

Purification and Characterization of A Cell Wall Hydrolyzing Enzyme Produced by An Alkalophilic *Bacillus* sp. BL-29

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A strain BL-29, which produces a extracellular lytic enzyme on *E. coli* was isolated from the soil. The strain was identified as belonging to the genus *Bacillus* sp. The lytic enzyme was purified to homogeneity by ion exchange chromatography and gel filtration. Specific activity of the purified enzyme was 28,850 U/mg protein and yield of the enzyme was 5%. The purified enzyme showed a single band on SDS-PAGE and its molecular weight was estimated to be 31,000 by SDS-polyacrylamide gel electrophoresis and gel filtration column chromatography. The optimum temperature and pH were 55°C and pH 10.0, respectively. The enzyme was stable at 45°C but enzyme activity was reduced by up to 50% when the temperature was raised to 55°C for 15 min. Stable range of pH was from 5.0 to 11.0. but Enzyme activity was inhibited by lead-acetate, mercuric chloride, ethylene glycol-bis-[β -aminoethyl ether]-N,N,N',N'-tetraacetic acid (EGTA), and ethylenediamine tetraacetic acid (EDTA), but not affected considerably by treatment with other chemical reagents.

Bacteriolytic enzyme is an enzyme which catalyzes the hydrolysis of a structurally important linkage in the bacterial cell wall peptidoglycan resulting in the lysis of the bacterial cells (4, 27). These bacteriolytic enzymes are produced by various microorganisms under many circumstances (15, 25). These include autolysins (17, 23, 26), virolysins (3), extracellular enzymes (2, 11, 15) and sporelysins (8). Bacterial species may contain one or more autolysins. It has been suggested that autolysins are involved in important biological processes such as cell wall turn over (16), cell separation (5), competence for genetic transformation, formation of flagella (24) and sporulation. Bacteriolytic enzymes that catalyze the lysis of bacterial cells have been characterized and classified as N-acetylmuramidase, N-acetylglucosaminidase, N-acetylmuramyl-L-alanine amidase, endopeptidase, transglycosylase. Bacteriolytic enzymes have been used to elucidate the cell wall structure of microorganisms (7, 13, 20) and to investigate microbial spoilage prevention because of its antimicrobial potential (1, 12) and have also been used for the isolation of the cytosolic fraction by removal of cell walls.

Recently we isolated a bacterial strain BL-29, which is an excellent producer lytic enzyme. In this study we describe the isolation and identification of

the bacterium BL-29, the purification, and some properties of the extracellular lytic enzyme.

MATERIALS AND METHODS

Screening of Bacteria

Bacteria were grown at 30°C on the agar plates of alkaline basal medium ((g. l) soluble starch, 10.0; polypeptone, 5.0; yeast extract, 5.0; K₂HPO₄, 1.0; MgSO₄ 7H₂O, 0.2; Na₂CO₃, 10.0; agar, 18.0) containing 0.2% autoclaved and lyophilized *E. coli* KCTC 1682. Na₂CO₃ solution was sterilized separately and added to the medium (10). After incubation at 30°C for 48 h, microorganisms which made a clear zone around the colony were selected as lytic enzyme producing microorganisms. In order to identify bacterial cell wall hydrolytic activity, colonies formed on the above plates were inoculated in test tubes containing 5 ml of alkaline basal liquid medium as described above. after incubation at 30°C for 30 h with continuous shaking, the culture supernatant was added to the purified cell wall suspension of *E. coli* KCTC 1682, and the decrease in turbidity was measured.

Identification of Bacteria

Morphological properties of the isolate were observed by electron microscopy and cultural characteristics were investigated on various agar media. Its biochemical and physiological characteristics were

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Key words: cell wall hydrolase, *Bacillus* sp.

examined according to Methods for General and Molecular Bacteriology (6).

Preparation of Crude Enzyme

For enzyme preparation, strain BL-29 isolated from soil was cultured at 30°C in a basal medium containing 20 g.l⁻¹ soluble starch, 5.0 g polypeptone, 5.0 g yeast extract, 1.0 g K₂HPO₄, 0.2 g MgSO₄·7H₂O, 10 g Na₂CO₃. After incubation, cells were removed by centrifugation at 10,000 g for 20 min at 4°C and the supernatant was used as a source of crude enzyme.

Preparation of *Escherichia coli* Peptidoglycan

The cell wall of *E. coli* KCTC 1682 was prepared by the method of Potvin *et al* (22). *E. coli* was grown in LB at 37°C and cells from the late exponential phase were recovered by centrifugation at 8,000 rpm for 15 min at 4°C. The pellet was washed in 80 ml of 25 mM Tris-HCl, pH 8.0 for 30 min at 4°C and cells were recovered as before. The pellet (1 g) was resuspended in 80 ml of 4% (w/v) SDS and shaken for 90 min on a shaker at room temperature and sonicated on ice at maximum power for 5 min. The sonicated extract was heated at 100°C for 15 min and centrifuged at 12,000 rpm for 15 min at room temperature. The pellet was resuspended in 80 ml of 0.1% (v/v) purified Triton X-100 and incubated for 30 min at room temperature with gentle shaking. The suspension was centrifuged as before and the pellet washed four times with distilled water.

Assay of Enzyme Activity

Lytic enzyme activity was measured as described by Kiyoshi Hayashi (9). The autoclaved and lyophilized cells of *E. coli* were suspended in 0.05 M glycine-NaOH buffer (pH 10.0) to give an initial absorbance of 1.0 at 660 nm with spectrophotometer (Shimadzu UV-265 FW). To 2 ml of this suspension, 0.1 ml of enzyme solution was added and the reaction mixture was incubated at 45°C for 10 min. Reduction of turbidity in the reaction mixture was measured at 660 nm. One unit of the enzyme activity was defined as the amount of enzyme necessary to decrease OD₆₆₀ of 0.001 in 1 min.

Protein Determination

Protein was monitored by A₂₈₀ during the purification processes. Protein concentration was determined by the method of Lowry *et al.* (20), using bovine serum albumin as standard.

Polyacrylamide Gel Electrophoresis

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (19) with 12.5% polyacrylamide gel containing 0.1% SDS. The protein was stained with Coomassie Brilliant Blue R-250.

Determination of Molecular Weight

The molecular weight of native enzyme was estimated by gel filtration on Sephadex G-100. The standard proteins used for calibration were as follows; Albumin, Bovine (66 kDa), Carbonic anhydrase (29 kDa) and Cytochrome-C (12.4 kDa). SDS-PAGE was also employed for the determination of the molecular weight of subunit. The following size markers were used.; Albumin, Bovine, (66 kDa), Albumin, Egg (45 kDa), Glyceraldehyde-3-phosphate dehydrogenase (36 kDa), Carbonic anhydrase (29 kDa), Trypsinogen (24 kDa), Trypsin inhibitor (20.1 kDa), α -Lactalbumin (14.2 kDa).

Purification of Lytic Enzyme

All procedures were carried out at 4°C. The Chromatographic procedures are described in the results.

RESULTS AND DISCUSSIONS

Screening of Microorganism Producing Bacteriolytic Enzyme

About 200 strains of bacteria, which made a clear zone by lysis of *E. coli* cell used as substrate were tentatively selected as microorganisms producing lytic enzyme. In order to identify bacterial cell wall hydrolytic activity, enzyme solutions of these strains were added to the purified peptidoglycan suspension of *E. coli* KCTC 1682 and the decrease of turbidity was measured. Among these, isolate BL-29 showed the largest clear zone of lysis for *E. coli* used as substrate (Fig. 1) and cell wall hydrolytic activity.

Identification of Isolate

The isolate BL-29 was motile, Gram positive, spore forming, and rod-shaped. Cells were 0.7 μ m wide and 2.1 μ m long when measured under transmission elec-

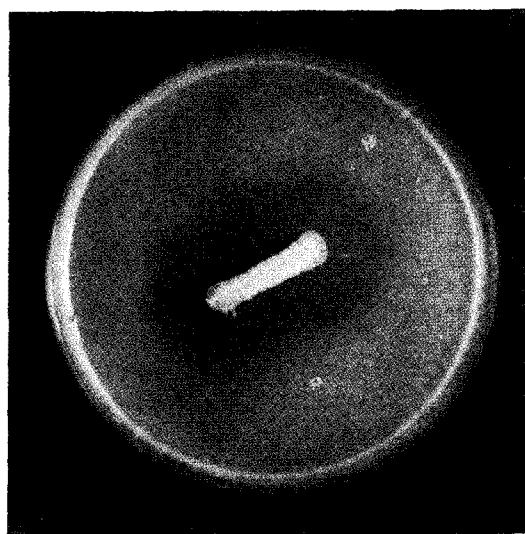


Fig. 1. Lysis of *E. coli* cell by *Bacillus* sp. BL-29 on agar plate containing *E. coli* as substrate.

tron microscopy (Fig. 2). The cultural and physiological characteristics are summarized in Table 1 and 2 respectively. The isolate showed obligate aerobe, catalase positive, oxidase positive bacterium. These results indicated that isolate BL-29 is a genus *Bacillus* sp. So, isolate BL-29 was named *Bacillus* sp. BL-29

Enzyme Production

Bacillus sp. BL-29 was cultured at 30°C on a rotary shaker in a basal medium with 20 g.l⁻¹ soluble starch, 5 g polypeptone, 5 g yeast extract, 1 g K₂HPO₄, 0.2 g MgSO₄·7H₂O, 10 g Na₂CO₃. The seed culture was incubated at 30°C overnight with continuous shaking be-

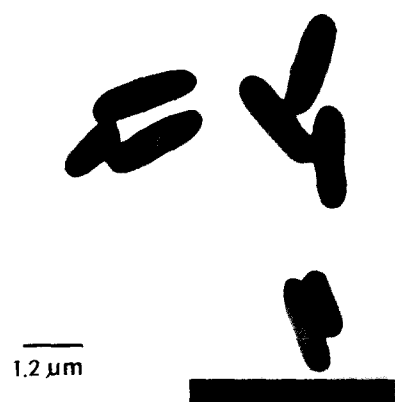


Fig. 2. Electron micrograph of the isolated strain BL-29 ($\times 8,000$). The bar represents 1.2 μm .

Table 1. Morphological and Cultural characteristics of the strain BL-29.

Characteristics	Strain BL-29	
1. Morphological characteristics		
Cell shape	Rods	
Motility	Motile	
Flagella	Positive	
Spore	Positive	
Gram stain	Positive	
Colony on the alkaline basal medium		
Colony - Form	Circular	
- Surface	Smooth	
- Elevation	Convex	
- Margin	Entire	
2. Cultural characteristics		
	pH 7.0	pH 10.2
MacConkey agar	-	ND
EMB agar	+	ND
Nutrient agar	+	++
Glucose nutrient agar	+	++
Alkaline basal medium	+	++
3. pH and Temperature for growth		
pH range for growth	7.0~11.0	
Temperature for growth	16°C~45°C	
NaCl tolerance for growth	≤2%	

fore inoculated at a concentration of 1% (v/v). The growth of the cell, enzyme production and pH variation of the medium dependent on time were as

Table 2. Physiological and Biochemical properties of the strain BL-29.

Characteristics	Strain BL-29
Oxygen relation	obligative aerobe
Hydrolysis of casein	+
starch	+
lipid(olive oil)	-
Hydrolysis of hippurate	-
Liquefaction of gelatin	-
Nitrate reduction to nitrite	-
N ₂ gas from nitrate	-
H ₂ S production	+
Production of indole	-
Methyl red test	-
Voges-proskauer reaction	+
Catalase	+
Oxidase	+
Urease	-
Utilization of citrate	+
Triple sugar iron agar	acid slant/acid butt
Diamino pimelic acid in cell wall peptidoglycan	mesoDAP
Assimilation of nitrogen	
arginine, asparagine	+
methionine,o	+
histidine, cystidine, valine	-
phenylalanine, hydroxyproline, DL- α -amino-n-butyric acid,threonine	-
Acid from	
glucose, arabinose, fructose, mannitol	+
Gas from glucose	-

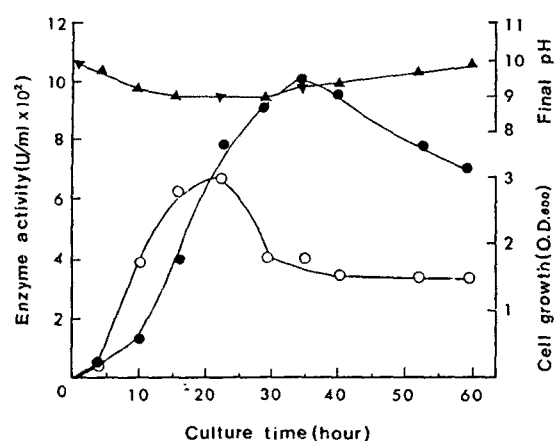


Fig. 3. Time course of the cell wall lytic enzyme production.

Bacillus sp. BL-29 was grown at 30°C under continuous shaking (105 rpm) in the medium composed of 2% soluble starch, 0.5% polypeptone, 0.5% yeast extract, 0.1% K₂HPO₄, 0.02% MgSO₄·7H₂O, 1% (w/v) Na₂CO₃. The overnight seed culture was inoculated at a concentration of 1% (v/v). ●; Enzyme activity, ○; Cell growth, ▲; pH.

shown in Fig. 3. Cell growth increased until 22 h after inoculation. The pH of the culture medium was gradually decreased to pH 9.0 from initial pH 10.0 and then it was increased to pH 10.0. Enzyme production reached a maximum (1.2×10^3 U/ml) at 36 h.

Enzyme Purification

To the culture supernatant, ammonium sulfate was added to give 75% saturation and the resulting pellet was obtained by centrifugation at 12000 g for 20 min. The pellet was dissolved in 0.05 M sodium phosphate buffer (pH 8.0) and dialyzed against the same buffer. The dialyzed protein was applied to DEAE-Cellulose column (3.5×40 cm) previously equilibrated with 0.05 M sodium phosphate buffer (pH 8.0) and eluted with a linear gradient of sodium chloride from 0 to 1 M in the same buffer. Active fractions eluted from the column were pooled and concentrated. The concentrated enzyme solution was applied to a CM-Sephadex A-50 (2.7×30 cm) column equilibrated with 0.05 M sodium acetate buffer (pH 6.0). The column was washed with

the same buffer and then eluted with a 500 ml linear gradient of NaCl from 0 to 1 M in the same buffer. The flow rate was 60 ml/h and fractions of 7 ml were col-

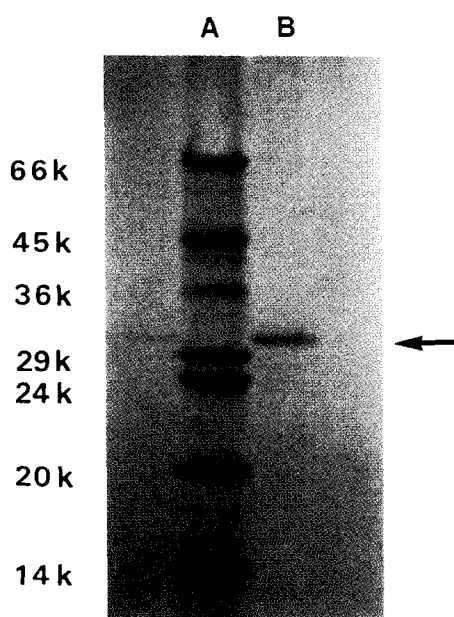


Fig. 4. SDS-polyacrylamide gel electrophoresis of the purified cell wall hydrolase.

Lane A; molecular weight standard, Lane B; purified cell wall hydrolase.

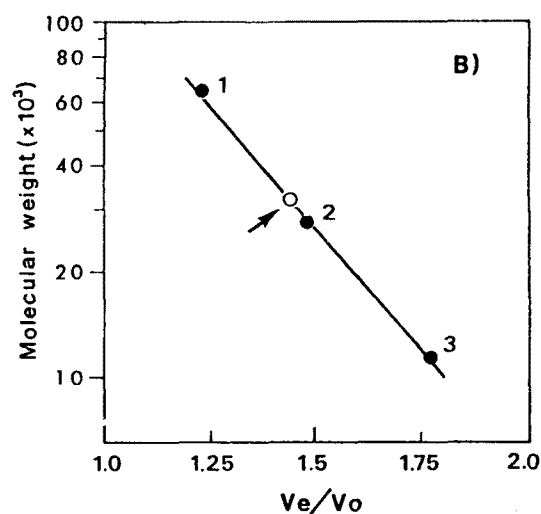
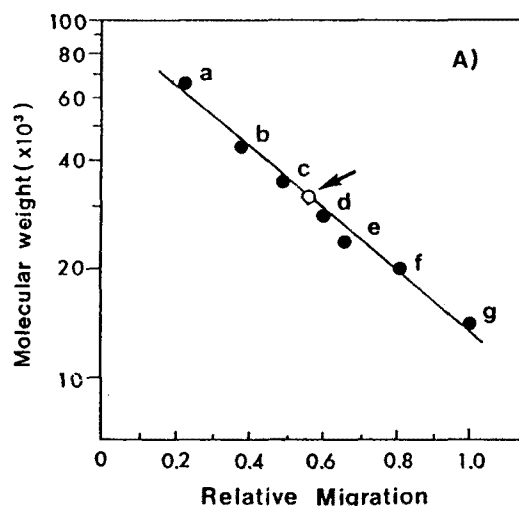


Fig. 5. Molecular weight determination of purified enzyme by SDS-PAGE (A) and gel filtration (B).

Albumin, Bovine (a, 66 kDa), Albumin, Egg (b, 45 kDa), Glyceraldehyde-3-phosphate dehydrogenase (c, 36 kDa), Carbonic anhydrase (d, 29 kDa), Trypsinogen (e, 24 kDa), Trypsin inhibitor (f, 20.1 kDa), and α -Lactalbumin (g, 14.2 kDa) were used as size standards in 12.5% SDS-polyacrylamide gel electrophoresis. Albumin, Bovine (1, 66 kDa), Carbonic Anhydrase (2, 29 kDa) and Cytochrome-C (3, 12.4 kDa) were used as size standards in the Sephadex G-100 gel filtration.

Table 3. Purification of the extracellular lytic enzyme of *Bacillus* sp. BL-29.

Purification step	Total activity (U)	Amt. of protein (mg)	Specific activity (U/mg)	Yield (%)	Purification (fold)
Culture supernatant	7.9×10^6	17.135	461	100	1.0
Ammonium sulfate (75%)	5.45×10^6	926	5,889	69.5	12.7
CM-Sephadex A-50	1.9×10^6	118	16,678	24.9	36.2
Sephadex G-75	4.9×10^5	18.48	26,959	6.3	58.5
Sephadex G-100	4.0×10^5	13.97	28,850	5.1	62.6

lected. The active fractions (42-50) were pooled and concentrated and applied to a Sephadex G-75 (2.5×53 cm) column equilibrated with 0.05 M sodium phosphate buffer (pH 8.0). The column was eluted with same buffer, and then applied to Sephadex G-100 (1.7×53 cm). The column was eluted with 0.05 M sodium phosphate buffer. SDS-PAGE of the purified enzyme preparation showed a single band of protein (Fig. 4). Table 3 summarizes the increases in the specific activity and the yield during the purification steps. The enzyme preparation of the final stage was purified about

63 fold with 5% yield, and specific activity was 28,850 U/mg of protein.

Estimation of Molecular Weight

The molecular weight of the purified enzyme was estimated as 31,000 dalton by SDS-PAGE (Fig. 5A) and gel filtration (Fig. 5B). From this result, the lytic enzyme of *Bacillus* sp. BL-29 is composed of a single peptide with a molecular mass of 31,000 dalton. The molecular weight of lytic enzymes from *Staphylococcus aureus* (28), *Bacillus* sp. (15), *Bacillus licheniformis* (21), and *Bacillus subtilis* (18) were reported to be 50,000, 27,000, 39,000, and 29,900, respectively.

Effect of pH on Activity and Stability

The lytic enzyme activity was measured at various pHs from pH 4.0 to pH 11.0 using various buffer solutions. The optimum pH for the enzyme was near pH 10.0 (Fig. 6). The pH dependence of enzyme stability was determined from the residual activity after 12 hours preincubation at various pHs at 37°C. The purified enzyme remained comparatively stable over a wide pH range, from pH 5.0 to pH 11.0 (Fig. 7). The most marked differences between the lytic enzyme of *Bacillus* sp. BL-29 and other lytic enzymes seemed to be the optimum pH for the lytic activity. The optimum pH of the lytic enzyme from *Bacillus* sp. BL-29 was pH 10.0, while those of lytic enzymes from *Staphylococcus aureus* (28), *Bacillus* sp. (15), *Bacillus licheniformis* (21), and *Bacillus subtilis* (18) were pH 6.0, 7.0, 7.5, and 6.8 respectively.

Effect of Temperature on Activity and Stability

Enzyme activities were assayed at various temperatures ranging from 20 to 70°C. at pH 10.0. The

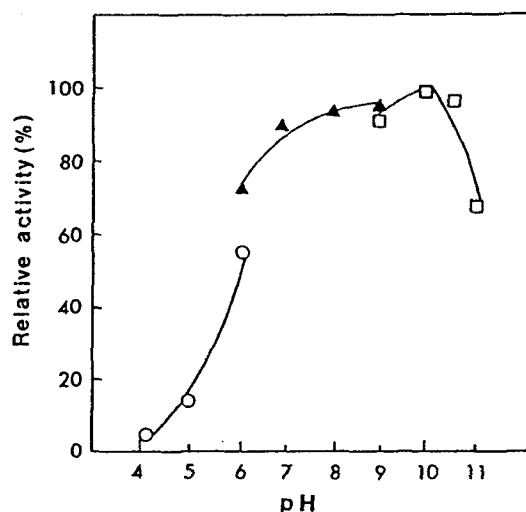


Fig. 6. Effect of pH on activity of the cell wall hydrolase. The following buffers were used: 50 mM sodium acetate buffer (○), 50 mM sodium phosphate buffer (▲), 50 mM Glycine-NaOH buffer (□).

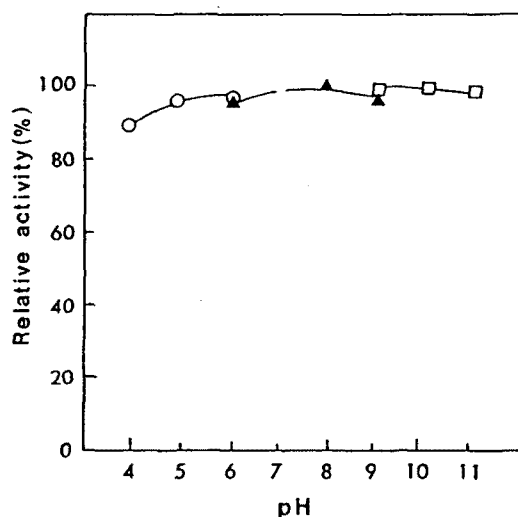


Fig. 7. Effect of pH on stability of cell wall hydrolase. To test pH stability, properly diluted enzyme solution was preincubated at various pHs for 12 h at 37°C. The remaining activity was measured under the standard condition. 50 mM sodium acetate buffer (○), 50 mM sodium phosphate buffer (▲), 50 mM Glycine-NaOH buffer (□).

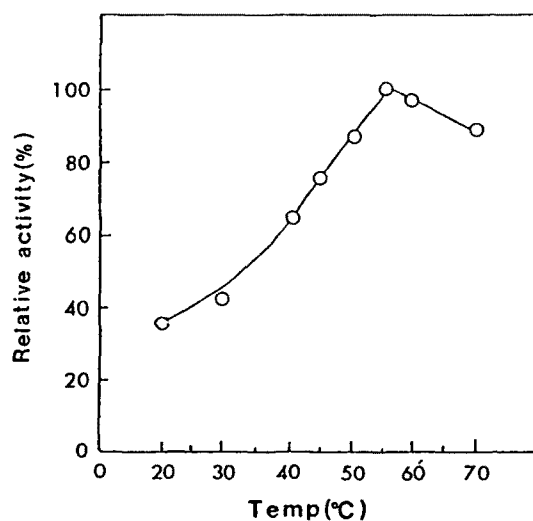


Fig. 8. Effect of temperature on activity of the cell wall hydrolase.

Enzyme activity was assayed at various temperatures at standard condition.

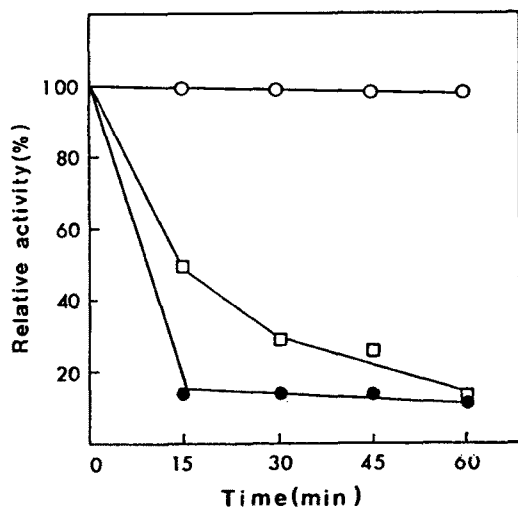


Fig. 9. Effect of temperature on the stability of the cell wall hydrolase.

The enzyme solution was preincubated at 45°C (○), 55°C (□), 65°C (●) for various intervals. After preincubation, the residual enzyme activity was measured.

Table 4. Effect of metal ions on the purified enzyme.

Metal ions (1 mM)	Relative activity (%)
AgNO ₃	70
BaCl ₂	94
CoCl ₂	97
CuCl ₂	90
CaCl ₂	95
CdCl ₂	89
FeSO ₄	98
HgCl ₂	53
KCl	100
LiCl	97
MgCl ₂	94
MnCl ₂	87
NaCl	100
Pb-acetate	30
None	100

optimum for enzyme activity was around 55°C as shown in Fig. 8.

The thermostability of the lytic enzyme was measured after incubation for predetermined periods ranging from 15 to 60 min at each temperature. The residual activities as a function of heat treatment time are shown in Fig. 9. The enzyme was stable up to 45°C, but 50% of the original activity was lost after treatment at 55°C for 15 min.

Effect of Metals Ions and Inhibitors on Enzyme Activity

The effects of some metal ions and inhibitors on enzyme activity were investigated (Table 4, 5). The enzyme was preincubated with various reagents for 30 min at 37°C at final concentrations of 1 mM and 10

Table 5. Effect of various reagents on enzyme activity.

Chemicals	Relative activity (%)	
	1 mM	10 mM
β-mercaptoethanol	92	83
Na-oxalate	85	96
Dithiothreitol	83	90
L-cysteine	84	80
EDTA	15	5
Iodoacetic acid	79	81
EGTA	15	10
p-CMB	86	70
None	100	100

mM before the enzyme activities were determined. The enzyme activity was decreased by treatment with mercuric chloride, lead-acetate, ethylene glycol-bis-[β-aminoethyl ether]-N,N,N',N'-tetraacetic acid (EGTA), and ethylenediamine tetraacetic acid (EDTA) but enzyme activity was not particularly affected by treatment with other metal ions and inhibitors. This result suggests that the lytic enzyme of *Bacillus* sp. BL-29 is metal containing enzyme.

REFERENCES

1. Banks, J. G., R. G. Board, and N. H. Sparks. 1986. Natural antimicrobial systems and their potential in food preservation in the future. *Biotechnol. Appl. Biochem.* **8**: 103-147.
2. Croux, C., B. Canard, G. Goma, and P. Soucaille. 1992. Purification and characterization of an extracellular muramidase of *Clostridium* acetylated peptidoglycan. *Appl. Environ. Microbiol.* **58**: 1075-1081.
3. Diaz, E., R. Lopez, and J. L. Garcia. 1992. Ej-1, a temperate bacteriophage of *Streptococcus pneumoniae* with a myoviridae morphotype. *J. Bacteriol.* **174**: 5516-5525.
4. Dolinger, D. L., L. Daneo-moore, and G. D. shockman. 1989. The second peptidoglycan hydrolase of *Streptococcus faecium* ATCC 9790 covalently binds penicillin. *J. Bacteriol.* **171**: 4355-4361.
5. Fein, J. E. and H. J. Rogers. 1976. Autolytic enzyme-deficient mutants of *Bacillus subtilis* 168. *J. Bacteriol.* **127**: 1427-1442.
6. Gerhardt, P., R. G. E. Murray, W. A. Wood, and N. R. Krieg. 1994. *Methods for General and Molecular Bacteriology*. American Society for Microbiology. Washington, D. C.
7. Ghuysen, J. M. 1968. Use of bacteriolytic enzyme in determination of wall structure and their role in cell metabolism. *Bacteriol. Rev.* **32**: 425-464.
8. Guinand, M., G. Michel, and D. J. Tipper. 1974. Appearance of gamma-D-glutamyl-(L) meso-diaminopimealate peptidoglycan hydrolase during sporulation in *Bacillus sphaericus*. *J. Bacteriol.* **120**: 173-84.
9. Hayashi, K. and T. Kasumi. 1981. Purification and

- characterization of the lytic enzyme produced by *Streptomyces rutgersensis* H-46. *Agric. Biol. Chem.* **45**: 2289-2300.
10. Horikoshi, K. and T. Akiba. 1982. *Alkalophilic Microorganisms*. Japan Scientific Societies Press, Tokyo.
 11. Huff, E., C. S. Silverman, N. J. Adams, and W. S. Awkard. 1970. Extracellular cell wall lytic enzyme from *Staphylococcus aureus*: purification and partial characterization. *J. Bacteriol.* **103**: 761-769.
 12. Hughey, V. L. and E. A. Johnson. 1987. Antimicrobial activity of lysozyme against bacteria involved in food spoilage and food-borne disease. *Appl. Environ. Microbiol.* **53**: 2165-2170.
 13. Inoue, M., S. Hamada, S. Kotani, and K. Kato. 1979. Enzymic lysis and structure of the cell walls of the oral *Streptococcus mutans* BH1. *Arch. Oral. Biol.* **24**: 529-37.
 14. Inoue, M., S. Hamada, T. Ooshima, S. Kotani, and K. Kato. 1979. Chemical composition of *Streptococcus mutans* cell walls and their susceptibility to *Flavobacterium* I-11 enzyme. *Microbiol. Immunol.* **23**: 319-328.
 15. Jung, M. H., I. S. Kong, D. H. Bai, and J. H. Yu. 1991. Purification and characterization of a bacteriolytic enzyme from Alkalophilic *Bacillus* sp. *J. Microbiol. Biotechnol.* **1**: 102-110.
 16. Kochs, A. L. and R. J. Doyle. 1985. Inside-to-outside growth and turnover of the cell wall of gram-positive rods. *J. Theor. Biol.* **117**: 137-157.
 17. Kuroda, A. and J. Sekiguchi. 1990. Cloning, sequencing genetic mapping of a *Bacillus subtilis* cell wall hydrolase gene. *J. Gen. Microbiol.* **136**: 2209-2216.
 18. Kuroda, A., M. Imazeki, and J. Sekiguchi. 1991. Purification and characterization of a cell wall hydrolase encoded by the *cwla* gene of *Bacillus subtilis*. *FEMS Microbiol. Lett.* **65**: 9-13.
 19. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680-685.
 20. Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.
 21. Oda, Y., R. Nakayama, A. Kuroda, and J. Sekiguchi. 1993. Molecular cloning, sequence analysis, and characterization of a new cell wall hydrolase, *cwlL*, from *Bacillus licheniformis*. *Mol. Gen. Genet.* **241**: 380-388.
 22. Potvin, C., D. Leclerc, G. Tremblay, A. Asselin, and G. Bellemare. 1988. Cloning, sequencing and expression of a *Bacillus* bacteriolytic enzyme in *Escherichia coli*. *Mol. Gen. Genet.* **214**: 241-248.
 23. Rogers, H. J., C. Taylor, S. Rayter, and J. B. Ward. 1984. Purification and properties of autolytic endo- β -N-acetylglucosaminidase and the N-Acetylmuramyl-L-alanine Amidase from *Bacillus subtilis* Strain 168. *J. Gen. Microbiol.* **130**: 2395-2402.
 24. Rogers, H. J., H. R. Perkins, and J. B. Ward. 1980. *Cell walls and membranes*. Chapman and Hall, London.
 25. Salton, M. R. J. 1955. Isolation of *Streptomyces* spp. capable of decomposing preparations of cell walls from various microorganisms and a comparison of their lytic activities with those of certain actinomycetes and myxobacteria. *J. Gen. Microbiol.* **12**: 25-30.
 26. Shockman, G. D., T. Kawamura, J. F. Barrett, and D. L. Dolinger. 1985. The autolytic peptidoglycan hydrolases of *Streptococcus faecium*. *Ann. Inst. Pasteur. Microbiol.* **136A**: 63-66.
 27. Strominger, J. L. and J. M. Ghuysen. 1967. Mechanism of enzymatic bacteriolysis. *Science* **156**: 213-221.
 28. Wang, X., N. mani, P. A. Pattee, B. J. Wilkinson, and R. K. Jayaswal. 1992. Analysis of a peptidoglycan hydrolase gene from *Staphylococcus aureus* NCTC 8325. *J. Bacteriol.* **174**: 6303-6306.

(Received March 29, 1995)