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# Multiple Catalases in *Bacillus subtilis*

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**Vegetative cells of *Bacillus subtilis* in logarithmic growth phase produced one catalase, labeled catalase 1, with a nondenatured molecular weight of 205,000. As growth progressed, other activity bands with slower electrophoretic mobilities on polyacrylamide gels appeared, including a series of bands with a common nondenatured molecular weight of 261,000, collectively labeled catalase 2, and a minor band, with a molecular weight of 387,000, labeled catalase 3. Purified spores contained only catalase 2, and it was not produced in *spo0A*- or *spo0F*-containing mutants. Strains deficient in catalase 1 or catalase 2 or both were selected after mutagenesis. Sensitivities of the two main catalases to NaCN, $NaN_3$, hydroxylamine, and temperature were similar, but the apparent $K_m$s for $H_2O_2$ differed, being 36.6 and 64.4 mM, respectively, for catalase 1 and catalase 2. The levels of catalase 1 increased 15-fold during growth into stationary phase and could be increased 30-fold by the addition of $H_2O_2$ to the medium. Catalase 2, which was not affected by $H_2O_2$, appeared only after the cells had reached stationary phase, and the maximum levels were only half of the basal level of catalase 1.**

Catalase (EC 1.11.1.6), which catalyzes the dismutation of hydrogen peroxide into $O_2$ and $H_2O$, has been purified from a variety of organisms and characterized. Many organisms, including *Escherichia coli* (2, 3, 15), *Saccharomyces cerevisiae* (20, 21), maize (19), and *Micrococcus radiodurans* (8), produce two unique catalases, while other organisms, including *Neurospora crassa* (11) and *Proteus mirabilis* (12), produce a single catalase. The catalases from most organisms, including those from *P. mirabilis* (12) and *S. cerevisiae* (21), physically resemble the mammalian catalase in being a tetramer of 60,000-dalton subunits containing four protoheme IX groups. However, both catalases from *E. coli*, the single catalase from *N. crassa*, and catalase A from *S. cerevisiae* (20) differ in several respects, including subunit composition, subunit size, and heme content.

The various *Bacillus* species are quite diverse in the levels of catalase produced. For example, *Bacillus coagulans* produces a 1,000-fold-higher level than does *Bacillus laterosporus* (24). Catalase is found in both vegetative cells and spores of *B. subtilis* (W. G. Murell, Ph.D. dissertation, University of Sydney, Sydney, Australia, 1955), and a significant increase in catalase is observed during the transition to stationary phase and at the onset of sporulation (6). The catalase activities in cells and spores were shown to possess similar properties, giving rise to the suggestion that the same enzyme is present in both states (Murell, Ph.D. dissertation). Subsequently, four catalase activity bands were observed after gel electrophoresis of crude extracts (10), but whether the bands were charge variants or discrete enzymes was not determined.

This study describes a reinvestigation of the catalase species present in *B. subtilis*. The results confirm that the earlier observations of similar properties for catalases from cells and spores are correct, but the present study also presents biochemical and genetic evidence that at least two distinct catalases are produced.

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## MATERIALS AND METHODS

**Strains.** The strains used are described in Table 1.

**Growth media.** Modified Schaeffer medium, 2× SG (13), contained 16 g of nutrient broth (GIBCO Laboratories), 0.5 g of $MgSO_4 \cdot 7H_2O$, and 2 g of KCl per liter to which was added after autoclaving 0.1 ml of 10 mM $FeSO_4$, 1 ml of 1 M $Ca(NO_3)_2$, 3.3 ml of 30% (wt/vol) glucose, and 0.1 ml of 1 M $MnCl_2$. LB medium (16) contained 10 g of tryptone (Difco Laboratories), 5 g of yeast extract (Difco), and 10 g of NaCl per liter. The minimal medium of Spizizen (22) contained 2 g of $(NH_4)_2SO_4$, 14 g of $K_2HPO_4$, 6 g of $KH_2PO_4$, 1 g of sodium citrate, and 0.2 g of $MgSO_4 \cdot 7H_2O$ per liter to which was added after autoclaving 0.3% (wt/vol) glucose or other carbon sources. Penassay broth (Difco) was prepared with Antibiotic Medium 3 (Difco) as directed by the manufacturer. Solid media were prepared by including 1.5% (wt/vol) agar. Tryptose blood base agar (GIBCO) was prepared as directed by the manufacturer. Flasks of liquid media were shaken at 37°C, and growth was monitored by using a Klett-Summerson colorimeter with a blue filter (red filter for 2× SG medium). Any samples with a Klett reading greater than 150 were diluted in the same medium to obtain an accurate value. The relationship of 0.26 mg (dry cell weight) per ml at 100 Klett units in 2× SG medium was determined by drying culture samples and correcting for the weight contribution from the medium.

**Catalase assays.** Catalase activity was determined by the method of Rorth and Jensen (18) in a Gilson oxygraph equipped with a Clark electrode. One unit of catalase is defined as the amount that decomposes 1 μmol of $H_2O_2$ in 1 min at 37°C. Catalase activity in colonies on agar plates was assayed by applying 1 drop of 30% $H_2O_2$ to the edge of a colony by using a syringe. Colonies with normal catalase activity vigorously evolved oxygen.

**Visualization of catalase and peroxidase activities on polyacrylamide gels.** Catalase and peroxidase activities were separated on 9% polyacrylamide gels, run as described by Davis (5) but with the separation gel prepared at pH 8.1 rather than pH 8.9. The peroxidase activity was visualized

TABLE 1. Description of *B. subtilis* strains

| Strain | Genotype | Source (reference) |
|---|---|---|
| 168 | *trpC2* | D. Stahly |
| 1S10 | *trpC2 spo0A12* | BGSC[a] |
| 1S19 | *trpC2 pheA1 spo0F221* | BGSC |
| UM1001 | *trpC2 kat-1 spo* | 168 + NG[b] |
| UM1005 | *trpC2 kat-5* | 168 + NG |
| UM1013 | *trpC2 kat-1 kat-6 spo* | UM1001 + NG |
| 1A603 | *trpC2 thiA*::Tn*917* | BGSC (23) |
| UM1015 | *trpC2 kat-6 thiA*::Tn*917 spo* | 1A603 × UM1013 → Tn*917 kat*[+][c] |

[a] BGSC, *Bacillus* Genetic Stock Center.
[b] NG, Cells were exposed to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.
[c] ×, Transductional cross.

by the method of Gregory and Fridovich (8), and catalase activity bands were stained by the method of Clare et al. (4) but with 20 mM $H_2O_2$ for better contrast.

**Native-molecular-weight analysis.** The method of Hedrick and Smith (9) was used in which protein was electrophoresed as described by Davis (5), except in pH 8.1 Tris hydrochloride, on a series of gels of various acrylamide concentrations from 6 to 14%. The $R_f$ of the protein relative to bromphenol blue tracking dye was determined for each gel of a different percentage. The retardation coefficient for each protein was determined from the slope of a graph of 100 · log(100 $R_f$) against polyacrylamide concentration. The logarithm of the retardation coefficient was then plotted against the logarithm of the molecular weight of the protein. The gels were stained for protein with Coomassie brilliant blue dye or for catalase as described above.

**Mutagenesis and isolation of catalase-deficient strains.** *B. subtilis* 168 was grown to early exponential phase ($10^8$ cells per ml) in 5 ml of 2× SG medium, collected by centrifugation, and suspended in 10 ml of citrate buffer (pH 5.5) containing 100 μg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine per ml. After being shaken gently at 37°C for 15 min, the cells were collected by centrifugation, washed once in 10 ml of SM medium (16) containing 0.02 M Tris hydrochloride (pH 7.6), 0.1 M NaCl, and 1 mM $MgSO_4$, and suspended in 5 ml of 2× SG medium. The culture was grown overnight at 37°C and plated on tryptose blood base agar plates suitably diluted to result in approximately 50 to 60 colonies per plate after overnight growth at this temperature. With a syringe, 1 drop of 30% $H_2O_2$ was applied to the edge of each colony, and catalase-deficient strains, which evolved oxygen at a much slower rate, were immediately streaked on a new plate. Approximately one colony in 2,000 to 3,000 was found to be deficient in catalase synthesis. The selected mutants were grown overnight, retested with $H_2O_2$ to confirm the deficiency, and then grown at 37°C in 5 ml of Penassay broth for oxygraph analysis of the catalase activity. Extracts were prepared by growing 50-ml cultures in 2× SG for specified times, collecting the cells by centrifugation, and after suspension in 1 ml of SM medium, sonicating the cells in four 1-min pulses with 5 g of glass beads. The cell debris and beads were removed by centrifugation.

**Spore purification.** Cultures were grown for 40 to 44 h in 2× SG medium, when examination in the microscope revealed maximum sporulation. The spores were collected by centrifugation, washed once with water, and separated from vegetative cells and germinated spores as described by Doi (7) by using a two-phase system prepared by mixing 11.2 g of polyethylene glycol, 34.1 ml of 3 M potassium phosphate (pH 7.0), and spore suspension to a final volume of 100 ml. The mixture was shaken and allowed to separate, and the bottom layer and interface containing the vegetative cells and germinated spores were removed. The spores were collected by centrifugation and washed with water. The procedure was repeated until no cells were visible at the interface.

## RESULTS

**Definition of catalase species.** The different catalase activities present in crude extracts of cultures grown for various times were separated by electrophoresis on native polyacrylamide gels and visualized (Fig. 1a). Only one predominant catalase band, labeled catalase 1, was present in the extracts of cells grown for 5 h. As growth progressed, changes occurred, with a second slower migrating band of catalase, labeled catalase 2, appearing in cells grown for 20 h. This was replaced by a series of isoenzyme bands with even slower mobilities at 30 and 40 h. Purified spores contained only the series of catalase 2 isoenzyme bands (Fig. 1b). In all extracts except that from cells grown for 5 h, a faint but discrete catalase band was evident migrating slower than the isoenzyme bands, and we labeled it catalase 3. No peroxidase activity bands were visible when the gel was stained for that activity.

The nondenatured molecular weights of the various catalase bands were determined by electrophoresis on acrylamide gels of different percentages (Fig. 2). Catalase 1 was found to have a molecular weight of 205,000, whereas all of the bands migrating slower than catalase 1, except for catalase 3, exhibited the same molecular weight of 261,000. These results suggest that the activity bands labeled catalases 1 and 2 are different species, justifying the different names. Furthermore, all of the isoenzyme bands collectively

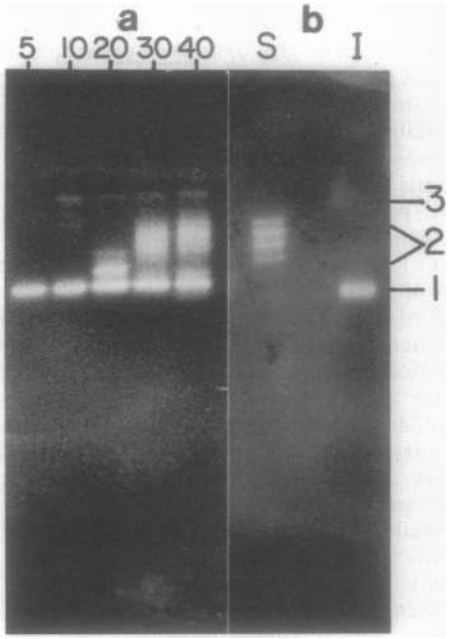


FIG. 1. (a) Catalase activities in crude extracts of *B. subtilis* prepared at 5, 10, 20, 30, and 40 h postinoculation and separated on native polyacrylamide gels. The numbers 1, 2, and 3 indicate the locations of catalases 1, 2, and 3, respectively. Approximately 0.85 U of catalase activity was run in each lane. (b) Catalase activity in an extract of purified spores from strain 168 (lane S) and in an extract of strain 168 induced with 0.25 mM $H_2O_2$ added at 0, 10, and 20 min and harvested at 45 min (lane I).

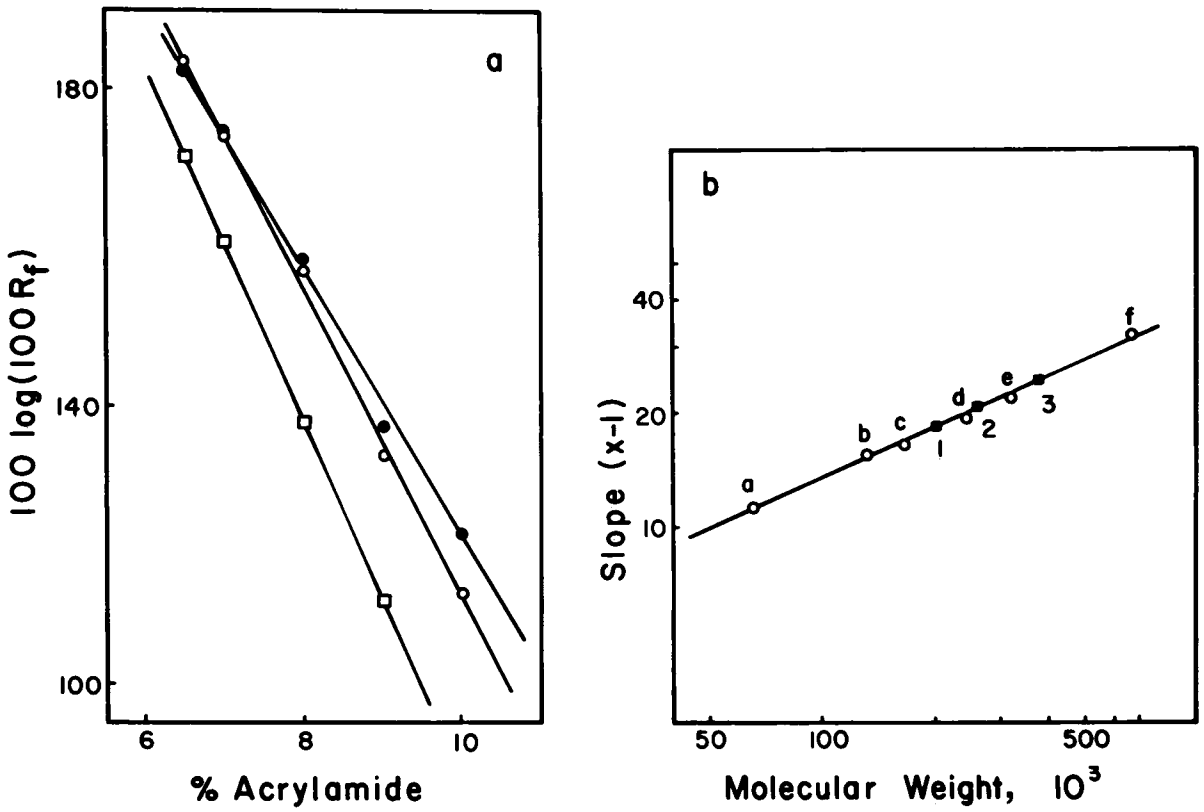


FIG. 2. Determination of nondenatured molecular weights of catalase isoenzymes of *B. subtilis* by electrophoresis on native polyacrylamide gels. (a) Effect of different acrylamide concentrations on the mobility of catalases 1 (●), 2 (○), and 3 (□). (b) Determination of the molecular weights of the three catalases from the slopes determined in panel a. The proteins used as molecular weight standards were as follows: a, bovine plasma albumin (monomer) (66,000); b, bovine plasma albumin (dimer) (132,000); c, HPI of *E. coli* (170,000); d, bovine catalase (240,000); e, thyroglobulin (dimer) (335,000); and f, thyroglobulin (tetramer) (669,000). The molecular weights determined were as follows: catalase 1, 205,000 (1); catalase 2, 261,000 (2); and catalase 3, 387,000 (3).

labeled catalase 2 had the same apparent molecular weight, confirming that they are related, differing only in electrophoretic mobilities, possibly the result of limited proteolysis. The slowest catalase, catalase 3, had a molecular weight of 387,000.

The effect of known sporulation mutations on catalase patterns was investigated. Strains containing *spo0A12* (Fig. 3) and *spo0F221* (data not shown) produced only catalase 1. Even cells grown for 36 h did not contain catalase 2, although a faint band of catalase 3 was evident. The phenomenon of defects early in sporulation affecting the synthesis of catalase 2 but not of catalase 1 suggests that the two enzyme activities are encoded by separate genes subject to different control mechanisms.

**Isolation and characterization of mutants deficient in catalase.** To confirm the presence of more than one catalase species in *B. subtilis*, mutants deficient in one of the main catalases were isolated. The most common type of phenotype created by nitrosoguanidine was exhibited by UM1005, which evolved a small amount of oxygen in the $H_2O_2$ drop test on agar plates. When cultures were assayed, catalase was undetectable in log-phase cultures and only low levels were found in stationary-phase cells and spores (Table 2). Another mutant, UM1001, which had a catalase phenotype similar to that of UM1005, had also lost the ability to sporulate (Table 2). Visualization of the catalase activities in UM1005 (Fig. 3) and UM1001 (data not shown) revealed the isoenzyme bands of catalase 2 but no catalase 1.

A further nitrosoguanidine treatment of UM1001 was carried out in an attempt to eliminate the remaining catalase 2 activity. In addition to strains in which the original mutation had reverted to the wild type, evolving wild-type levels of oxygen and exhibiting normal patterns of catalases 1 and 2 on gels, mutants that evolved no detectable oxygen in the $H_2O_2$ drop test were found. One of these mutants, UM1013, was chosen for further characterization. Assays of cultures and crude extracts for catalase activity confirmed its complete absence (Table 2). As expected, no catalase bands were visible after electrophoretic separation of crude extracts from cells grown for 16 and 36 h (Fig. 3).

Because of the predominance of catalase 1, it was not possible to directly isolate a mutant deficient in catalase 2 when catalase 1 was still present, and it was necessary to generate such a strain by other methods. Reversion of the mutation affecting catalase 1 synthesis in UM1013 by nitrosoguanidine treatment was one method, and bacteriophage PBS1-mediated transduction was a second method. One strain, UM1015, was selected, with the desired phenotype of evolving apparently wild-type levels of oxygen in the drop test. Visualization of the catalase activities revealed only catalase 1 in crude extracts (Fig. 3). At this point, strains had been generated containing just catalase 1 (UM1015) or just catalase 2 (UM1005) or neither enzyme (UM1013), which also suggested that the two main catalases in *B. subtilis* were affected by distinct loci. The appearance and disappearance of catalase 3 did not seem to be linked to the appearance or

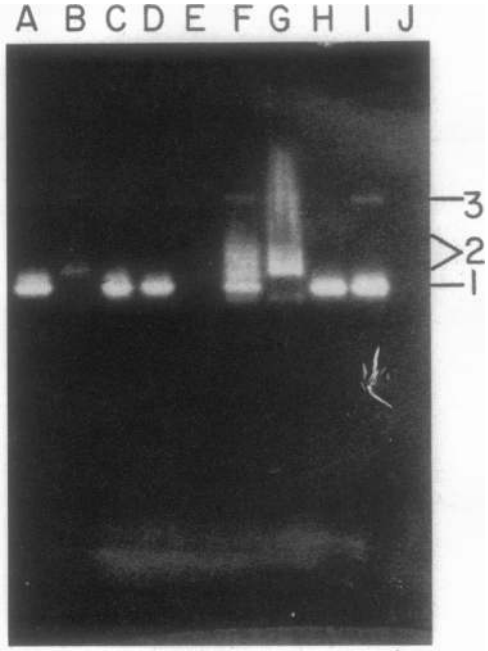


FIG. 3. Catalase activities in crude extracts of various strains of *B. subtilis*. Cells were harvested at 16 h (lanes A to E) and 36 h (lanes F to J) and disrupted by sonication. The numbers indicate the locations of catalases 1, 2, and 3, respectively. Extracts from the following strains were run: lanes A and F, strain 168; lanes B and G, UM1005; lanes C and H, UM1015; lanes D and I, 1S10; and lanes E and J, UM1013. Between 0.8 and 2.0 U of catalase was run in each lane, except for lane B, which contained 0.1 U, and lanes E and J, which contained no detectable activity in 160 μg of protein.

disappearance of either catalase 1 or 2, and its very low levels complicated further study.

The various mutants were assayed for sensitivity to $H_2O_2$ (Table 3), revealing the following order of increasing sensitivity: wild-type strain 168 = UM1015 (catalase 2 deficient) < UM1001 or UM1005 (catalase 1 deficient) = UM1013 (catalase 1 and 2 deficient). This order of sensitivity was consistent with the relative levels of catalase in exponentially growing cells (Table 2).

**Properties of the catalases.** It was possible to determine some of the properties of an individual catalase in crude extracts in the absence of the other catalases by choosing appropriate extracts (Table 4). For example, catalase 1 was the only catalase present in UM1015 and in 5- and 10-h extracts of strain 168, whereas catalase 2 was the only activity in purified spores and in UM1005. The apparent $K_m$s for $H_2O_2$ were determined to be 36.6 and 64.4 mM, respectively, for catalases 1 and 2. Both enzymes were 97% inactivated by heating to 100°C, and at 62°C, catalase 2 was found to be only slightly more stable. Both enzymes were inhibited by hydroxylamine, cyanide, and azide, although catalase 2 was slightly more sensitive. In whole spores, catalase 2 was more resistant to heat, azide, and cyanide but not to hydroxylamine. These results are similar to those reported by Murrell (Ph.D. dissertation), but whereas that worker concluded that there was only one enzyme in *B. subtilis*, the additional electrophoretic and genetic data suggest the presence of more than one catalase.

TABLE 2. Levels of catalase in extracts of various *B. subtilis* strains

| Strain | Catalase (U/mg [dry cell weight]) | |
|---|---|---|
| | Mid-log phase (grown 5 h) | Stationary phase (grown 24 h) |
| 168 | 7.5 | 123.7 |
| UM1001 | ND[a] | 3.9 |
| UM1005 | ND | 3.7 |
| UM1013 | ND | ND |
| UM1015 | 10.9 | 89.3 |

[a] ND, Not detectable.

TABLE 3. Sensitivity of *B. subtilis* strains to 0.5 mM $H_2O_2$

| Strain | Catalase deficiency | Cells killed in 5 min (%) |
|---|---|---|
| 168 | | 63.0 |
| UM1001 | 1 | 83.9 |
| UM1013 | 1 and 2 | 86.7 |
| UM1015 | 2 | 65.8 |

**Changes in catalase levels.** Changes in the levels of catalase have been observed during growth and spore development in *B. subtilis* (6). Catalase levels in strain 168 remained near basal levels during log-phase growth, but as the growth rate slowed, there was a five- to sixfold increase in catalase (Fig. 4a). The catalase levels remained high for approximately 15 h and then decreased as spores appeared in the medium. Only catalase 1 was present during this stage of growth (Fig. 1) and must be the species responsible for the increase. This was confirmed by assaying catalase levels during growth of UM1015 (Fig. 4b). This strain produced only catalase 1, and the increase in catalase as the cells grew into late stationary phase was similar to what was observed for strain 168. Strain UM1005, containing only catalase 2, exhibited much lower levels of catalase, and while there was an increase in catalase during the shift into stationary phase and the onset of developmental changes, the final level was only 1/10 of that in strains 168 or UM1015 (Fig. 4c).

In *E. coli*, catalase HPII levels increased as cells grew into stationary phase in a manner similar to what we have observed for catalase 1. The increase in catalase in *E. coli* was related to the turn-on of the tricarboxylic acid (TCA) cycle metabolism, wherein growth on any TCA cycle intermediate caused high levels of HPII (14). A similar comparison of catalase levels in *B. subtilis* grown on various carbon sources is shown in Table 5, and the results are quite different from those reported for *E. coli*. Growth of *B. subtilis* on glucose resulted in the highest catalase levels as compared with growth on TCA cycle intermediates and other nonfermentable carbon sources. Clearly, the turn-on

TABLE 4. Effect of various treatments on catalase activity in crude extracts of *B. subtilis* 168

| Treatment | Catalase (U/ml) (% inhibiton) | | |
|---|---|---|---|
| | Mid-log cell extract (catalase 1) | Sport extract (catalase 2) | Intact spores |
| None | 0.31 | 0.23 | 0.54 |
| NaCN (1 mM) | 0.03 (81) | ND[a] (100) | 0.08 (85) |
| $NaN_3$ (1 mM) | 0.01 (97) | ND (100) | 0.06 (89) |
| $NH_2OH$ (1 mM) | 0.01 (97) | ND (100) | ND (100) |
| 100°C, 15 min | 0.01 (97) | 0.01 (96) | 0.10 (82) |
| 62°C, 15 min | 0.01 (97) | 0.02 (92) | 0.20 (63) |
| 37°C, 15 min | 0.32 (0) | 0.24 (0) | 0.54 (0) |

[a] ND, Not detectable.

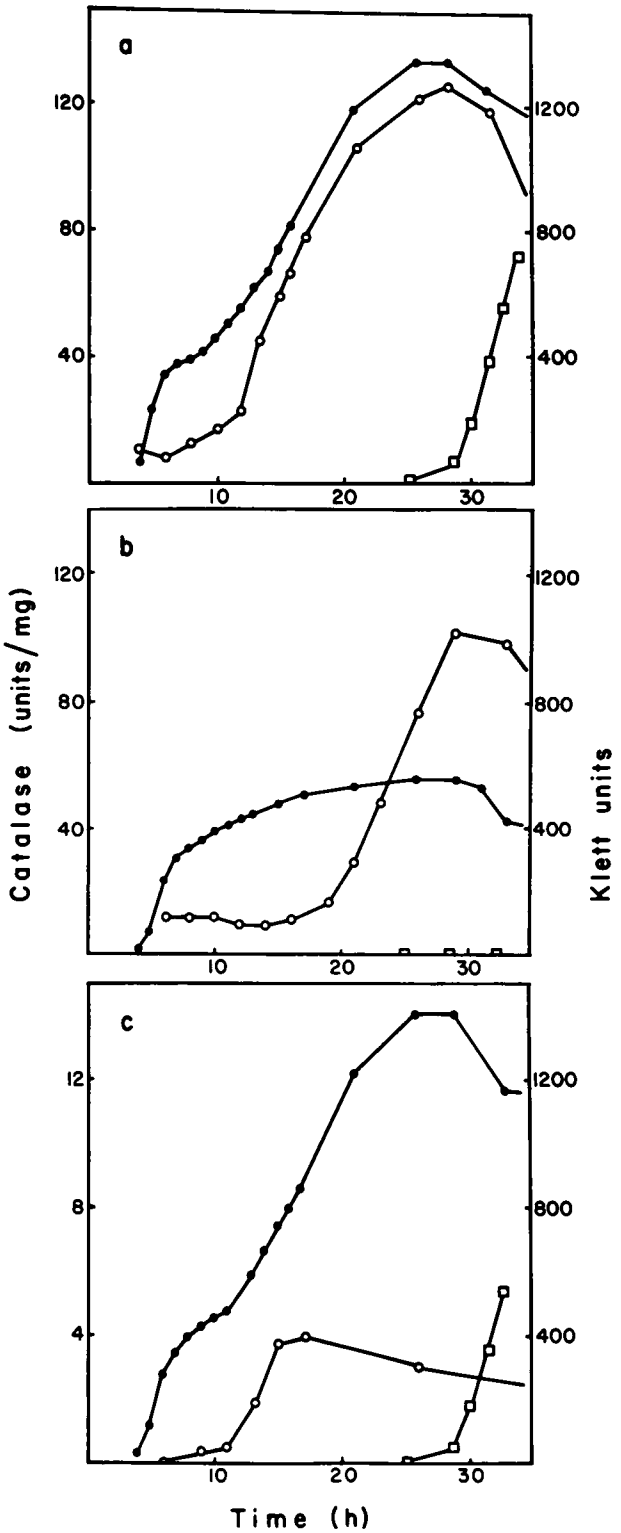


FIG. 4. Changes in catalase levels in various *B. subtilis* strains during growth. Growth (●), catalase levels (○), and spore appearance (□) were monitored. Catalase activity is given in units per milligram (dry cell weight). The following strains were used: a, strain 168; b, UM1015; and c, UM1005. Note the 10-fold-lower activity scale in panel c.

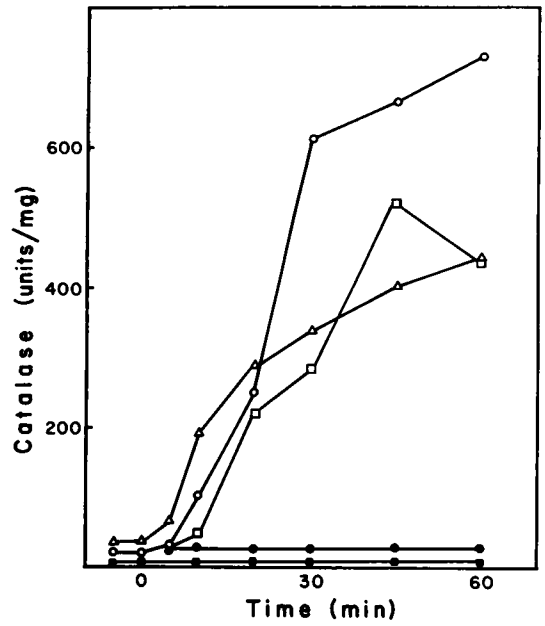


FIG. 5. Induction of catalase synthesis by $H_2O_2$ or ascorbate. Three additions of 0.25 mM $H_2O_2$, at 0, 10, and 20 min, were made to 2× SG medium containing, at a cell density of 50 Klett units, mid-log-phase cultures of strain 168 without (○) and with (●) 0.1 mM chloramphenicol, UM1015 (□), and UM1005 (■). Ascorbate (10 mM) was added at 0 min to a culture of strain 168 in 2× SG medium (△). Catalase activity is expressed as units per milligram (dry cell weight).

of catalase 1 synthesis was not linked to activation of the TCA cycle.

Developmental changes commenced about the same time that the increase in catalase 1 occurred. Strain UM1015 was sporulation defective but showed the change in catalase 1. Unfortunately, the sporulation defect in UM1015 was undefined, making it impossible to draw any conclusions regarding the influence of sporulation on changes in catalase 1. Strain 1S10 contains the pleiotropic *spo0A12* locus, which blocks sporulation at stage zero, effectively stopping the sporulation process at a very early stage. Despite this block, the strain showed an increase in catalase 1 at the same stage of growth as did strains 168 and UM1015, revealing that developmental changes were not responsible for the increase in catalase 1 (data not shown).

**Induction of catalase synthesis by $H_2O_2$.** The induction of catalase synthesis by $H_2O_2$ has been reported in a number of organisms, including *B. subtilis* (6, 10). Because it was impossible to repeat the experiments reported earlier (10) by

TABLE 5. Catalase levels in *B. subtilis* 168 grown in minimal medium supplemented with various carbon sources

| Carbon source | Catalase (U/mg [dry cell weight]) | |
|---|---|---|
| | Mid-log phase (grown 7.5 h) | Stationary phase (grown 24 h) |
| Glucose | 35.2 | 191.0 |
| Glucose + cAMP[a] | 29.5 | 195.6 |
| Fumarate | 21.7 | 73.0 |
| Glycerol | 32.6 | 129.0 |
| Gluconate | 8.0 | 155.3 |

[a] cAMP, Cyclic AMP.

using the details provided, a direct comparison of catalase patterns on polyacrylamide gels in this work with those in the earlier work was precluded and it was necessary to reinvestigate whether the synthesis of catalase 1 or catalase 2 or both was induced by $H_2O_2$. To ensure efficient induction, it was necessary to add the $H_2O_2$ in small volumes at 10-min intervals. This protocol resulted in a 30-fold increase in catalase levels (Fig. 5) in log-phase cultures of strain 168. When extracts of an induced culture were visualized after electrophoresis, only catalase 1 was present (Fig. 1b). Chloramphenicol prevented the increase in catalase activity, confirming that protein synthesis was responsible for the increase. The addition of $H_2O_2$ to a culture of UM1005, which produces only catalase 2, did not result in any increase in catalase levels (Fig. 5). The addition of ascorbate to a culture of strain 168 caused an 11-fold increase in catalase levels (Fig. 5), confirming that *B. subtilis*, like *E. coli*, can be induced by ascorbate.

## DISCUSSION

The presence of at least two unique catalases in *B. subtilis* was confirmed on the basis of the enzymes having different electrophoretic mobilities, different molecular weights, different apparent $K_m$s for $H_2O_2$, and different genetic origins. The original conclusion that *B. subtilis* produced only one catalase was based on the limited observations of similar properties for catalases in both vegetative cells and spores (Murrell, Ph.D. dissertation). These observations were confirmed and expanded upon in this report, resulting in the identification of multiple catalases. The existence of multiple catalases had been suggested earlier by the observation of several activity bands on polyacrylamide gels (10), but the precise pattern of catalase bands seen earlier (10) could not be reproduced by using the details provided. The current characterization was based on the pattern of activity bands that changed as growth progressed. Catalase 1, a major band, and catalase 3, a minor band, appeared reproducibly as discrete bands with significantly different molecular weights. The grouping of several activity bands with similar electrophoretic mobilities under the name catalase 2 was justified on the basis of similar molecular weights and common disappearance following mutagenesis or in *spo0*-containing mutants. In possessing two main species of catalase, *B. subtilis* is therefore similar to *E. coli* (2, 3, 15), *M. radiodurans* (8), and *S. cerevisiae* (20, 21).

The catalase present in the greatest amounts in vegetative cells and the only catalase in mid-log-phase cells was catalase 1. The basal levels were 3-fold higher than the basal levels of catalase in *E. coli* (17), and the fully induced levels were 20-fold higher. During growth into stationary phase, *B. subtilis*, like *E. coli* (14), induced the synthesis of a catalase, specifically catalase 1. In *E. coli*, this late turn-on of synthesis was linked to the turn-on of nonfermentative metabolism, although the mechanism was not determined. There was no such link in the case of *B. subtilis*, and furthermore, the increase in catalase could not be linked to the onset of spore development because *spo0* mutations did not affect the increase. Catalase 1 synthesis could be induced over 30-fold by $H_2O_2$, suggesting that the in vivo increase in catalase 1 could be the result of $H_2O_2$ generated in stationary-phase cells. The nature of the protein mediating the $H_2O_2$ effect, whether a positive effector like OxyR protein, which activates the transcription of *katG* as well as other genes in *E. coli* and *Salmonella typhimurium* (1), or a negative effector remains to be determined.

The synthesis of catalase 2 was tied very closely to the sporulation process. It was the predominant activity found in purified spores, and mutations blocking sporulation at stage zero prevented its synthesis. One mutant with an undefined block in sporulation still produced catalase 2, suggesting that blocks in sporulation at later stages may not block enzyme synthesis. A screening of a series of defined mutants blocked in sporulation at later stages should define the precise stage at which catalase 2 synthesis is initiated. The converse relationship, whether sporulation could occur in the absence of catalase 2, could not be determined from the strains constructed, and an answer will have to await either the isolation of a sporulation-proficient catalase 2 mutant or the demonstration of genetic cotransfer of a sporulation-deficient phenotype with a mutation in the catalase 2-encoding gene. The levels of catalase 2 remained very low, reaching only 50% of the basal level of catalase 1, and were unaffected by $H_2O_2$. The production of catalase 2 would seem to be redundant because of its close similarity to the more abundant catalase 1, and there must be a very strong selective process that preferentially absorbs catalase 2 into spores rather than catalase 1, which is present in 40-fold greater amounts.

## ACKNOWLEDGMENT

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