Genetic mapping of *katB*, a locus that affects catalase 2 levels in *Bacillus subtilis*

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A locus affecting the synthesis of spore-specific catalase 2 in *Bacillus subtilis* was mapped using two- and three-factor transductional crosses at 342° between *hsrE* and *iol*. It was named *katB*. Strains lacking catalase 2 remained sporulation proficient, but blockage of sporulation at stage IV or earlier affected the electrophoretic mobility of the native enzyme.

Key words: *Bacillus subtilis*, catalase 2, *katB*, sporulation, mapping.

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Un locus qui affecte la synthèse de la catalase 2, une enzyme spécifique aux spores de *Bacillus subtilis*, a été cartographié à l'aide de croisements transductionnels bifactoriels et trifactoriels vers 342°, entre *hsrE* et *iol*. Ce locus a été désigné *katB*. Chez les souches dépourvues de catalase 2, la sporulation s'est amorcée normalement mais, au stade IV ou antérieurement, un blocage de la sporulation a affecté la mobilité électrophorétique de l'enzyme native.

Mots clés : *Bacillus subtilis*, catalase 2, *katB*, sporulation, cartographie.

Bacillus subtilis produces two different catalases: catalase 1, which is found in vegetative cells, and catalase 2, which is the only catalase found in purified spores (Loewen and Switala 1987*a*). Catalase 1 has been characterized as a hexamer of identical 65-kDa subunits with one protoheme IX per subunit (Loewen and Switala 1987*b*). A gene affecting the synthesis of catalase 1 has been mapped tightly linked to *thiA* at 70° on the genome (Loewen and Switala 1987*c*). Catalase 2 has been characterized as a hexamer of identical 81-kDa subunits with one heme *d*-like group per subunit (Loewen and Switala 1988). A mutant deficient in catalase 2 has been isolated (Loewen and Switala 1987*a*) and this paper describes the use of this mutant to map a locus affecting catalase 2 synthesis.

The genotypes and sources of the strains used in this work are listed in Table 1. Cultures were grown in Penassay broth prepared with antibiotic medium 3 as directed (DIFCO), on tryptose blood-base (TBB) agar plates as directed (GIBCO), in modified Schaeffer medium (Leighton and Doi 1971), and in the minimal medium of Spizizen (1958) containing specific amino acids at 20 μg/mL and adenosine at 50 μg/mL as required. The isolation of catalase-deficient mutants has been described (Loewen and Switala 1987*a*). Strains resistant to arginine hydroxamate were selected on minimal medium agar plates containing 200 μg/mL arginine hydroxamate (Mountain and Baumberg 1980). Glucose was replaced with 0.1% (w/v) sodium gluconate or inositol in minimal medium to score for *gnt* and *iol*, respectively (Fujita and Fujita 1983). The *hsrE* locus was scored by streaking on TBB plates previously spread with φ105C (Ikawa *et al.* 1981). The procedures used for transduction with bacteriophage PBS1 were as described by Sun and Takahashi (1982). 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 (Youngman *et al.* 1983). 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 indicated different activities. For example, the presence of catalase 1 resulted in a very rapid burst of bubbles that masked the slower rate of bubble formation caused by catalase 2. Consequently, screening for catalase 2 activity had to be carried out in *katA*-containing mutants that lack catalase 1. Catalase activity was visualized after electrophoretic separation on an 8.5% polyacrylamide gel run as described by Davis (1964), except that Tris–HCl, pH 8.1, was used and on slab gels (15 × 15 cm) by the method of Clare *et al.* (1984), but using 20 mM H_2O_2 for better contrast. Catalase activity was quantitated by the method of Rorth and Jensen (1967) and protein was determined after the method of Layne (1957).

The Dedonder strains (Dedonder *et al.* 1977) could not be used for the preliminary mapping of the locus affecting catalase 2 because the high activity of catalase 1 present in these strains masked the much lower activity of catalase 2. An alternative approach was to transfer selectable markers into mutants lacking both catalases, followed by scoring for catalase 2 activity cotransducing with the markers. Bacteriophage PBS1 lysates of a series of strains containing transposon Tn*917* insertions at regular intervals around the genome described by Vandeyar and Zahler (1986) were used to transduce UM1013, selecting for MLS^r conferred by the transposon and scoring for catalase activity. The locations of the transposons in the strains scored were at 0° (CU4147), 11° (CU4120), 54° (CU4121), 70° (CU4123), 96° (CU4124),

TABLE 1. *Bacillus subtilis* strains used in this study

Strain	Genotype	Source or reference
168	*trpC2*	BGSC
QB944 (kit 1)	*purA16, cysA14, trpC2*	Dedonder *et al.* 1977
QB123 (kit 9)	*sacA321, ctrA1, trpC2*	Dedonder *et al.* 1977
CU4120 (1A600)	*cym-84*::Tn*917, trpC2, SPβc2*	BGSC, Vandeyar and Zahler 1986
CU4121 (1A601)	*purB83*::Tn*917, trpC2, SPβc2*	BGSC, Vandeyar and Zahler 1986
CU4124 (1A603)	*thiA84*::Tn*917, trpC2, SPβc2*	BGSC, Vandeyar and Zahler 1986
CU4127 (1A604)	*metD83*::Tn*917, trpC2, SPβc2*	BGSC, Vandeyar and Zahler 1986
CU4129 (1A609)	*pyr-82*::Tn*917, trpC2, SPβc2*	BGSC, Vandeyar and Zahler 1986
CU4132 (1A612)	*gltA81*::Tn*917, trpC2, SPβc2*	BGSC, Vandeyar and Zahler 1986
CU4134 (1A614)	*serA84*::Tn*917, trpC2, SPβc2*	BGSC, Vandeyar and Zahler 1986
CU4136 (1A616)	*nic-82*::Tn*917, trpC2, SPβc2*	BGSC, Vandeyar and Zahler 1986
CU4142 (1A622)	*argA85*::Tn*917, trpC2, SPβc2*	BGSC, Vandeyar and Zahler 1986
CU4143 (1A623)	*alaA84*::Tn*917, trpC2, SPβc2*	BGSC, Vandeyar and Zahler 1986
CU4145 (1A625)	*mth-84*::Tn*917, trpC2, SPβc2*	BGSC, Vandeyar and Zahler 1986
CU4146 (1A626)	*hisA82*::Tn*917, trpC2, SPβc2*	BGSC, Vandeyar and Zahler 1986
CU4147 (1A627)	*zaa-84*::Tn*917, trpC2, SPβc2*	BGSC, Vandeyar and Zahler 1986
CU4164 (1A644)	*zii-83*::Tn*917, trpC2, SPβc2*	BGSC, Vandeyar and Zahler 1986
CU4165 (1A645)	*zif-85*::Tn*917, trpC2, SPβc2*	BGSC, Vandeyar and Zahler 1986
61668	*iol-6, trpC2, metC7*	Fujita and Fujita 1983
61656	*Δigf, hisA1, leuA8, metB5, trpC2*	Fujita and Fujita 1983
YF100	*Δigf, hsrE*$^+$	Fujita and Fujita 1983
YF127	*gnt-4, trpC2, metC7*	Fujita and Fujita 1983
Ahr2-52	*ahrA2-52*	Mountain and Baumberg 1980
1S10	*trpC2, spo0A12*	BGSC
1S86	*trpC2, spoIIA1*	BGSC
1S48	*trpC2, spoIIIB2*	BGSC
1S46	*trpC2, spoIVA178*	BGSC
1S50	*trpC2, spoVA89*	BGSC
1S88	*trpC2, spoVIA513*	BGSC
UM1005	*trpC2, katA5*	Loewen and Switala 1987*a*
UM1013	*trpC2, katA1, spo, katB6*	Loewen and Switala 1987*c*
UM1015	*trpC2, katB6, spo, zif-85*::Tn*917*	CU4124 × UM1013 → MLS^r, *kat*$^+$
UM1018	*trpC2, katA1, spo*	CU4165 × UM1013 → MLS^r, *kat*$^+$
UM1019	*trpC2, cysA14, purA16, zif-85*::Tn*917*	CU4165 × QB944 → MLS^r, *purA*
UM1023	*trpC2, purA16, spo, katA1, katB6, zif-85*::Tn*917*	UM1019 × UM1013 → MLS^r, *purA katB*
UM1026	*trpC2, metC7, iol-6, zif-85*::Tn*917*	CU4165 × 61668 → MLS^r, *iol*
UM1027	*trpC2, metC7, gnt-4, zif-85*::Tn*917*	CU4165 × YF127 → MLS^r, *gnt*
UM1028	*ahrA2-52, zif-85*::Tn*917*	CU4165 × Ahr2-52 → MLS^r, *ahr*
UM1036	*trpC2, katA5, katB6, zif-85*::Tn*917*	UM1023 × UM1005 → MLS^r, *kat*
UM1043	*trpC2, katA1, spo, katB6, iol-6, zif-85*::Tn*917*	UM1026 × UM1013 → MLS^r, *iol*

NOTE: BGSC, *Bacillus* Genetic Stock Center; D. R. Zeigler, curator.

115° (CU4127), 137° (CU4129), 180° (CU4132), 208° (CU4134), 245° (CU4136), 260° (CU4142), 281° (CU4143), 290° (CU4145), 305° (CU4146), and 342° (CU4165). Catalase activity cotransduced with the transposon in only three lysates: CU4123 (96% cotransduction), CU4124 (0.8% cotransduction), and CU4165 (81% cotransduction). The transductants from CU4123 and CU4124 exhibited very high levels of catalase indicative of catalase 1 (Loewen and Switala 1987*c*). The transductants from CU4165 exhibited much lower catalase levels, similar to the levels in UM1005, which contains only catalase 2. The presence of only catalase 2 was confirmed by visualizing the catalase activities following electrophoretic separation of a crude extract of UM1018 (Fig. 1). This indicated that a locus affecting catalase 2 synthesis was located in proximity to 342°, a location very different from *katA* at 70°. This new locus was named *katB*.

Other markers in the vicinity of 340° include *purA* (355°), *gnt* (344°), *iol* (343°), *ahrA* (342°), and *hsrE* (337°). The results of two- and three-factor PBS1-mediated transductional crosses mapping *katB* relative to these markers are shown in Table 2 and the relationship of *katB* to the surrounding loci is summarized in Fig. 2. The relationship of *ahrA* to the *iol-gnt-fdp-hsrB* grouping is reversed from the published orientation (Piggot and Hoch 1985) in which the assignment was based on a comparison of linkages of *ahrA* (Mountain and Baumberg 1980) and *iol* (Fujita and Fujita 1983) to *purA* determined in separate experiments. These results also reveal a 100% linkage between *hsrE* and *zif-85*::Tn*917*, suggesting either a direct insertion of the transposon in *hsrE* or a deletion covering *hsrE* (Vandeyar and Zahler 1986).

In extracts of wild-type strains, catalase 2 migrates as a series of electrophoretically separable bands on nondenaturing polyacrylamide gels and is the only catalase found in spores (Loewen and Switala 1987*a*). The effect of the sporulation process on the electrophoretic pattern of catalase 2 was investigated using a series of sporulation defective mutants blocked at various stages in the sporulation process. Extracts of the various mutants were prepared and the catalase activities visualized following electrophoresis (Fig. 1). A single band of catalase activity with a slower mobility than catalase 1 was

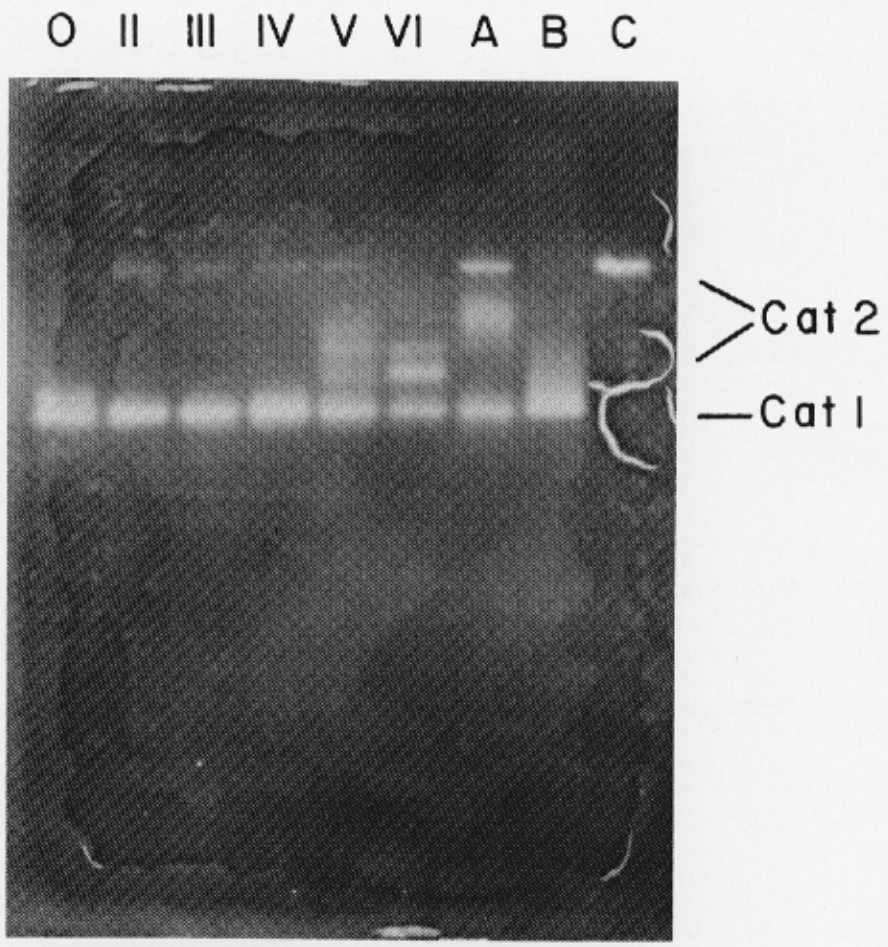

FIG. 1. Visualization of catalase in crude extracts of various strains after electrophoresis on an 8.5% polyacrylamide gel run under non-denaturing conditions. Extracts from the following strains were used: lane 0, 1S10 (*spo0*); lane II, 1S86 (*spoII*); lane III, 1S48 (*spoIII*); lane IV, 1S46 (*spoIV*); lane V, 1S50 (*spoV*); lane VI, 1S88 (*spoVI*); lane A, 168 (*wild type*); lane B, UM1015 (*katB*); lane C, UM1018 (*katA*).

evident in extracts of mutants with sporulation blocked at stages 0, II, III and IV. The expected mutliple bands of catalase 2 were absent, but the single slow band (only faintly visible in the *spo0* mutant) exhibited the same apparent molecular weight of 260 000 as catalase 2 (Loewen and Switala 1987*a*). In extracts of mutants with sporulation blocked at stages V and VI, the single slow band was supplemented with the multiple bands characteristic of catalase 2, all exhibiting apparent molecular weights of 260 000 on native gels. The conversion of the single band of catalase 2 into multiple electrophoretically separable bands involves a change in the charge of the protein and this is most likely a result of the removal of a small number of amino acids by a protease expressed during stage V of the sporulation process although some other form of modification cannot be ruled out.

The strain UM1013 used in this study contained mutations in both *katA* and *katB* and was sporulation defective, raising the question of the importance of the catalases in the sporulation process. The parent of UM1013, UM1001, lacked only catalase 1 because of a lesion in *katA* and was sporulation defective. By contrast, UM1005, which also lacked only catalase 1, remained sporulation proficient, suggesting that the defect in sporulation in UM1001 and UM1012 was not the result of the deficiency in catalase 1. The importance of the spore-specific catalase 2 in the sporulation process was investigated by transducing the mutation in *katB* into UM1005, generating UM1036, which lacked all assayable catalase. Despite the lack of both catalases, the strain remained sporulation proficient, indicating that neither catalase was required for sporulation.

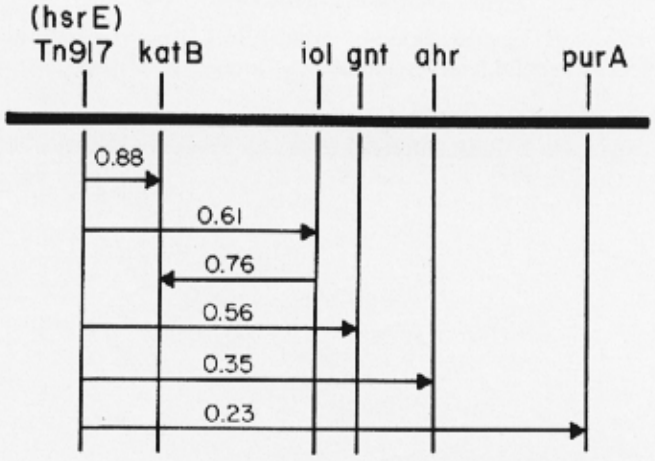

FIG. 2. Genetic map orienting *katB* relative to nearby genes. The gene locations were determined by transduction and the numbers represent the cotransduction frequencies.

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

Donor	Recipient	Selected marker	Unselected marker(s) Class	No.(%)
CU4165	UM1013	MLS^r	*kat*	95(16)
zif-85::Tn*917*	*katB6*		*kat*$^+$	513(84)
UM1019	UM1013	MLS^r	*pur, kat*$^+$	84(18)
zif-85::Tn*917*	*katB6*		*pur, kat*	23(5)
purA			*pur*$^+$, *kat*$^+$	328(70)
			pur$^+$, *kat*	36(7)
UM1026	UM1013	MLS^r	*iol, kat*$^+$	53(52)
zif-85::Tn*917*	*katB6*		*iol, kat*	9(9)
iol			*iol*$^+$, *kat*$^+$	36(36)
			iol$^+$, *kat*	3(3)
UM1027	UM1013	MLS^r	*gnt, kat*$^+$	88(53)
zif-85::Tn*917*	*katB6*		*gnt, kat*	4(3)
gnt			*gnt*$^+$, *kat*$^+$	60(36)
			gnt$^+$, *kat*	13(8)
UM1028	UM1013	MLS^r	*ahr*$^+$, *kat*$^+$	226(30)
zif-85::Tn*917*	*katB6*		*ahr*$^+$, *kat*	40(5)
ahrA			*ahr*$^-$, *kat*$^+$	403(54)
			ahr$^-$, *kat*	75(11)
UM1028	61668	MLS^r	*ahr*$^+$, *iol*$^+$	153(32)
zif-85::Tn*917*	*iol*		*ahr*$^+$, *iol*	6(1)
ahrA			*ahr*$^-$, *iol*$^+$	86(18)
			ahr$^-$, *iol*	235(49)
UM1028	YF100	MLS^r	*ahr*$^+$, *hsr*	190(40)
zif-85::Tn*917*	*Δigf, hsrE*$^+$		*ahr*$^+$, *hsr*$^+$	0(0)
ahrA			*ahr*$^-$, *hsr*	290(60)
			ahr$^-$, *hsr*$^+$	0(0)
Qβ944	UM1043	*iol*$^+$	*kat*	39(24)
	iol, katB		*kat*$^+$	126(76)

NOTE: *ahr*$^+$ denotes resistance to arginine hydroxamate or *ahrA* while *ahr*$^-$ denotes sensitivity to the reagent.

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