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Glycosidase Activities of *Bacillus anthracis*

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Bacillus anthracis could be distinguished from the taxonomically related species *B. cereus*, *B. mycoides*, and *B. thuringiensis* by a comparison of glycosidase activities. All the bacilli tested possessed α-glucosidase activity, as evidenced by the hydrolysis of *p*-nitrophenyl-α-D-glucoside. In *B. anthracis*, the glucosidase activity could be enhanced by the addition of agents which damage cellular surface structures. Treatment of *B. anthracis* strains with toluene, Triton X-100, or mutanolysin or cellular disruption by sonication resulted in higher rates of α-glucoside hydrolysis than were accomplished by cells suspended in buffer. It is suggested that intact *B. anthracis* cells have a limited permeability to the glucosidase substrate. In contrast to the results obtained for *B. anthracis*, Triton X-100 markedly diminished the enzymatic hydrolysis of *p*-nitrophenyl-α-D-glucoside by strains of *B. cereus*, *B. mycoides*, and *B. thuringiensis*. Triton X-100 also enhanced the α-maltosidase activity of *B. anthracis* but not that of the other bacilli. *B. mycoides* possessed an apparently inducible *N*-acetylglucosaminidase, although the enzyme was absent in *B. anthracis*. The glucosaminidase was inducible in the presence of *p*-nitrophenyl-*N*-acetylglucosamine in the absence of conventional nitrogen sources. Chloramphenicol prevented the induction of the glucosaminidase in *B. mycoides*. In several *B. cereus* and all *B. thuringiensis* strains, the glucosaminidase was constitutive. The results suggest a means for the rapid laboratory differentiation of *B. anthracis* from other closely related bacilli. Assays for α-glucosidase and α-maltosidase, in the presence and absence of Triton X-100, can be used to distinguish *B. anthracis* from *B. cereus*, *B. mycoides*, and *B. thuringiensis*. Similarly, the hydrolysis of *p*-nitrophenyl-β-*N*-acetylglucosamine induced by *B. mycoides* but not by *B. anthracis* provides an additional means for differentiating these similar bacilli.

The taxonomic positions of the pathogen *Bacillus anthracis* and the nonpathogens *B. cereus*, *B. mycoides*, and *B. thuringiensis* are close. Present technology does not permit the rapid identification of *B. anthracis* by means of serological technology (7, 12, 16), metabolic activities (8, 13, 14), nucleic acid relatedness (11), or determination of composition of intact cells (10). Gordon (9) has reviewed many of the difficulties in the identification of members of the genus *Bacillus*. Recently, Cole et al. (5) observed that soybean agglutinin would agglutinate members of *B. anthracis* and *B. mycoides*, whereas a lectin from *Helix pomatia* would agglutinate only *B. mycoides*. Other members of the genus *Bacillus* were not agglutinated by either lectin. Because *B. anthracis* and *B. mycoides* strains have a tendency to autoagglutinate, lectin reactivity may not be entirely reliable for rapid laboratory identifications of these organisms.

In the genus *Bacillus*, many glycosidases have been found and partially characterized (reviewed in reference 17). These include amylases, mannanases, fucosidases, galactanases, chitanases, and many others. Furthermore, bacilli seem to possess autolysins, such as glucosaminidases (3, 4), which could possibly be used for diagnostic purposes. This work was designed to study glucosidase activities in *B. anthracis*, *B. cereus*, *B. mycoides*, and *B. thuringiensis*, with the goal of describing methods for the routine identification of the pathogen *B. anthracis*. The results show that the organism can be readily differentiated from its close taxonomic and normally nonpathogenic neighbors by the use of selected nitrophenyl saccharides as substrates.

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MATERIALS AND METHODS

Chemicals and reagents. *ortho*-Nitrophenyl-β-D-galactopyranoside (βONPG), *p*-nitrophenyl-α-D-galactopyranoside (αPNPG), *p*-nitrophenyl-α-D-mannopyranoside, *p*-nitrophenyl-β-D-mannopyranoside, *p*-nitrophenyl-α-D-glucoside (αPNPGL), *p*-nitrophenyl-β-D-glucoside, *p*-nitrophenyl-α-L-fucopyranoside, *p*-nitrophenyl-β-D-fucopyranoside, *p*-nitro-phenyl-α-D-maltoside (αPNPML), *p*-nitrophenyl-β-D-maltoside, *p*-nitrophenyl-β-D-lactoside, *p*-nitrophenyl-*N*-acetyl-β-D-glucosamine (PNPAG), Triton X-100, sodium dodecyl sulfate, Tween 80, and *N*-acetylglucosamine (GlcNAc) were products of Sigma Chemical Co., St. Louis, Mo. Salts, solvents, and routine reagents were obtained from Fisher Scientific Co., Louisville, Ky. Mutanolysin (19) was a gift from K. Yokogawa, Dainippon Ltd., Osaka, Japan.

Bacteria and growth conditions. Some of the species and strains of *Bacillus* employed in this study have been described by Cole et al. (5). These include *B. anthracis* ATCC 14185, ATCC 11966, UMRL, Ames, Colorado, V-770, Vollum 1-B, and Sterne. In addition, *B. anthracis* V770-NPI-R (U.S. Army Medical Research Institute for Infectious Diseases culture collection) was used in the present study. *B. cereus* T, ATCC 246, ATCC 6464, 9620, 9634, ATCC 23260, ATCC 7064, ATCC 14579, ATCC 11778, and ATCC 19637, *B. mycoides* ATCC 6462 and MWC, and *B. thuringiensis* 4040, 4041, 4042B, 4045, 4055, and 4060 were from the previous investigation (5). The stock cultures were maintained as spores on AK sporulation agar (BBL Microbiology Systems, Cockeysville, Md.). For experimental purposes, the bacteria were cultured overnight at 37°C (except for *B. mycoides* ATCC 6462, which was incubated at room temperature) on tryptic blood agar base (TBAB; Difco Laboratories, Detroit, Mich.).

Assay for glycosidase activity. Bacterial cell suspensions for demonstration of glycosidase activity were prepared by transferring the cells to TBAB plates and incubating the organisms overnight at 37°C. The bacterial colonies were suspended in a small volume of phosphate buffer (10 mM NaH_2PO_4, adjusted to pH 7.0 with 0.1 N NaOH) to an optical density of 0.5 at 450 nm on a Coleman Junior II spectrophotometer. Each glycosidase substrate was made in a 3 mM concentration and stored in the freezer until used. To each well of microtiter plates, 200 μl of bacterial cell suspension and 100 μl of the glucosidase substrate were added. The bacterial cell controls in each assay consisted of 200 μl of bacterial cell suspension and 100 μl of buffer. The controls for the glycosidase substrate were 200 μl of buffer and 100 μl of the glucosidase substrate. Each assay was run in duplicate. The plates were incubated at 37°C and read at 1, 3, and 6 h and after overnight incubation. A yellow color indicated a positive reaction (6).

Quantitative determinations of the products of glycosidase activity were made by use of a Coleman Junior spectrophotometer. For *o*-nitrophenol a wavelength of 420 nm was used, whereas for *p*-nitrophenol a wavelength of 405 nm was used.

Sonication of bacteria. Cells washed and suspended in phosphate buffer were sonicated with a Sonifier cell disruptor (model W140; Branson Sonic Power Co., Danbury, Conn.) at an output of 6. Heating of the cells during sonication was avoided by use of an ice bath. Usually, 2-min sonic treatments were employed, which resulted in the reduction of the turbidity of the suspensions by 40 to 50%. No effort was made to determine the absolute extent of cellular breakage.

RESULTS

Glycosidase activities in *B. anthracis* and related bacilli. When suspensions of *Bacillus* species were incubated with nitrophenol-containing saccharides, *B. anthracis* exhibited α-glucosidase and α-maltosidase activities. No significantly high reactions were observed for nitrophenol-containing α,β-fucosides, βONPG, α, β-mannosides, β-glucoside, β-maltoside, or β-lactoside. Table 1 shows typical results. Furthermore, the *B. cereus* and *B. thuringiensis* strains exhibited metabolic heterogeneity. The *B. anthracis* strains, *B. cereus* ATCC 246, ATCC 7064, ATCC 11778, and ATCC 23260, the *B. mycoides* strains, and *B. thuringiensis* exhibited 4060 would not cleave the β-glucoside, although all the other bacilli exhibited β-glucosidase activities. Furthermore, when *N*-acetylglucosaminidase activities were considered, only *B. cereus* ATCC 14579 was highly active, although some other strains of *B. cereus* as well as *B. thuringiensis* were weakly active. There was no consistent means of designating one enzyme as belonging to only one species.

Some important results of diagnostic significance emerged from this survey, however. The *B. anthracis* strains did not exhibit color reactions with PNPAG after 3 h, whereas all the *B. thuringiensis* strains did. Moreover, all the *B. anthracis* strains were strongly active against an α-maltoside, but none of the *B. thuringiensis* strains was similarly active. The results therefore suggest a rapid means of distinguishing *B. anthracis* from many of the related bacilli.

***p*-Nitrophenyl-*N*-acetyl-β-D-glucosaminidase in *B. mycoides*.** Figure 1 shows the time course of the hydrolysis of PNPAG by *B. mycoides*. When cells were grown on TBAB, there was a considerable delay in hydrolysis of the substrate.

TABLE 1. Glycosidase activities in *Bacillus* species

Organism	Glycosidase activity (absorbance units) for the following substrate[a]:											
	αPNPF	βPNPF	βONPG	αPNPG	αPNPGL	βPNPGL	PNPAG	βPNPL	αPNPML	βPNPML	αPNPM	βPNPM
B. anthracis ATCC 14185	0.00	0.01	0.02	0.00	0.53	0.00	0.00	0.00	0.20	0.04	0.00	0.00
B. anthracis ATCC 11966	0.02	0.01	0.01	0.00	0.19	0.00	0.00	0.00	0.35	0.05	0.03	0.00
B. mycoides ATCC 6462	0.00	0.00	0.01	0.00	0.23	0.00	0.01	0.00	0.09	0.00	0.00	0.00
B. cereus T	0.01	0.01	0.00	0.00	0.32	0.13	0.02	0.01	0.17	0.01	0.00	0.00
B. cereus ATCC 246	0.00	0.00	0.00	0.01	0.18	0.01	0.01	0.04	0.13	0.00	0.00	0.00
B. cereus ATCC 6464	0.00	0.01	0.01	0.00	0.26	0.07	0.00	0.04	0.09	0.01	0.00	0.01
B. cereus 9620	0.00	0.06	0.01	0.02	1.05	0.11	0.21	0.04	0.83	0.00	0.02	0.02
B. cereus 9634	0.00	0.00	0.03	0.00	0.34	0.08	0.00	0.04	0.06	0.00	0.01	0.00
B. cereus ATCC 23260	0.00	0.01	0.00	0.00	0.18	0.10	0.00	0.04	0.03	0.00	0.00	0.00
B. cereus ATCC 7064	0.00	0.00	0.00	0.00	0.25	0.00	0.02	0.04	0.05	0.01	0.00	0.00
B. cereus ATCC 14579	0.00	0.02	0.02	0.02	0.64	0.09	0.22	0.01	0.19	0.00	0.03	0.00
B. cereus ATCC 11778	0.00	0.01	0.02	0.01	0.10	0.00	0.10	0.01	0.05	0.02	0.00	0.00
B. cereus ATCC 19637	0.01	0.00	0.04	0.01	0.66	0,08	0.01	0.02	0.10	0.01	0.00	0.02
B. thuringiensis 4040	0.02	0.00	0.03	0.01	0.18	0.51	0.18	0.03	0.06	0.06	0.00	0.02
B. thuringiensis 4041	0.00	0.01	0.00	0.01	0.30	0.53	0.27	0.04	0.03	0.04	0.00	0.00
B. thuringiensis 4042B	0.01	0.01	0.03	0.00	0.50	0.09	0.24	0.02	0.10	0.02	0.00	0.00
B. thuringiensis 4045	0.01	0.00	0.00	0.01	0.28	0.49	0.26	0.03	0.03	0.03	0.01	0.00
B. thuringiensis 4055	0.01	0.00	0.04	0.01	0.34	0.08	0.32	0.02	0.02	0.00	0.00	0.00
B. thuringiensis 4060	0.01	0.00	0.00	0.01	0.33	0.03	0.15	0.03	0.06	0.03	0.00	0.01

[a] Color was assessed by spectrophotometry after 3 h of incubation of the cells at 37°C in phosphate buffer. Substrate concentrations were 1.0 mM. Densities of cell suspensions (final optical density) were 0.25 to 0.30 at 450 nm and 1 cm. For *B. anthracis* UMRL, Ames, Colorado, Vollum 1-B, V770-NPI-R, V-770, and Sterne, strong α-glucosidase (net absorbance increases from 0.11 to 0.90) and α-maltosidase (absorbance increases from 0.1 to 0.6) activities were also observed. In addition, these *B. anthracis* strains were devoid of detectable *N*-acetylglucosaminidase activity. Abbreviations: αPNPF, *p*-nitrophenyl-α-D-fucopyranoside; βPNPF, *p*-nitrophenyl-β-D-fucopyranoside; βONPG, *o*-nitrophenyl-β-D-galactopyranoside; αPNPG, *p*-nitrophenyl-α-D-galactopyranoside; αPNPGL, *p*-nitrophenyl-α-D-glucoside; βPNPGL, *p*-nitrophenyl-β-D-glucoside; PNPAG, *p*-nitrophenyl-*N*-acetyl-β-D-glucosamine; βPNPL, *p*-nitrophenyl-β-D-lactoside; αPNPML, *p*-nitrophenyl-α-D-maltoside; βPNPML, *p*-nitrophenyl-β-D-maltoside; αPNPM, *p*-nitrophenyl-α-D-mannopyranoside; βPNPM, *p*-nitrophenyl-β-D-mannopyranoside.

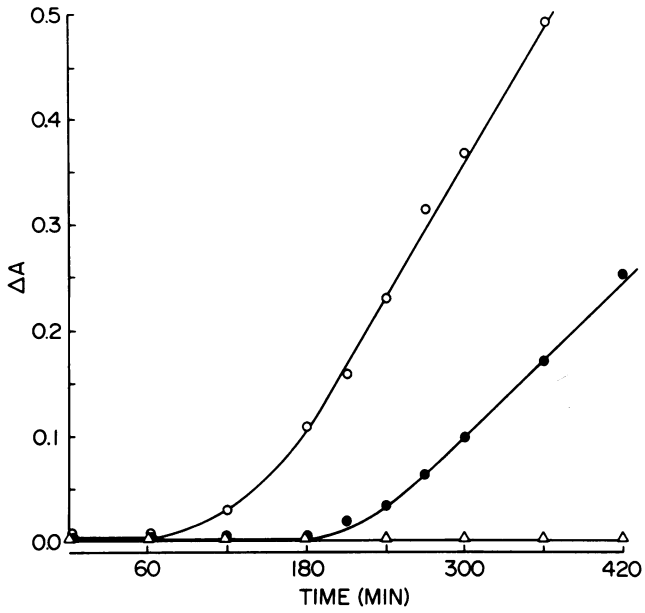

FIG. 1. Time course for the hydrolysis of PNPAG by *B. mycoides* 6462 and *B. anthracis*. Symbols: ●, *B. mycoides* 6462 grown on TBAB; ○, *B. mycoides* 6462 grown on TBAB containing 100 μg of GlcNAc per ml, △, *B. anthracis* 11966 grown on TBAB, ΔA, Change in absorbance.

Growth of the cells, however, in the same type of agar but containing 100 μg of GlcNAc per ml resulted in a cell preparation capable of hydrolyzing the PNPAG much more quickly. It appears, therefore, that one of the products of *N*-acetylglucosaminidase activity, GlcNAc, induces the enzyme at least partially or promotes the production of the enzyme after the substrate is added to the washed cells. It is also important to note that protein synthesis was required for the hydrolysis of PNPAG, regardless of the growth medium. When PNPAG and chloramphenicol (final concentration, 50 μg/ml) were added to suspensions of the bacteria, no hydrolysis of the substrate occurred, even after 24 h of incubation at 37°C. Moreover, the absence of a nitrogen source in the suspension medium suggests that the glucosaminidase had to be synthesized from preexisting cellular proteins. Finally, when various strains of *B. anthracis* were incubated in PNPAG, there was no evidence of hydrolysis. Growth of *B. anthracis* in the presence of GlcNAc did not result in the production of the glucosaminidase.

α-Glucosidase activities in *B. mycoides* and *B. anthracis*. The observation that strains of *B. anthracis* and *B. mycoides* produced generally high α-glucosidase levels (Table 1) suggested a possible diagnostic use of αPNPGL. The problem was to define conditions that would result in either enhanced or repressed enzyme activity in *B. anthracis* but not in the other bacteria. Accordingly, suspensions of *B. anthracis* were subjected to various treatments and then were mixed with αPNPGL. Agents such as toluene which increase permeability of bacterial cell membranes (18) may permit rapid diffusion of the substrate into the cell. Similarly, a mild chaotrope, Triton X-100, may cause enough cellular disorganization to result in ready access to the enzyme by the substrate. The same rationale holds for cell breakage and for incubation with mutanolysin. The results (Fig. 2) reveal that *B. anthracis* readily hydrolyzed αPNPGL when the cells had been disrupted by sonication. In addition, Triton X-100 also markedly enhanced the hydrolysis of αPNPGL in *B. anthracis*. Rates of hydrolysis exceeding that of the untreated cells were also observed when the suspensions were interacted with mutanolysin or toluene. Control experiments showed that mutanolysin alone had no effect on αPNPGL. One reagent, sodium dodecyl sulfate, completely destroyed the ability of *B. anthracis* to hydrolyze the substrate. The results show that it is a simple matter to greatly enhance the activity of α-glucosidase in *B. anthracis*.

When strains of *B. mycoides* were subjected to sonication or were treated with the various agents, the rates of hydrolysis of αPNPGL were generally lower than that of the control cells. Figure 3 shows that Triton X-100 markedly diminished the ability of *B. mycoides* ATCC 6462 to hydrolyze αPNPG. This result is in marked contrast to that of *B. anthracis*.

For diagnostic microbiology it is desirable to use stable and reliable reagents. For this reason, the decision was made to investigate the effects of Triton X-100 on enzyme activities in *Bacillus* species. In the case of *B. anthracis*, Triton X-100 always stimulated α-glucosidase activities. Triton X-100 diminished the α-glucosidase activities of other bacilli that were tested (Table 2). It is concluded that the monitoring of α-glucosidase activities in the presence and absence of Triton X-100 is a valuable diagnostic acid for *B. anthracis*.

α-Maltosidase activities in *B. anthracis* and *B. mycoides*. The data thus far suggest that the hydrolysis of both PNPAG and αPNPG would be useful in the rapid laboratory identification of *B. anthracis*. An additional enzyme which could be stimulated or inactivated specifically in *B. anthracis* would greatly enhance the potential utility of glycosidases as diagnostic aids. Triton X-100 markedly stimulated α-maltosidase activity in *B. anthracis*, whereas in *B. mycoides* ATCC 6462, the activity was destroyed (Fig. 4). Similar results were obtained for all *B. anthracis* strains. *B. mycoides* MWC did not react with substrate. The effects of Triton X-100 on the α-maltosidase activities in other bacilli were similar to that

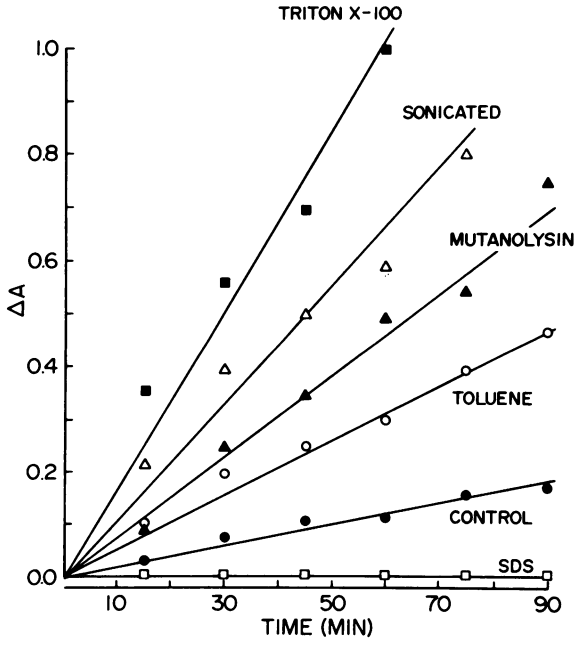

FIG. 2. Hydrolysis of αPNPGL by *B. anthracis* UMRL. ΔA, Change in absorbance; SDS, sodium dodecyl sulfate.

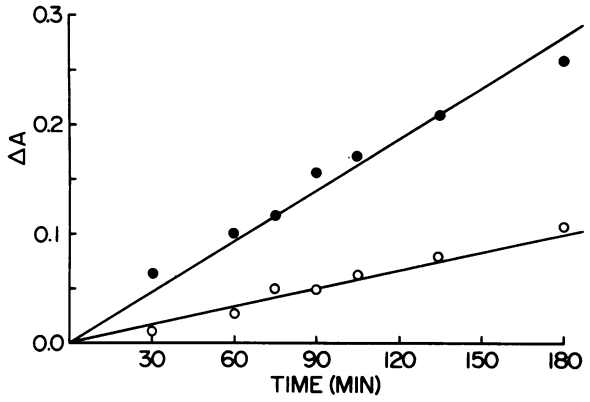

FIG. 3. Hydrolysis of αPNPGL by *B. mycoides* 6462. Symbols: ●, control cells; ○, cells in the presence of 1% Triton X-100. ΔA, Change in absorbance.

observed in *B. mycoides* ATCC 6462 (Table 3). Incubation of cells with trypsin or subtilisin did not modify the rates of activities of either α-maltosidases or α-glucosidases, suggesting that the enzymes were not surface exposed (data not shown).

DISCUSSION

As far as is known, this is the first study to survey the closely related species *B. anthracis*, *B. mycoides*, *B. cereus*, and *B. thuringiensis* for glycosidase activities. The objective of the research has been to establish conditions in which *B. anthracis* can be readily distinguished from the other bacilli. The results show that it is possible to use glucosaminidase, α-glucosidase, and α-maltosidase activities to identify *B. anthracis*. Even though all the bacilli exhibited a constitutive α-glucosidase activity, only *B. anthracis* was stimulated by Triton X-100. The exact reason why α-glucosidase activity can be stimulated in *B. anthracis* is obscure, but the results suggest that substrate availability is important. For example, cells disrupted by sonication rapidly hydrolyzed αPNPGL (Fig. 2). It is likely that cellular damage exposed the α-glucosidase. Support for this premise derives from the observation that toluene could also enhance the hydrolysis of αPNPG by *B. anthracis* (Fig. 2). Toluene is known to create holes in the membranes of bacteria, which permit the entry of small molecules into the cells but do not permit the exit of proteins or nucleic acids (18). The denaturant sodium dodecyl sulfate completely eliminated the α-glucosidase activities in all the bacilli, presumably by unfolding the enzymes. The labilities of the α-glucosidases of *B. mycoides*, *B. cereus*, and *B. thuringiensis* (Table 2) are exactly opposite

TABLE 2. Effect of Triton X-100 on *p*-nitrophenyl-α-D-glucosidase activity in *Bacillus* species[a]

Organism	Δ Absorbance	
	Control	Triton X-100
B. anthracis ATCC 14185	0.36	0.51
B. anthracis UMRL	0.11	0.48
B. cereus T	0.13	0.12
B. cereus ATCC 14179	0.02	0.02
B. mycoides ATCC 6462	0.06	0.04
B. mycoides MWC	0.07	0.04
B. thuringiensis 4040	0.05	0.01
B. thuringiensis 4045	0.06	0.01

[a] Cells were suspended to an optical density of 0.25 in 10 mM sodium phosphate (pH 7.0) buffer or in buffered 1% Triton X-100 and incubated at 37°C. The substrate (final concentration, 1.0 mM) was α-PNPGL. Values shown represent absorbance changes after 60 min of incubation.

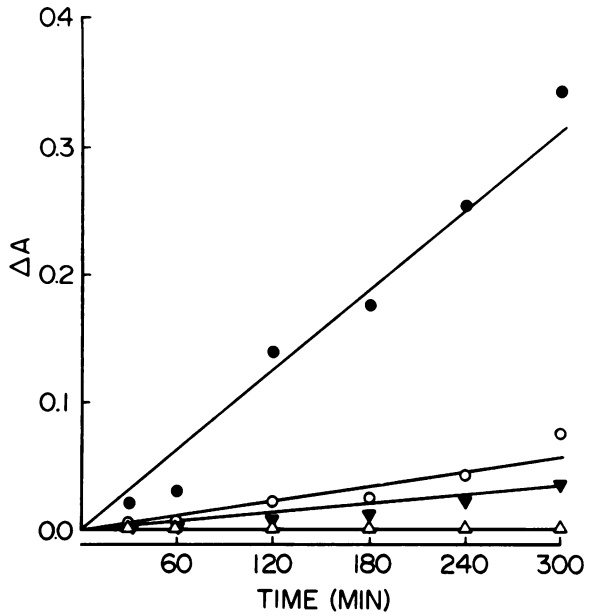

FIG. 4. Effects of Triton X-100 on α-maltosidase activity of *B. anthracis* and *B. mycoides* 6462. Symbols: ○, *B. anthracis* 11966; ●, *B. anthracis* 11966 plus 1% Triton X-100; ▼, *B. mycoides* 6462; △, *B. mycoides* 6462 plus 1% Triton X-100. Cell densities were 0.2 absorbance unit. ΔA, Change in absorbance.

TABLE 3. Effect of Triton X-100 on α-maltosidase activities in *Bacillus* species[a]

Organism	Δ Absorbance	
	Control	Triton X-100
B. anthracis ATCC 14185	0.05	0.19
B. anthracis ATCC 11966	0.04	0.18
B. anthracis UMRL	0.04	0.14
B. mycoides ATCC 6462	0.02	0.00
B. mycoides MWC	0.00	0.00
B. cereus ATCC 246	0.01	0.01
B. cereus USAMRIID 9620	0.81	0.78
B. cereus ATCC 23260	0.00	0.00
B. cereus ATCC 7064	0.02	0.00
B. cereus ATCC 14579	0.23	0.15
B. cereus ATCC 11778	0.04	0.03
B. cereus ATCC 19637	0.06	0.06
B. thuringiensis 4040	0.02	0.00
B. thuringiensis 4041	0.00	0.00
B. thuringiensis 4042-B	0.04	0.00
B. thuringiensis 4045	0.01	0.00
B. thuringiensis 4055	0.02	0.00
B. thuringiensis 4060	0.00	0.00

[a] Cell suspensions (final optical density, 0.20 to 0.25) were mixed with αPNPML (final concentration, 1 mM) in phosphate buffer or in phosphate buffer–Triton X-100 (final concentration, 1%, vol/vol). Values shown represent the net change in absorbance measured at 405 nm at 180 min.

that observed for the α-glucosidase of *B. anthracis*. It is likely that the enzymes in the species closely related to *B. anthracis* are easily disorganized or denatured by Triton X-100 and the other treatments, such as sonication. The data showing that Triton X-100 enhanced the hydrolysis of α-maltosides in *B. anthracis* qualify Triton X-100 as an additional reagent for the identification of that bacterium. It is likely that the α-maltosidase of *B. anthracis* is distinct from the enzyme which hydrolyzes αPNPGL, although the bacterium did slowly hydrolyze αPNPGL. The effect of Triton X-100 on *B. anthracis* can be used to distinguish the bacterium from *B. mycoides*, *B. cereus*, and *B. thuringiensis*.

The observation that mutanolysin (19) could enhance the hydrolysis of αPNPG by *B. anthracis* may be explained (Fig. 2). Control experiments established that mutanolysin could not hydrolyze αPNPGL. It is also unlikely that a protease contaminant in the mutanolysin would have resulted in the higher rate of hydrolysis of αPNPGL by *B. anthracis*, as neither trypsin nor subtilisin modified the reaction rates. Mutanolysin only slowly lyses *B. anthracis* cells (G. Zipperle, personal communication). The extent of lysis induced by mutanolysin in the time course of the experiment (Fig. 2) was no more than 10%. It is likely, therefore, that mutanolysin weakened surface structures to such an extent that the cells became more permeable to the substrate, thereby permitting a faster rate of hydrolysis of the αPNPGL.

Results showing that *B. mycoides* can be readily adapted to cleave the glycosidic linkage in PNPAG are interesting. First, the lack of chromophore formation when *B. anthracis* was incubated with PNPAG, whereas the other bacilli did hydrolyze the substrate, shows a distinct difference between *B. anthracis* and the related bacilli. The glucosaminidase activity required protein synthesis, because chloramphenicol prevented chromophore development. Moreover, GlcNAc, a product of the action of the enzyme, seemed to prime the cells for later induction of the enzyme by the substrate. The requirement for protein synthesis by the *B. mycoides* cells for hydrolysis of PNPAG may reveal useful information about the physiology of the organism. The cells were suspended in PNPAG-buffer solution in the absence of an obvious nitrogen source. It is possible that the turnover of preexisting proteins provides a source of amino acids. This would be much different from current concepts about cell protein turnover in bacteria (15), as turnover is not generally regarded as a common event. The nitrogen could have come from PNPAG itself. There are reports of deacetylases and deaminases in *B. subtilis* (1, 2), but little is known about their function or regulation. Significant use of the substrate, however, to provide a source of nitrogen would limit substrate availability and hence reduce chromophore development rather than stimulate it. Further work will be necessary to gain an understanding of the role of glucosaminidases in the physiology of *B. mycoides*.

In terms of possible glucosaminidase activity in *B. anthracis*, there is no compelling reason to conclude that the bacterium needs the enzyme for growth. The cell wall of *B. anthracis* is almost completely *N* unacetylated (G. Zipperle, J. Ezzell, and R. J. Doyle, Can. J. Microbiol., in press). An acetylglucosaminidase would therefore be an unlikely autolysin. The present results are compatible with this view.

It seems likely that α-glucosidases, α-maltosidases, and β-*N*-acetylglucosaminidase will play important roles in the laboratory identification of *B. anthracis*. The enhancement of α-glucosidase and α-maltosidase activities in *B. anthracis* by Triton X-100 and the lack of glucosaminidase activity in the bacterium provide substantial tools for diagnostic purposes. Clinical testing will be required for confirmation of the use of glycosidases for the identification of *B. anthracis*.

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