposon Tn*917* insertion in *thiA* contained in strain CU4123 (20) was chosen as a suitable probe to further define the location of *kat-1*. A PBS1 lysate of CU4123 was used to transduce UM1001 by selecting for MLS^r encoded by the transposon. A screening of the selected colonies for catalase activity revealed that 98.2% exhibited a wild-type catalase

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TABLE 1. *B. subtilis* strains used

Strain	Genotype	Source or reference
168	*trpC2*	D. Stahly
QB944 (kit 1)	*purA16 cysA14 trpC2*	4
QB928 (kit 2)	*aroI906 purB33 dal-1 trpC2*	4
QB934 (kit 3)	*tre-12 metC3 glyB133 trpC2*	4
QB943 (kit 4)	*pyrD1 ilvA1 thyA1 thyB1 trpC2*	4
QB922 (kit 5)	*gltA292 trpC2*	4
QB935 (kit 6)	*aroD120 lys-1 trpC2*	4
QB936 (kit 7)	*leuA8 aroG932 ald-1 trpC2*	4
QB917 (kit 8)	*hisA1 thr-5 trpC2*	4
QB123 (kit 9)	*sacA321 ctrA1 trpC2*	4
CU4123 (1A603)	*thiA84*::Tn*917 trpC2* SPβ *c2*	BGSC[a]; 21
1A301	*trpC2 hisH2 recH342*	BGSC
1A84	*trpC2 glyB133 metD1*	BGSC
UM1001	*trpC2 katA1 spo*	168 + NG[b] → Kat$^-$; 10
UM1002	*trpC2 katA2 spo*	168 + NG → Kat$^-$
UM1003	*trpC2 katA3 spo*	168 + NG → Kat$^-$
UM1005	*trpC2 katA5*	168 + NG → Kat$^-$
UM1008	*tre-12 metC3 trpC2 katA1*	UM1001 × QB934 → *gly*$^+$ Kat$^-$
UM1011	*trpC2 katA1 spo thiA84*::Tn*917*	CU4123 × UM1001 → MLS^r
UM1012	*trpC2 spo thiA84*::Tn*917*	CU4123 × UM1001 → MLS^{r}Kat$^+$
UM1013	*trpC2 katA1 spo kat-6*	UM1001 + NG → Kat$^-$
UM1014	*trpC2 katA1 spo kat-6 thiA84*::Tn*917*	CU4123 × UM1013 → MLS^r
UM1015	*trpC2 spo kat-6 thiA84*::Tn*917*	CU4123 × UM1013 → MLS^r

[a] BGSC, *Bacillus* Genetic Stock Center, D. R. Zeigler, curator.
[b] NG, *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine, 100 μg/ml.

phenotype (Table 2), indicative of a very tight linkage between *thiA* and *kat-1*. Of 360 MLS^r transductants scored for thiamine requirement, all required the vitamin for growth, confirming the presence of just one transposon and its location in the *thiA* gene.

One of the few transductants retaining the catalase-deficient phenotype was saved as UM1011, and a PBS1 lysate was prepared. Using this lysate, it was possible to carry out a three-factor transduction with strain 1A84 containing *glyB* as recipient (Table 2). This experiment established the probable gene order *kat-thiA-glyB*. Strain UM1013, lacking both catalase 1 and catalase 2 activities (10), was transduced with a lysate of strain CU4123, and an MLS^r Kat$^-$ transductant, UM1014, was retained. A PBS1 lysate of strain UM1014 was used to transduce 1A84 and QB934 by selecting for either MLS^r or *gly*$^+$ colonies which were then scored for the other two markers to confirm the *kat-thiA-glyB* order (Table 2). To confirm that only catalase 1 was present in the Kat$^+$ transductants, one Kat$^+$ MLS^r recombinant, UM1015, was chosen from the CU4123 transduction of UM1013 and an extract was prepared for visualization of the catalase activity after electrophoresis.

TABLE 2. Mapping of *kat-1* relative to adjacent genes by two- and three-factor transductional crosses

Donor	Recipient	Selected marker	Unselected marker(s)	
			Class	No. (%)
UM1001 (*kat-1*)	QB934 (*glyB*)	*gly*$^+$	*kat*	52 (7)
			kat$^+$	646 (93)
CU4123 (*thiA*::Tn*917*)	UM1001 (*kat-1*)	MLS^r	*kat*$^+$	1193 (98)
			kat	22 (2)
CU4123 (*thiA*::Tn*917*)	UM1001 (*kat-1*)	MLS^r	*kat*$^+$	429 (97)
			kat	12 (3)
UM1011 (*thiA*::Tn*917 kat-1*)	1A84 (*glyB*)	MLS^r	*kat gly*$^+$	14 (5)
			kat gly	71 (89)
			kat$^+$ *gly*$^+$	2 (3)
			kat$^+$ *gly*	2 (3)
UM1014 (*thiA*::Tn*917 kat-1*)	1A84 (*glyB*)	*gly*$^+$	*kat* MLS^r	10 (4)
			kat MLS^s	0 (0)
			kat$^+$ MLS^r	7 (3)
			kat$^+$ MLS^s	215 (93)
UM1014 (*thiA*::Tn*917 kat-1*)	QB934 (*glyB*)	*gly*$^+$	*kat* MLS^r	33 (13)
			kat MLS^s	0 (0)
			kat$^+$ MLS^r	10 (4)
			kat$^+$ MLS^s	218 (83)
UM1014 (*thiA*::Tn*917 kat-1*)	1A301 (*recH*)	MLS^r	*rec*$^+$ *kat*	399 (77)
			rec$^+$ *kat*$^+$	23 (4)
			recH kat	89 (17)
			recH kat$^+$	10 (2)

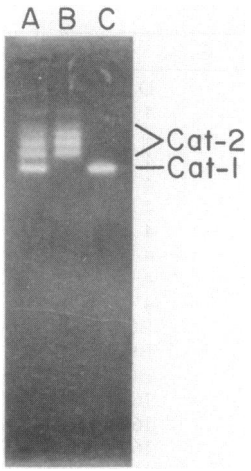

FIG. 1. Visualization of catalase 1 (Cat-1) and catalase 2 (Cat-2) in crude extracts of various strains after electrophoresis on a native 8.5% polyacrylamide gel. Extracts from the following strains were run: lane A, 168; lane B, UM1008; lane C, UM1015.

As shown in Fig. 1, only one band of activity corresponding to catalase 1 was present, thereby confirming that the *kat-1* locus affected only catalase 1 and that the absence of catalase 2 caused by *kat-6* was unaffected.

The only marker close to *thiA* on the side opposite *glyB* was *recH* (16), which had been determined to be only 63% linked to *thiA* (15). A lysate of UM1014 was used to transduce strain 1A301 containing *recH*, and MLS^r transductants were selected. Subsequent scoring for *recH*, on plates containing 0.15 μg of mitomycin C per ml, and *kat-1* suggested the order *recH-thiA-kat* because of the twofold greater number of *rec*$^+$ *kat*$^+$ recombinants compared with *recH kat*$^+$ recombinants. The discrepancy between this order and the order determined by using *glyB* may have been the result of the poor plate assay for *recH*-containing cells, which favored the scoring of *rec*$^+$ colonies even with a second screening. This would have resulted in an artificially higher number of apparent *rec*$^+$ *kat*$^+$ recombinants, possibly accounting for the small difference between the two classes of recombinants relative to the total number. Another manifestation of the difficulties with the *rec*$^+$ scoring arose in carrying out the converse experiment of selecting for *rec*$^+$ and scoring for *kat* and *thiA*, which was not successful despite several attempts. Because of this scoring problem, the gene order *recH-kat-thiA-glyB*, determined by using the more reliably scored *glyB* locus, has been taken to be correct. Subsequent cloning of the *kat-1 thiA* region currently in progress will serve to physically define the relative orientations of these two closely linked genes which, according to the equation of Wu (22), should be no more than 6,000 base pairs apart.

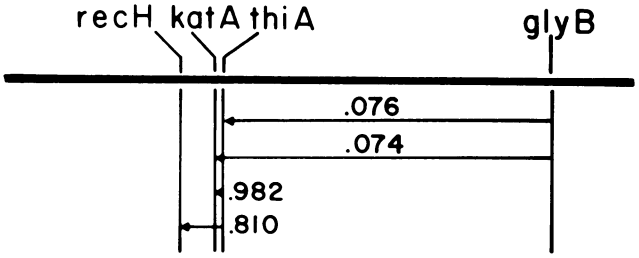

FIG. 2. Genetic map orienting *katA* relative to nearby genes. The gene locations were determined by transduction. The numbers represent the cotransduction frequencies.

Similar analyses were carried out with three other independently isolated mutants containing *kat-2*, *kat-3*, and *kat-5* isolated from different nitrosoguanidine mutagenesis selections. In all three cases, the linkages of the *kat* mutations to *glyB* and *thiA* were the same as for *kat-1*, indicating that all four mutations were alleles of the same locus. Because this is the first report of a locus affecting catalase levels in *B. subtilis* (16), the locus has been named *katA* and the alleles studied in this paper have been named *katA1*, *katA2*, *katA3*, and *katA5*. It is expected that at least one independent locus, *kat-6*, affecting the activity of catalase 2 in UM1013 and UM1015 (Fig. 1) will eventually be characterized and mapped at a location distinct from *katA*. The location of *katA* relative to the surrounding genes and some of the transduction data used in determining the positions are summarized in Fig. 2.

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