EXTRACTION, PURIFICATION AND CHARACTERIZATION OF A PEPTIDOGLYCAN HYDROLASE FROM VEGETATIVE CELLS OF *CLOSTRIDIUM PERFRINGENS* TYPE A

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A peptidoglycan hydrolase (PGH), which causes the germination and lysis of the coat-stripped spores of *Clostridium perfringens*, was extracted from *perfringens* vegetative cells. The enzyme was partially purified and showed an MW of 125,000 and a *pI* of 7.6. It was sensitive to a variety of monovalent and divalent cations and was inhibited by sulfhydryl-active agents. The sites of PGH action, the cell wall and the spore-cortex, were determined by the release of free amino groups from cell wall fragments and the release of soluble fluorescent products from fluorescamine-labelled cortical fragments of the *C. perfringens* spores. The enzyme appears distinct from the extracellular initiation protein produced by *C. perfringens*. PGH may be an autolysin with corticolytic activity.

Clostridium perfringens is a common cause of human food poisoning in Western countries. Illness results from the ingestion of large numbers of vegetative cells which sporulate and produce an enterotoxin in the intestine (30). The organism is commonly found in raw meat and poultry, frequently in the spore state. Temperature abuse of food allows germination and subsequent multiplication of vegetative cells.

In previous papers we have described the extraction and isolation of a spore-lytic enzyme (SLE) from whole spores of *C. perfringens* (15, 16). And we have previously isolated and characterized an extracellular spore-lytic (germination) enzyme from vegetative cells of this organism (12, 25, 27, 34, 35). The latter enzyme causes the germination of coatless spores and hydrolysis of cortical fragments of this organism. The enzyme was termed initiation protein (IP) by Cassier and Sebald (6) who first detected it by its ability to promote recovery of heat-injured spores of this organism, presumably by replacing the inactivated, native, spore-germination enzyme.

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Several cell wall hydrolases active on *Micrococcus* cell walls are reportedly present in *C. perfringens* (28). Enzymes which cause the germination of spores of *Bacillus cereus* (5), *Bacillus megaterium* (11) and *C. perfringens* (1, 16) have also been reported. The latter enzymes were obtained by extraction of the respective spores. We report here the presence and extraction of another peptidoglycan lytic enzyme from *C. perfringens*, this one associated with the vegetative cell.

MATERIALS AND METHODS

Bacterial strains. The organism used was *C. perfringens* type A strains of NCTC 8798. Cells of two asporogenic stage 0 mutants of NCTC 8798, strains 8-16, and 8-62 were also used. All strains were originally obtained from C. Duncan and were maintained in cooked meat medium (Difco).

Media. Cells were grown at 37°C in a complex (DS) sporulation medium (7) using raffinose (0.4%) as the carbohydrate (26). The medium was inoculated (1% inoculum) with cells grown overnight in fluid thioglycollate medium (Difco). In preliminary experiments the effectiveness of starch (0.4%) vs. raffinose was compared. For preparation of cell walls, cells were grown in a medium composed of 1.5% casitone, 0.5% yeast extract, 0.8% dextrose, 0.05% sodium thioglycollate and 1% sodium phosphate (dibasic).

Preparation of cells. Six h cells were harvested from DS medium by centrifugation. To remove any contaminating extracellular enzymes and proteins these pellets were washed twice at 5°C with 50 mM Tris-HCl buffer, pH 8.0 containing 250 mM NaCl then twice with the same buffer without NaCl. The washed cells were used for subsequent experiments.

Buffers. Buffer A consisted of 50 mM sodium citrate, pH 6.5; buffer B contained 50 mM Tris-HCl, pH 8.0 at 5°C, 0.15 M NaCl and protease inhibitors (1.8×10^{-3} mM phenylmethane sulfonylfluoride and 2.1 mM ethylenediaminetetraacetic acid). Buffer C consisted of 50 mM glycine-hydroxide, pH 10.0, and buffer D was composed of 50 mM sodium phosphate, pH 6.5.

Peptidoglycan hydrolase (PGH) assay. The assay for peptidoglycan hydrolase was based on the ability of the enzyme to cause the germination of spores of the same strain whose coats had been chemically removed. The procedure, done in duplicate, was similar to that described by Franceschini and Labbé (12). To remove coat protein cleaned spores were suspended in sodium dodecyl sulfate (0.5%) plus dithiothreitol (50 mM) in buffer C for 2 h at 37°C. The treated spores were centrifuged ($10,000 \times g$, 15 min) washed three times with distilled water and resuspended in a sufficient volume of buffer A to result in an absorbance (600 nm) of 0.4–0.5. The assay mixture consisted of 2 ml of spore suspension and 0.1–0.5 ml of enzyme, the volume of enzyme added depending on the enzyme activity. The decrease in absorbance (600 nm) of the spore suspension which accompanies loss of spore refractility was measured in a Beckman model 25 spectrophotometer equipped with a kinetic system. One unit of activity was defined as the amount producing a decrease

in absorbance of 0.001/min/ml. In most cases results are expressed as U/ml of extracted cells. To standardize results, washed cells were resuspended on a weight basis before extraction (see below). Results in tables are given as means of duplicate experiments.

Cell disruption: sonication. The intracellular spore-lytic enzyme was extracted from washed cells by sonication or autolysis. In the case of sonication a 25% suspension (w/v, wet weight) of cells was prepared in buffer B, placed on ice and sonicated intermittently until few intact cells remained. The disrupted cell suspension was centrifuged at $16,000 \times g$ for 20 min. The supernatant fluid was kept. In one series of experiments the pellet was resuspended in the same initial volume of buffer B and extracted four more times. Extracts obtained by using high salt concentrations were dialyzed against several changes of buffer B before assay.

Cell disruption: autolysis. A 25% (w/v) suspension of washed cell pellet was prepared using buffer B. The suspension was incubated in a 37°C water bath until autolysis was complete (about 2 h). The autolysate was centrifuged at $16,000 \times g$ for 20 min and the supernatant fluid was saved as crude extract.

Inactivation of spore-lytic enzyme. Cellulose phosphate-purified spore lytic enzyme (110 U/ml) was heated at 100°C for 10 min or treated with Pronase (200 g/ml, Calbiochem) for 30 min at 37°C. Activity was assayed as described above.

Preparation of fluorescamine-labelled cortical fragments. Fluorescamine-labelled cortical fragments and time course studies of their hydrolysis with PGH was performed as previously described for initiation preprotein (34).

Preparation of cell walls. Late log phase cells (5 l) were collected by centrifugation at $10,000 \times g$ for 20 min. The pellet was autoclaved for 10 min to inactivate lytic enzymes and suspended in 200 ml of 50 mM sodium phosphate buffer, pH 7.0. The cells were disrupted by sonication with glass beads (710–1,180 μm , Sigma). Crude wall fragments were collected by centrifugation at $27,000 \times g$ for 30 min, resuspended in 0.05 M glycine-hydroxide buffer, pH 10, containing 1% sodium dodecyl sulfate. The mixture was stirred at 37°C for 16 h. The cell walls were collected by centrifugation and resuspended in 100 ml of 50 mM sodium phosphate buffer, pH 7.0. Trypsin and ribonuclease (final concentrations each: 0.5 mg/ml) were added and the mixture was incubated with stirring at 37°C for 4 h. The treated walls were washed consecutively with cold distilled water four times, 2 M sodium chloride twice and distilled water twice. The walls were then heated for 10 min in a boiling water bath to inactivate the trypsin and ribonuclease. After two further washings with distilled water the pelleted walls were collected by centrifugation and stored at -20°C .

Hydrolysis of cell wall fragments. Thawed cell wall fragments were suspended in 10 mM sodium phosphate buffer, pH 6.5, sonicated for 1 min to disrupt clumps and adjusted to an absorbance (600 nm) of 0.45 to 0.50. For time course studies 1.5 ml of cellulose-phosphate purified PGH (initial activity 2,000 U/ml) were added to 15 ml of prewarmed (45°C) cell wall fragments. Samples (2 ml) were removed periodically and the reaction was stopped by boiling for 7 min. Residual wall

fragments were removed by centrifugation at $13,000 \times g$ for 5 min and the supernatant fluid was passed through a $0.2 \mu\text{m}$ filter prior to analysis of free amino groups. As a control PGH was heated at 100°C for 10 min before assay. Hydrolysis of wall fragments at 45°C was followed for 2 h and was monitored as loss in absorbance at 600 nm.

Chemical assays. Protein determination was done by the method of Bradford (3) using bovine serum albumin as a standard. Free amino groups were determined by the method of Ghuysen et al. (13) and reducing sugars by the ferricyanide reduction method of Park and Johnson (31).

pH optimum determination. Fifty mM buffers ranging in pH from 4.5 to 9.0 were prepared as described by Gomori (17). Buffer was mixed with a small volume of coatless spores (suspended in distilled water) to give a final volume of 2.0 ml and a final absorbance (600 nm) of 0.4–0.5. The enzyme in Buffer D was mixed with the prewarmed (45°C) buffer/substrate mixture and assayed for activity.

Effect of cations. Divalent cations (Ca^{2+} , Mg^{2+} , Co^{2+} , Mn^{2+}) were added to the assay mixture at final concentrations of 1 and 10 mM. All were in chloride form except Mn^{2+} which was in sulfate form. Univalent cations (Li^+ , Na^+ , K^+ , NH_4^+ , all chloride form) were added at final concentrations of 1.0–5.0 mM. The reactions, done in duplicate in a final volume of 2.0 ml, were performed by first incubating enzyme ($50 \mu\text{l}$) and ions at 45°C for 10 min before adding substrate.

Polyethyleneimine precipitation. Polyethyleneimine (Sigma) was used to precipitate nucleic acids and acidic proteins from crude extracts. A 10% stock solution was prepared and adjusted to pH 8.0 using concentrated HCl as described by Jendrisak and Bruggess (23). Polyethyleneimine was added to the crude extract at a final concentration of 0.015% (v/v). The mixture was stirred for 10 min and centrifuged at $16,000 \times g$ for 15 min. The supernatant fluid was saved.

Ammonium sulfate precipitation. The polyethyleneimine-treated crude extract was brought to 70% saturation with solid ammonium sulfate and stirred gently at 5°C overnight. The precipitate was collected by centrifugation ($16,000 \times g$, 25 min) and redissolved in 1/3 of the starting volume using buffer B. The material was then dialyzed at 4°C against several changes of a 20-fold larger volume of appropriate column-equilibration buffer.

DEAE-Sephacel (anion) chromatography. The redissolved and dialyzed ammonium sulfate precipitate was applied to a column ($1.7 \times 20 \text{ cm}$) of DEAE-Sephacel equilibrated with buffer B. Fractions containing unbounded activity were applied to a cellulose-phosphate column after extensive dialysis against buffer D plus 0.02% sodium azide.

Cellulose phosphate (cation) chromatography. Cellulose phosphate was hydrated and charged as described by Himmelhoch (22). After packing, the column ($2.5 \times 17 \text{ cm}$) was washed with 10 bed volumes of 0.5 M sodium phosphate, pH 6.5 then equilibrated with 10 bed volumes of run buffer, i.e. buffer D plus 0.02% sodium azide. The pooled and dialyzed DEAE-Sephacel eluate was applied to the column at 0.5 ml/min. After application of enzyme, the column was washed with run buffer

until 280 nm-absorbing material in the eluate was negligible. Enzyme was eluted using a 250 ml linear gradient of 0–0.3 M NaCl in buffer D. Two ml fractions were collected and assayed for protein (A_{280}) and enzyme activity. Fractions with a specific activity of 15% of maximum or greater were kept.

Hydroxyapatite (adsorption) chromatography. The pooled cellulose phosphate eluate was applied to a column (1.7 × 9 cm) of hydroxyapatite previously equilibrated with buffer D. The column was then washed with three to four bed volumes of buffer until the eluate was free of 280 nm-absorbing material. The enzyme was eluted with a linear gradient of 0.05–0.3 M sodium phosphate buffer, pH 6.5. Fractions of two ml were collected and assayed for enzyme activity and protein (A_{280}). Fractions containing enzyme with a specific activity of 50% of maximum or greater were collected and saved for characterization studies.

The pooled eluates from ion exchange and hydroxyapatite chromatography were concentrated by ultrafiltration using a PM 10 (Amicon) membrane.

Each of three thiol-active agents, mercuric chloride (Fisher Scientific, Fairlawn NJ, U.S.A.), *p*-chloromercuriphenylsulfonic acid (Sigma) and *N*-ethylmaleimide (Sigma) were added to the assay mixture at a final concentration of 10^{-4} to 10^{-1} M to examine their effect on enzyme activity. A small volume of coatless spore suspension was mixed with the above reagents in buffer A and prewarmed to 45°C. Then 50 µl of enzyme was added and assayed (in duplicate) for activity. The ability of sodium thioglycollate (10 mM) to reverse the inhibitory effect was determined by premixing it with enzyme for 2 min before adding it to the prewarmed spore/inhibitor mixture for assay.

Polyacrylamide gel electrophoresis. Disc gel (7.5% acrylamide) electrophoresis of basic proteins was performed in duplicate at pH 4.3 as described by Mauer (29) using Pyronin Y as the tracking dye. In these cases, the electrode connections to the power supply were reversed. One gel was stained with Coomassie blue G-250 and the other sliced (0.5 cm) to determine enzyme activity. Slices were crushed and extracted with 1 ml of buffer A overnight. Following cellulose phosphate chromatography only one gel band showed PGH enzyme activity. For SDS-PAGE, cellulose phosphate-purified PGH was extracted from 10 cylindrical gels (10% enzyme recovery). This material was concentrated using a Centricon 10 concentrator (Amicon) to about 1,000 U/ml (0.05 mg protein/ml) and used for molecular weight determination by SDS-PAGE.

Molecular weight determination by SDS-PAGE. The molecular weight of PGH was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (5% acrylamide) (21) of PGH electrophoretically-purified (by gel electrophoresis) following cellulose-phosphate chromatography. Molecular weight standards (8 to 10 µg; Sigma) were—galactosidase (116,000), phosphorylase b (97,400), bovine albumin (66,000), egg albumin (45,000), and carbonic anhydrase (29,000).

Analytical isoelectric focussing. Analytical isoelectric focussing was performed using 10 cm tubes of 7% acrylamide. A pH range of 3–10 was obtained

using Isolyte (Isolab, Inc.) ampholytes at a final concentration of 3%. The anode and cathode electrodes were composed of 0.003 M H_3PO_4 and 0.02 M NaOH plus 0.02 M $\text{Ca}(\text{OH})_2$ respectively. The cellulose-phosphate-purified, ultrafiltration-concentrated material (0.68 mg) was mixed with the acrylamide before polymerization. Focussing was carried out for 6.5 h at 200 V at 4°C. The gels were sliced in 0.4 cm sections and eluted in 1 ml deionized, distilled water or in 1 ml 50 mM citrate buffer, pH 6.5. Water extracts were used to determine pH. Buffer extracts were used to determine PGH activity.

RESULTS

Extraction of PGH

Preliminary results indicated that PGH was produced in large amounts in DS sporulation medium. To determine the necessity for sporulation, cells were grown in DS medium using two different carbohydrates each of which supports different levels of sporulation. Higher PGH levels were obtained using raffinose (78.4 U/ml) than starch (1.0 U/ml) in spite of lower sporulation levels (27% versus 90%) in raffinose. The results suggested that the amount of extractable SLE did not depend on the degree of sporulation but rather on the medium composition. To confirm this, PGH activity in vegetative cells and mutant strains was determined. Fluid thioglycollate is a glucose-rich medium in which sporulation does not occur. PGH activity was extractable from both exponential- and stationary-phase cells grown in this medium. Similarly enzyme activity was present in asporogenous mutants of strain NCTC 8798 whether cell disruption was achieved by sonication or autolysis. The use of a French press was less effective than sonication (data not shown).

The above extractions were performed at a low (0.15 M) NaCl concentration. When cells were extracted using high salt concentrations, the highest SLE activity was obtained using 3 M NaCl or 6 M LiCl, although a concentration of 0.15 M NaCl was quite effective, yielding 75% of the maximum obtained with 6 M LiCl (Table 1).

The first extraction of cells with low salt buffer did not remove all SLE activity (Table 2); 15–18% of the total activity could be obtained by each of three additional treatments. The likely source of the SLE in these cases was cells unaffected by the

Table 1. Effect of salt concentration on extraction of PGH from *C. perfringens* cells.

Salt	Percent of maximum activity
6 M LiCl	100
3 M LiCl	83
3 M NaCl	99
0.15 M NaCl	75

Salts were dissolved in 50 mM Tris-HCl buffer, pH 8.0 with protease inhibitors; cells were grown in DS-raffinose medium for 6 h, washed, and extracted by sonication.

Table 2. Successive extraction of PGH from *C. perfringens* cells.

Order of extraction	PGH activity recovered (total units)	Percent of total extracted
1st	105,000	45
2nd	41,800	18
3rd	36,600	17
4th	33,600	15
5th	14,000	6

Cells were grown in 20 l of DS-raffinose for 6 h then washed and extracted five times by sonication in buffer B.

Table 3. Summary of purification of *C. perfringens* peptidoglycan hydrolase.

Step	Total protein (mg)	Total activity (units $\times 10^{-3}$)	Sp. activity (units/mg)	-Fold purification	% yield
Crude extract	6,090	231	38	1	100
Polyethyleneimine supernatant	3,780	176.4	46	1.2	75.2
Ammonium sulfate	1,129	233.8	207	5.5	101
DEAE-Sephacel	110	290.2	2,586	68.2	125
Cellulose phosphate	8.0	100	12,500	330	43.3
Hydroxyapatite	1.7	87.0	51,176	1,347	37.6

preceding sonication treatments. It is likely that the efficiency of the latter is affected by the increased viscosity associated with the release of proteins and nucleic acids requiring their periodic removal by centrifugation.

Appearance of PGH during growth

The appearance of PGH during growth of strain NCTC 8798 in DS medium was followed in cells grown in DS medium. Enzyme activity from cells, resuspended on a weight basis before extraction, was highest at 6 h (Fig. 1). This reflected the achievement of a stationary phase at that time.

Purification and properties of PGH

The procedure used to purify the PGH is shown in Table 3. Polyethyleneimine was found to be a simple method for the removal of nucleic acids without adding RNase or DNase. PGH did not bind to DEAE-Sephacel but this medium did remove significant amounts of contaminating protein. An inhibitor was also apparently removed since total activity increased after this step. The elution profile of PGH following cellulose phosphate and hydroxyapatite chromatography is shown in Fig. 2. The final yield of the PGH was 37.6% with a 1,347-fold purification. The hydroxyapatite-purified material showed three bands one of which was associated with PGH activity (not shown). Attempts at further purification was unsuccessful

Fig. 1

Fig. 1. Growth and spore-lytic enzyme (peptidoglycan hydrolase) activity in *C. perfringens* NCTC 8798 grown in DS medium with raffinose as the carbohydrate source. Duplicate one hundred ml samples were taken at various times from a 1-l culture, washed and extracted by sonication as described in METHODS. Maximum sporulation occurred at 6 h. Results are from duplicate assays.

Fig. 2

Fig. 2. Chromatography of PGH on cellulose-phosphate and hydroxyapatite. (A) Unbound protein from a DEAE-Sephacel was applied to a cellulose phosphate column. (B) Fractions 20-40 were pooled and applied to a hydroxyapatite column. Pooled fractions indicated by a bar were analyzed by polyacrylamide gel electrophoresis.

due to the extreme lability of the hydroxyapatite-purified PGH.

The molecular weight of PGH was 125,000 as determined by SDS-PAGE. The isoelectric point was 7.6. Alkaline pI's have been reported for other bacterial lytic enzyme (2). A distinct pH optimum of 6.0 was obtained (not shown).

Neither divalent ions (1 to 10 mM) nor monovalent ions (10 to 50 mM) stimulated

Fig. 3

Fig. 3. Kinetics of release by PGH of soluble fluorescent products during hydrolysis of fluorescamine-labelled spore cortical fragments of *C. perfringens*.

Fig. 4

Fig. 4. Release of amino groups by PGH (●) during hydrolysis (■) of *C. perfringens* vegetative cell wall fragments.

PGH activity. On the contrary all except cobalt, which had no effect, inhibited PGH. This could be due to ionic strength effects since the inhibitory effects were concentration-dependent (not shown).

The thiol-active agents, mercuric chloride, *p*-chloromercuriphenylsulfonic acid, and *N*-ethylmaleimide completely inhibited activity at concentrations of 0.1, 10, and 100 mM respectively. Most of the inhibition was reversible by preincubation of the enzyme with sodium thioglycollate (10 mM, not shown). This suggests a role for sulfhydryl groups in maintaining enzyme activity. No enzyme activity remained if the preparation was treated for 10 min at 100°C or treated with pronase.

The enzyme also hydrolyzed fluorescamine-labelled cortical fragments. This was indicated by the release of soluble, fluorescent end products. Most of these labelled end products had appeared within 30 min (Fig. 3). None appeared if the PGH was boiled for 10 min before the assay. The results indicate that PGH causes spore germination because of its direct action on the spore cortex. This and the following characterization studies were performed using cellulose phosphate-purified material. Gel electrophoresis of this preparation revealed five bands, only one of which had PGH activity.

Treatment of cell wall fragments with PGH resulted in their hydrolysis and a concomitant release of free amino groups (Fig. 4). Within 1 h absorbance was reduced to about 75% of the initial value. No reducing sugars were detected. No enzyme activity was observed if PGH was boiled 10 min before assay. The results indicated that PGH is a peptidase or an amidase but not an *endo*- β -*N*-

acetylglucosaminidase or *endo-β-N*-acetylmuramidase.

DISCUSSION

In this study we have extracted and partially purified a lytic enzyme active on both spore cortex and vegetative cells of *C. perfringens*. The enzyme causes the germination of sensitized spores. Also, it has a molecular weight of 125,000, causes the release of soluble fluorescent products from fluorescamine-labelled cortical fragments, and hydrolyses cell wall fragments. The enzyme was inhibited by sulfhydryl agents as well as by various monovalent and divalent cations. In these respects it is similar to the extracellular spore-lytic enzyme of *C. perfringens*, Initiation Protein (27). The differences between IP and the PGH isolated here were, respectively, 1) a molecular weight of 100,000 vs. 125,000, 2) a broad pH optimum of between 6 and 7 vs. a distinct pH optimum of 6.0, 3) isoelectric points of 7.9 vs. 7.6, and 4) an ability to hydrolyze cortical fragments of *C. perfringens* (as determined by a loss in absorbance) vs. lack of such activity under the same assay conditions. It is likely that these two proteins are distinct. On the other hand in one *Bacillus* species it was shown that a cloned cell wall hydrolase gene could express several forms due to proteolytic processing (33). *C. perfringens* has both intracellular and extracellular proteases (Park and Labbé, unpublished (18)). This may indicate that IP and PGH represent the same gene product but with post-translational modifications. Amino acid sequence analysis could clarify this point. To date limited quantities of each enzyme have precluded such an analysis.

Our previous results dealing with germination enzymes from intact *C. perfringens* spores (15, 16) led to our present attempts at identifying similar lytic activity within sporulating cells of this organism. Such enzymes have been identified in *Bacillus thuringiensis* to be an *N*-acetylmuramyl-L-alanine amidase and an endopeptidase (24). Similar enzymes have also been reported in sporulating cells of *Bacillus sphaericus* (20) and *Bacillus subtilis* (19). However, in the work described here we found no association of extractable enzyme activity and sporulation. Both vegetative cells and asporogenic mutants contained PGH activity. This is the first report of a spore-lytic enzyme present within vegetative cells of spore-forming bacteria.

Although there was no association of PGH with sporulation of this organism we chose a sporulation medium for further work because of the higher enzyme yields from cells grown in it. The highest activity was obtained from early stationary-phase cells and decreased in later phases. At about 8 h of growth in DS medium, some autolysis begins. This may account for the fall in total activity at this time.

A high salt concentration was not necessary to extract the bulk of this enzyme, suggesting it is not tightly bound to the cell wall as has been reported for autolysins from *C. perfringens* and *Bacillus subtilis* vegetative cells for which 3 M or 5 M LiCl was used for extraction (4, 8, 36). PGH seems to be a soluble cytoplasmic enzyme.

The function of the PGH described here remains unclear. It is tempting to assign it a role in germination or sporulation. However it differs in size and other characteristics from the native germination enzyme extracted from spores of this organism (16). In addition it was produced during growth, in the absence of sporulation. It may be a lytic enzyme involved in cell division or cell wall turnover and happens to affect spore cortex the basic structure of which appears to be similar to vegetative wall peptidoglycan (35). Lytic enzymes from vegetative *Bacillus* sp. have been implicated in the turnover of cell wall peptidoglycan during growth (14, 32) and in the separation of daughter cells at the time of cell division (9, 10). Since PGH is not a glycosidase it is distinct from previously-reported autolysins from *C. perfringens* (36).

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