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Purification and Characterization of a Bacteriolytic Enzyme from Alkalophilic *Bacillus* sp.

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Alkalophilic *Bacillus* sp. YJ-451, which was isolated from soil at several area in Korea, produced a novel type of bacteriolytic enzyme (cell wall peptidoglycan hydrolase) extracellulary. The cell wall hydrolytic activity was identified as a clear zone on sodium dodecyl sulfate polyacrylamide gel electrophoresis containing 0.2% (w/v) cell wall of *Bacillus* sp. as substrate. This enzyme was successively purified 66 fold with 3.2% yield in culture broth by ammonium sulfate precipitation, CM-cellulose column chromatography, and gel filtration, followed by hydroxylapatite column chromatography. The molecular weight of the purified enzyme was estimated to be 27,000 by sodium dodecyl sulfate polyacrylamide gel electrophoresis and gel filtration column chromatography. The optimum pH and temperature for the activity of the enzyme were pH 10.0 and 50°C, respectively. The enzyme was stable between pH 5.0 and 10.0 and up to 40°C. Among the microorganisms used in this experiment the enzyme was active against most of gram negative strains and the genus *Bacillus* such as *B. megaterium, B. licheniformis, B. circulans, B. pumilus, B. macerans, B. polymyxa*. The release of dinitrophenylglutamic acid but not reducing group from cell wall peptidoglycan digested by the enzyme suggested that the enzyme is a kind of peptidase which hydrolyzes the peptide bond at the amino group of D-glutamic acid in the peptidoglycan.

Bacteriolytic enzyme (cell wall peptidoglycan hydrolase) is an enzyme which catalyzes the hydrolysis of a structurally important linkage in the bacterial cell wall peptidoglycan and as a result, this complex polymeric substance is solubilized (22). Many of these enzymes are bacteriolytic; they lyse intact bacterial cells. These bacteriolytic enzymes are produced by various microorganisms under many circumstances (22). These include autolysins (intracellular enzymes capable of degrading the cell walls of the same organism in which the enzyme is found, as well as those of others), virolysins (produced by bacteria on infection with bacteriophages or found as constituents of the virus itself), extracellular enzymes (sometimes excreted in large amounts by bacteria) and spore lysins (presumably produced or activated at the time of spore germination). It has been suggested that autolysins are involved in important biological processes such as cell-wall turnover (11), cell separation (2), competence for genetic transformation, formation of flagella (19). Four common types of bacteriolytic enzyme are recognized: (i) a lysozymelike enzyme hydrolyzing N-acetylmuramyl-β-1,4-N-acetylglucosamine bonds (muramidase); (ii) a β-N-acetylglucosaminidase liberating the free reducing groups of N-acetylglucosamine (glucosaminidase); (iii) an N-acetylmuramyl-L-alanine amidase (amidase) hydrolyzing the bond between N-acetylmuramyl acid and L-alanine and (iv) peptidases hydrolyzing the stem or bridge peptides. Several bacterial cell wall hydrolases have been easily detected because of *Micrococcus luteus* which is highly sensitive to bacteriolysis, provoked by dissolution of cell wall (20). The bacteriolytic enzyme is used for the research to elucidate the cell wall structure of microorganism (4) and the food preservation against microbial spoilage because of their antimicrobial potential (1) and also used for the isolation of cytosolic fraction by removing the cell wall with treatment of lytic enzyme (10).

From soil collected at several area in Korea, we have isolated a strain of alkalophilic bacterium YJ-451, which produces a novel bacteriolytic enzyme.

In this report, we described the screening of microorganism producing bacteriolytic enzyme and the purification and properties of the bacteriolytic enzyme from alkalophilic *Bacillus* sp. YJ-451.

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Key words: Alkalophilic *Bacillus* sp., cell wall peptidoglycan, bacteriolytic enzyme

MATERIALS AND METHODS

Screening of Bacteria

For isolating of alkalophilic microorganism producing lytic enzyme, a small amount of soil was suspended in sterilized water and then spread on the agar plates of alkaline basal medium containing 1% (w/v) soluble starch, 0.5% (w/v) polypeptone (Difco Laboratories, Detroit, Mich.), 0.5% (w/v) yeast extract (Difco), 0.1% (w/v) K_2HPO_4, 0.02% (w/v) $MgSO_4 \cdot 7H_2O$, 1% (w/v) Na_2CO_3 1.5% (w/v) agar (Difco). Na_2CO_3 solution was sterilized separately and added to the medium. After incubation at 37℃ for 2 days, colonies formed on the plate were inoculated in the test tube containing 5 m*l* of alkaline basal liquid medium described above, and incubated at 37℃ for 24 hours with continuous shaking. The 5 μ*l* of culture supernatant of each isolated microorganism was spotted on the plate containing autoclaved, lyophilized *Bacillus* sp. YC-335 (University stock) used as substrate for lytic enzyme activity. After incubation at 37℃ for 24 hours, microorganism which made a clear zone around spotted area was selected as microorganism producing lytic enzyme. In order to identify bacterial cell wall hydrolytic activity, enzyme solution of isolated microorganism was added to the cell wall suspension of *Bacillus* sp. YC-335, and the decrease of turbidity in the suspension was observed.

Detection of Cell Wall Hydrolytic Activity by SDS-PAGE

Cell wall hydrolytic activity was detected in situ by using an SDS-PAGE containing 0.2% (w/v) cell wall of *Bacillus* sp. YC-335 as described by Leclerc *et al.* (14). Crude enzyme suspended in 25 mM Tris-HCl (pH 8.0) containing 2 mM dithiothreitol (Sigma Chemical Co., St. Louis, Mo.), 2% sucrose, 1% SDS, and 0.5 mM phenylmethylsulfonylfluoride (Sigma) was heated for 2 min in boiling water prior to electrophoresis. After electrophoresis, the gel was incubated for 12 to 16 hours at 37℃ in 500 m*l* of 25 mM Tris-HCl (pH 8.0) containing 1% Triton X-100 (Sigma) to permit protein renaturation. Transparent band of lysis in the translucent gel was rendered more visible by staining with 1% methylene blue in 0.01% KOH prior to photograph.

Preparation of *Bacillus* sp. Cell Wall Peptidoglycan

The cell wall preparation of *Bacillus* sp. YC-335 and *B. megaterium* ATCC 14945 was prepared by the method of treatment with SDS followed by Triton X-100 described Potvin *et al.* (18).

Identification of Bacteria

Morphological properties of the isolate were observed by electron microscopy and cultural characteristics were investigated on nutrient agar and alkaline basal medium. It's biochemical and physiological characteristics were examined according to Bergey' manual of determinative bacteriology (12).

Assay for Lytic Enzyme Activity

Bacteriolytic enzyme activity was determined by the modified method of Kiyoshi Hayashi (9). The lyophilized cells of *Bacillus* sp. YC-335 were suspended in 50 mM glycine buffer (pH 10.0) to give an initial absorbance of 1.0 at 660 nm with spectrophotometer (Shimazu UV-120-02). To 2 m*l* of this suspension was added 0.1 m*l* of enzyme solution and the reaction mixture was incubated at 45℃ for 10 min. Reduction of turbidity in the reaction mixture was measured at 660 nm. One unit of lytic activity was defined as the amount of the enzyme which caused a decrease of 0.001 absorbance per minute.

Enzyme Production and Preparation of Crude Enzyme

Strain YJ-451 was cultured at 30℃ for 36 hours under continuous shaking in the medium composed of 2% (w/v) soluble starch, 0.5% (w/v) polypeptone, 0.5% (w/v) yeast extract, 0.1% (w/v) K_2HPO_4, 0.02% (w/v) $MgSO_4 \cdot 7H_2O$, 1% (w/v) Na_2CO_3. After incubation for 36 hours, the culture supernatant was obtained by centrifugation and ammonium sulfate was added to the culture supernatant to give 75% saturation. The precipitates formed were collected by centrifugation at 12,000×g for 20 min at 4℃.

Protein Determination

Protein from the columns was meaured by A_{280}. Protein concentration was determined by the method of Lowry *et al.* (15), using bovine serum albumin as standard.

SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (13) with 12.5% polyacrylamide gel containing 0.1% SDS and Tris-glycine buffer (pH 8.5) at a constant current of 20 mA. The protein was stained with coomassie brilliant blue R-250.

Determination of Molecular Weight

The molecular weight of the enzyme was estimated by high performance liquid chromatography on a protein-Pak I-250 gel filtration column. The standard proteins (Sigma) used for calibration were as followed; α-amylase (MW. 200,000), alcohol dehydrogenase (MW. 150,000), carbonic anhydrase (MW. 29,000). SDS-PAGE was also employed for the determination of the molecular weight of subunit. The following size markers (Sigma) were used; phosphorylase B (MW. 92,500), bovine serum albumin (MW. 66,200), ovalbumin (MW. 45,000), carbonic anhydrase (MW. 29,000), soy bean

trypsin inhibitor (MW. 21,500), lysozyme (MW. 14,400).

Purification of Lytic Enzyme

All procedure were carried out at 4℃. The chromatographic procedures were described in the results and the figure legends.

Lytic Action Spectra

All the microorganisms used in this experiment were cultivated at 30℃ for 24 hours in a medium composed of 0.5% (w/v) meat extract (Difco), 1% (w/v) pepton (Difco) and 0.5% (w/v) NaCl, pH 7.0. The cells were harvested in stationary phase of growth and washed twice with deionized water. Other conditions were the same as those of the assay for lytic enzyme activity. The activity of the enzyme indicated by decreasing percent of turbidity at 660 nm after 30 min reaction.

Studies of the Lytic Enzyme on the Cell Wall Peptidoglycan

The cell wall peptidoglycan (30 mg) of *B. megaterium* ATCC 14945 was suspended 4 m*l* of 25 mM Tris-HCl buffer (pH 8.0) to give an initial absorbance of 0.8 at 660 nm and purified enzyme (100 µg) was added and incubated at 45℃. Turbidity was measured at intervals at 660 nm. In order to identify the specific bond cleaved, 0.4 m*l* were removed during the incubation and boiled for 10 min. Undigested cell walls were removed by centrifugation and suitable samples of the supernatant were assayed for appearance of amino groups with 1-fluoro-2,4-dinitrobenzene (FDNB, Sigma) as described by Ghuysen *et al.* (5) and reducing groups by the modified method of Park and Johnsons (24). The released N-terminal amino acid was determined by reacting supernatant from digested cell wall peptidoglycan with FDNB, followed by hydrolysis and chromatography as described by Ghuysen *et al.* (5).

RESULT

Screening of Microorganism Producing Bacteriolytic Enzyme

From various kinds of isolated microorganisms, strain YJ-451 was found to form largest clear zone of lysis for *Bacillus* sp. YC-335 used as substrate (Fig. 1). In order to identify the cell wall hydrolytic activity, enzyme solution of the strain YJ-451 was added to the cell wall suspension of *Bacillus* sp. YC-335. Because of decrease in turbidity of the cell wall suspension (data not shown), strain YJ-451 was selected as microorganism producing bacteriolytic enzyme.

Detection of Cell Wall Hydrolytic Activity

For detection of cell wall hydrolytic activity, SDS-PAGE containing 0.2% (w/v) cell wall of *Bacillus* sp. YC-335 was performed. Active hydrolase, a polypeptide of apparent MW 27,000 was observed as clear zone

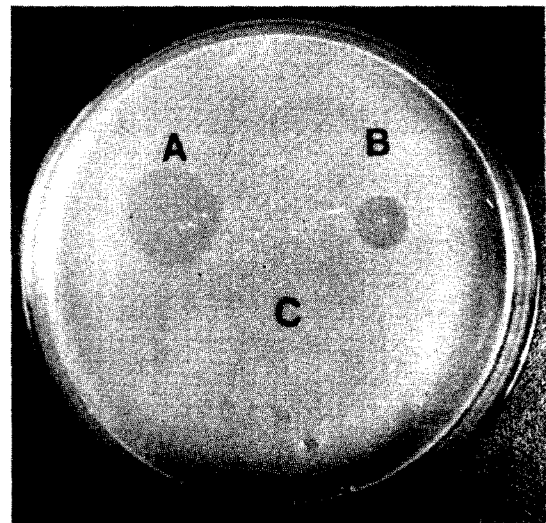

Fig. 1. Lysis of *Bacillus* sp. by the culture supernatant of strain No.451.
The 5 µ*l* of culture supernatant was spotted on the plate containing autoclaved, lysophilized *Bacillus* sp. YC-335 as substrate for lytic enzyme activity. Plate was incubated at 37℃ for 16 to 24 hrs, and lytic activity was shown by formation of a clear zone around spotted area. A, Strain No.451; B, lysozyme; C, *Escherichia coli* as control.

by staining with 1% methylene blue in 0.01% KOH (Fig. 2). This indicated that this hydrolytic activity resulted from cell wall hydrolase.

Identification of Strain YJ-451

The strain YJ-451 was an aerobic, spore forming, gram positive, motile, rod shape, catalase positive bacterium and was found to belong to the genus *Bacillus* (Fig. 3). The characteristic point of this strain is alkalophilic and the optimum pH for growth is about pH 10.2. Table 1 summarizes other physiological and biochemical properties of *Bacillus* sp. YJ-451.

Enzyme Production

Bacillus sp. YJ-451 was cultured at 30℃ on a rotary shaker in the medium composed of 2% (w/v) soluble starch, 0.5% (w/v) polypeptone, 0.5% (w/v) yeast extract, 0.1% (w/v) K_2HPO_4, 0.02% (w/v) $MgSO_4 \cdot 7H_2O$, 1% (w/v) Na_2CO_3. The seed culture was incubated at 37℃ for overnight with continuous shaking and then was 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 shown Fig. 4. The cell growth was increased until 18 hours after inoculation, reached stationary phase and after 18 hours it was decreased slowly. The pH of culture medium was gradually decreased to pH 8.6 from initial pH 10.2 during 24 hours incubation and then it was increased to pH 9.4 and it was maintained at pH 9.4 till 60 hours, which

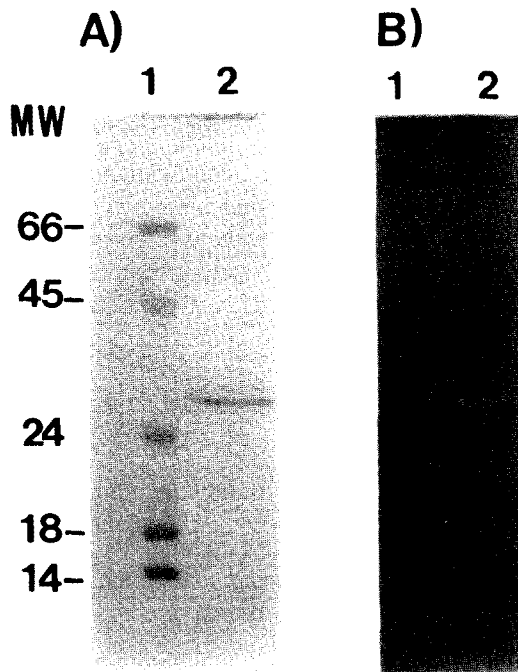

Fig. 2. Detection of cell wall hydrolylase activity by SDS-PAGE (12.5% polyacrylamide gel containing 0.2% (w/v) SDS-cell wall of *Bacillus* sp. YC-335 as substrate).

After electrophoresis, the gel was stained with coomassie brilliant blue R-250(A), and treated with 25 mM Tris-HCl (pH 8.0) containing Triton X-100 to renature protein and stained with methylene blue solution (1% methylene blue in 0.01% KOH) prior to being photographed (B). Lanes: 1, protein standard molecular weight markers; 2, crude enzyme of strain No.451. Arrows show a clear zone due to active lytic enzyme.

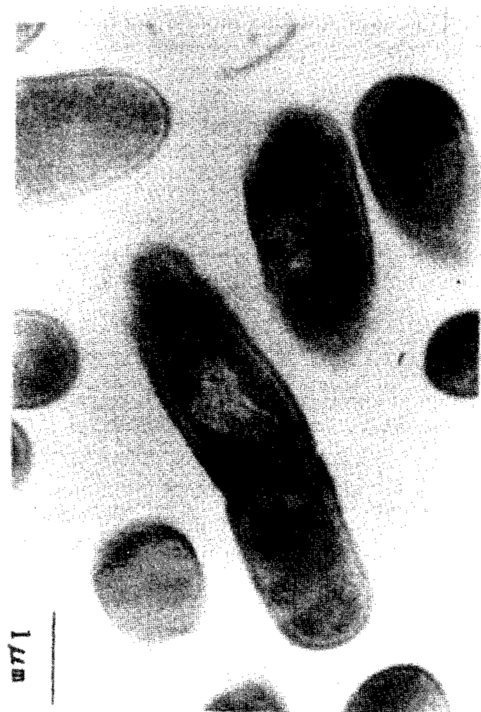

Fig. 3. Electron micrograph of strain No.451.

is known as one of the characteristics of alkalophilic bacterium fermentation. The enzyme was produced after 12 hours and showed maximum activity (1.2×10^3 U/ml) at 36 hours.

Purification of Bacteriolytic Enzyme

The crude enzyme prepared as described in Materials and Methods was dissolved in 0.05 M acetate buffer (pH 6.0). After dialysis against 100 volumes of the same buffer with several changes of the same buffer for 24 hours, the crude enzyme was applied to CM-cellulose column (4×12 cm) previously equilibrated with 0.05 M acetate buffer (pH 6.0). The enzyme was eluted with linear gradient of sodium chloride from 0 to 0.5 M in the same buffer. The elution profile is shown in Fig. 5. Active fractions eluted from the column were collected and concentrated to a final concentration of 31.5 mg/ml by ultrafiltration using Avantec Kit (UHP-43, toyo). The concentrated enzyme solution was applied to Sephadex

Table 1. Characteristics of strain No.451

Characteristics	Strain No.451
1. Morphological characteristics	
Form	rods
Motility	positive
Gram stain	positive
spores	positive
2. Culture characteristics	
Nutrient agar (pH 7.0)	−
Nutrient agar (pH 10.0)	+
Glucose nutrient agar (pH 7.0)	−
Glucose nutrient agar (pH 10.0)	+
Alkaline basal medium (pH 10.2)	++
Alkaline basal medium containing 10% NaCl	+
Growth at pH	pH 7.5-11.4
Growth temperature	up to 42℃
3. Biological characteristics	
Hydrolysis of starch	positive
Hydrolysis of casein	positive
Hydrolysis of gelatin	positive
VP test	negative
Catalase	positive
Oxidase	positive
Indole test	positive
Gelatin liquefacation	positive
Decomposition of tyrosine	positive
Deamination of phenylalanine	negative

−, no growth; +, normal growth; ++, abundant growth

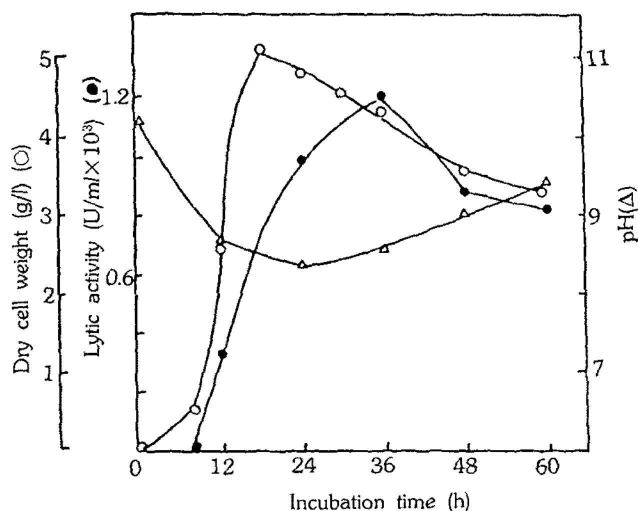

Fig. 4. Time course of the lytic enzyme production.
Bacillus sp. YJ-451 was grown at 30℃ under continuous shaking in the medium composed of 2% (w/v) soluble starch, 0.5% (w/v) polypeptone, 0.5% (w/v) yeast extract, 0.1% (w/v) K_2HPO_4, 0.02% (w/v) $MgSO_4 \cdot 7H_2O$, 1% (w/v) Na_2CO_3. The overnight seed culture was inoculated at a concentration of 1% (v/v).

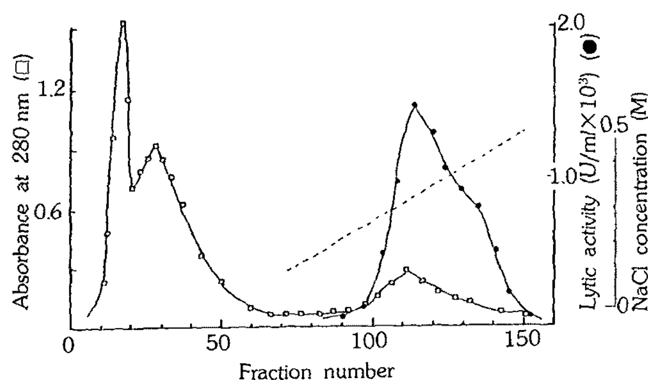

Fig. 5. Chromatography of the crude enzyme on CM-cellulose column.
Experimental conditions: flow rate, 60 ml/h; fraction volume, 9 ml; temperature, 4℃. Fractions 105 to 140 were pooled.

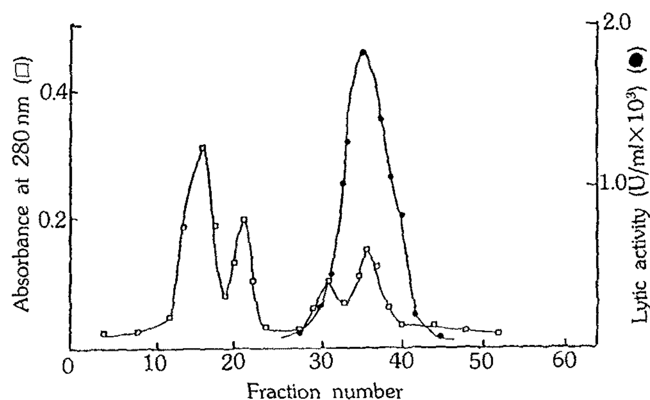

Fig. 6. Sephadex G-100 gel filtration.
Experimental conditions: flow rate, 30 ml/h; fraction volume, 4 ml; temperature, 4℃. Fractions 33 to 44 were pooled.

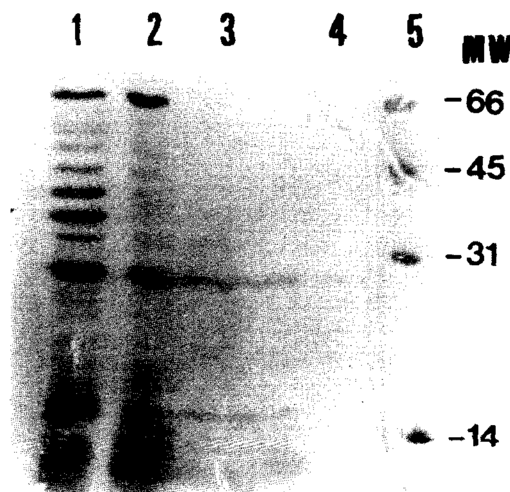

Fig. 7. Analysis of the lytic enzyme by SDS-PAGE.
SDS-PAGE was carried out according to the method of Laemmli. Lanes: 1, after ammonium sulfate precipitation; 2, after CM-cellulose column chromatography; 3, after Sephadex G-100 gel filtration; 4, after hydroxylapatite column chromatography; 5, protein standard molecular weight markers.

G-100 column (2×50 cm), equilibrated with 0.05 M glycine-NaOH buffer (pH 10.0) containing 0.2 M NaCl. The elution pattern thus obtained is shown in Fig. 6. The fractions from No. 33~44 were collected and concentrated by ultrafiltration. The concentrated enzyme solution was applied again to hydroxylapatite column equilibrated with 0.03 M phosphated buffer (pH 6.8) and eluted with linear gradient of 0.3 M phosphate buffer (pH 6.8). The enzyme preparation of final stage was purified about 66 folds with 3.2% yield, and specific activity was 18,285 unit/mg of protein. SDS-PAGE of the purified enzyme preparation showed a single band of protein (Fig. 7).

Estimation of Molecular Weight

The molecular weight of the enzyme was estimated as 27,000 by gel filtration (Fig. 8A) and SDS-PAGE (Fig. 8B). From this results, the lytic enzyme from *Bacillus* sp. YJ-451 is composed of single peptide with molecular mass of 27,000 dalton.

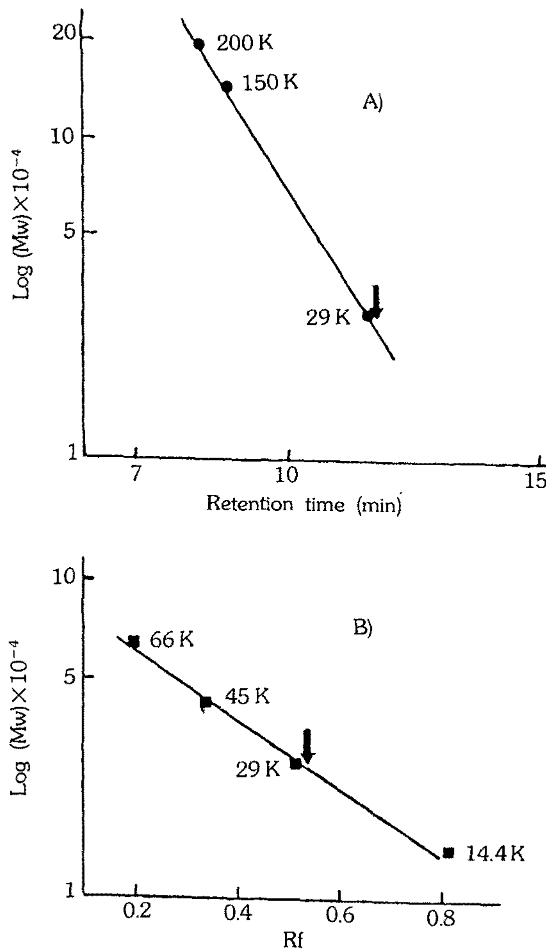

Fig. 8. Molecular weight determination of lytic enzyme (arrows) by gel filtration (A), and SDS-PAGE (B).
Gel filtration was performed using high performance liquid chromatography on a protein-Pak I-250 gel filtration column. The standard proteins (Sigma) used for calibration were as followed; α-amylase (MW. 200,000), alcohol dehydrogenase (MW. 150,000), carbonic anhydrase (MW. 29, 000). SDS-PAGE was carried out with standard mark proteins; phosphorylase B (MW. 92,500), bovine serum albumin (MW. 66,200), ovalbumin (MW. 45,000), carbonic anhydrase (MW. 29,000), soy bean trypsin inhibitor (MW. 21,500), lysozyme (MW. 14,400).

Effect of pH on Activity and Stability

The effect of pH on the activity of purified lytic enzyme was determined by activity assay method. As shown Fig. 9A, the optimum pH for reaction of this enzyme was appeared at pH 10.0. The enzyme was dissolved in various buffer solution and stood at 4℃ for 24 hours. After enzyme solution was brought to pH 10.0, the residual activity was measured under the standard assay method. The lytic enzyme was stable at the range of pH 5.0~10.0 under the tested condition (Fig. 9B).

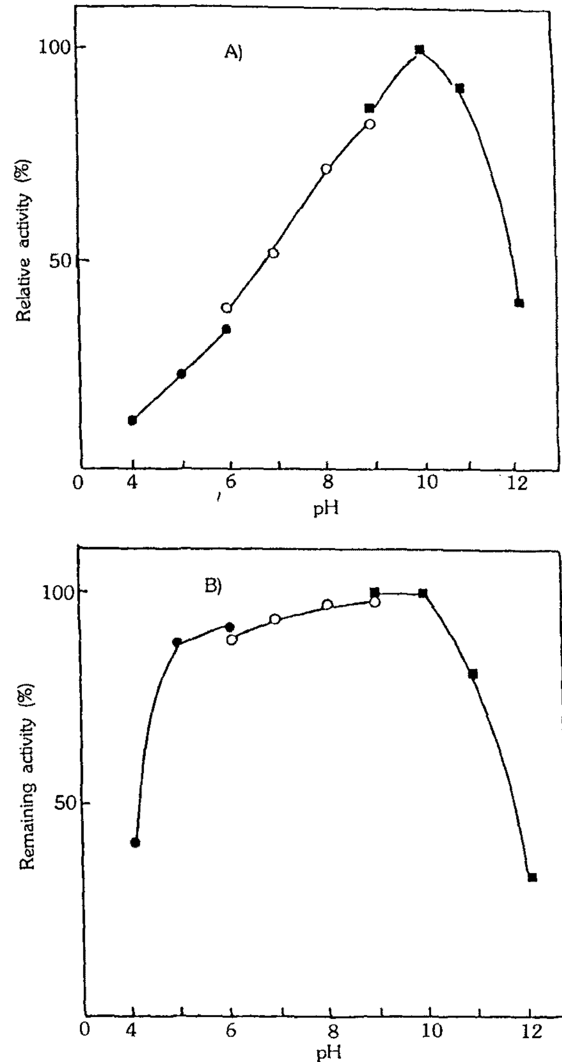

Fig. 9. Effect of pH on the lytic activity (A) and stability of lytic enzyme (B) in 50 mM acetate buffer (●), phosphate buffer (○) and glycine-NaOH buffer (■).

Effect of Temperature on Activity and Stability

The enzyme reaction was carried out at pH 10.0 at various temperature. The optimum temperature for the enzyme reaction was about 50℃ as shown in Fig. 10A. To check the thermal stability, the enzyme in 0.05 M glycine buffer (pH 10.0) was incubated at various temperature for 30 min. As shown in Fig. 10B, the enzyme was stable up to 40℃.

Lytic Action Spectra

Various test organisms were subjected to examining their susceptibility to the lytic enzyme. Among the substrate microorganisms used in this experiment, the lytic enzyme was mainly active against gram negative bacteria

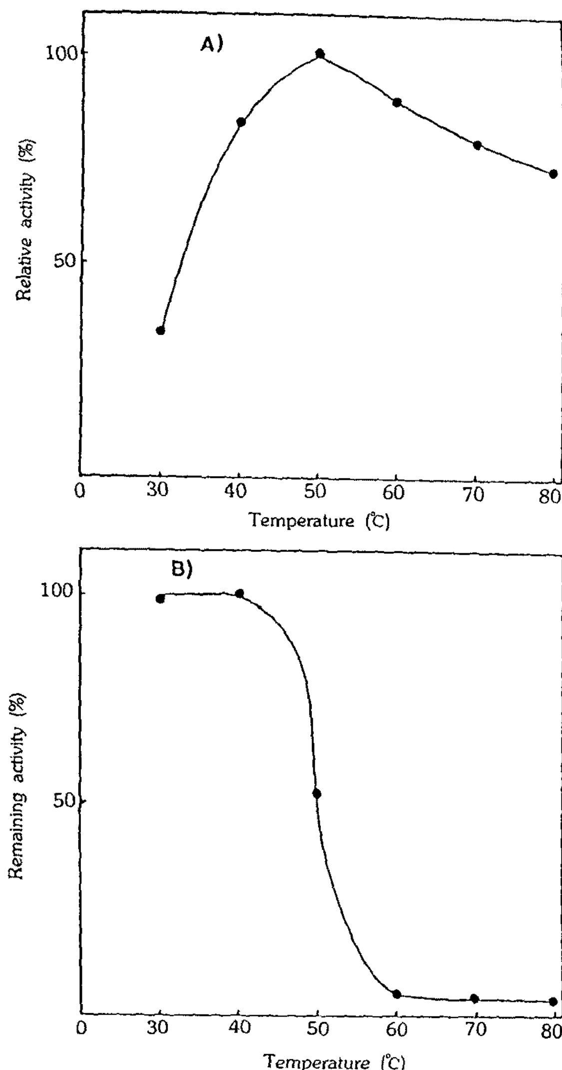

Fig. 10. Effect of temperature on the lytic activity (A) and stability of lytic enzyme (B). Assays were conducted with 50 mM glycine-NaOH buffer (pH 10.0).

Table 2. Lytic action spectra

Strains	Lysis(%)[a]
Pseudomonas aureofaciens ATCC 13985	80
Pseudomonas fluorescens ATCC 12648	85
Pseudomonas chlororaphis ATCC 9446	100
Pseudomonas putida ATCC 12633	100
Pseudomonas syringae IFO 3508	75
Pseudomonas testosteroni ATCC 11996	100
Proteus vulgaris ATCC 6059	100
Salmonella typhimurium ATCC 14028	100
Serratia marcescens ATCC 990	80
Escherichia coli ATCC 37122	100
Escherichia coli ATCC 31030	90
Xanthomonas citri IFO 12213	65
Azotobacter vinelandii IFO 12018	80
Brevibacterium ammoniagenes ATCC 6871	0
Brevibacterium helvolum ATCC 19390	0
Bacillus megaterium ATCC 14945	100
Bacillus licheniformis ATCC 9789	85
Bacillus circulans ATCC 9966	100
Bacillus pumilus ATCC 14884	75
Bacillus macerans ATCC 8514	100
Bacillus polymyxa ATCC 35411	80
Micrococcus luteus ATCC 9341	0
Corynebacterium glutamicum ATCC 31808	25
Staphylococcus aureus ATCC 10537	0
Streptococcus faecalis ATCC 29212	30
Leuconostoc mesenteroides IFO 3426	0

[a]The experimental conditions are described in Materials and Methods. The lysis indicated by decreasing percent of turbidity at 660 nm after 30 min reaction under standard assay method.

and the genus *Bacillus* such as *B. megaterium*, *B. licheniformis*, *B. circulans*, *B. pumilus*, *B. macerans*, *B. polymyxa*, but inert against *Micrococcus luteus* (Table 2). It has been known that the cell walls of bacteria were not composed with pure glycopeptide, and the affinity of a bacteriolytic enzyme for the sensitive linkages present in the peptidoglycan may be partially or completely abolished by nonglycopeptide components of the cell walls (5).

Action of the Lytic Enzyme on the Cell Wall Peptidoglycan

In order to elucidate the lytic action of the lytic enzyme, cell wall peptidoglycans purified from *Bacillus* sp. YC-335 and *B. megaterium* ATCC 14945 were used. A specimen of peptidoglycan of *B. megaterium* ATCC 14945 was suspended in 25 mM Tris-HCl (pH 8.0) and digested with the lytic enzyme. At intervals during incubation at 45℃, samples were removed to measure the decrease of optical density at 660 nm and the increase of free amino groups, reducing groups. Fig. 11 shows that an increase of free amino groups was paralled with a decrease of turbidity, and no reducing sugar was liberated. It suggested that the lytic enzyme of *Bacillus* sp. YJ-451 was an amidase or an endopeptidase, but not a glucosaminidase. To identify the N-terminal amino acid released, we determined the DNP-amino acid in the hydrolyzed peptidoglycan by thin layer chromatography on silica gel 60 F_{254} plate (Merck). DNP-glutamic acid was identified by thin layer chromatography (Fig. 12). And the amount of N-terminal amino acid in the hydrolyzed peptidoglycan of *B. megaterium* ATCC 14 945 was determined by amino acid analyzer after dinitrophenylation. The enzyme digestion reduced glutamic acid content to 18% of original in *Bacillus megaterium*

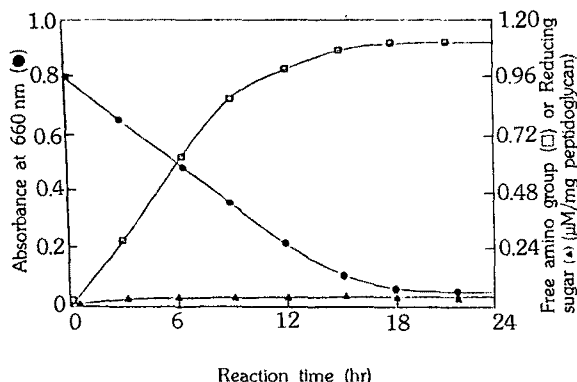

Fig. 11. Digestion of cell wall of *Bacillus megaterium* by purified lytic enzyme of *Bacillus* sp. YJ-451.
Purified cell walls were suspended in 25 mM Tris-HCl buffer (pH 8.0) at $OD_{660}=0.8$. To 4 m*l* of cell wall suspension, purified lytic enzyme was added, and samples were removed at various intervals for determination of turbidity at 660 nm (●) and release of reducing groups (▲) and free amino groups (□).

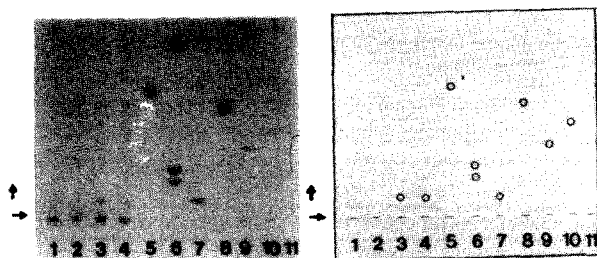

Fig. 12. Identification of DNP-amino acids by silica gel thin-layer chromatography.
After dinitrophenylation and acid hydrolysis, DNP-amino acids were extracted by ether and analyzed by chromatography. The plate was first developed solvent A (n-butanol-1% ammonia) at room temperature, and after drying it was developed with solvent B (chloroform-methanol-acetic acid, 85:14:1) at 4℃. The figure shows the tracings of DNP-amino acids on the chromatogram. Lanes: 1, *Bacillus* sp. YC-335 PCW (purified cell wall); 2, *Bacillus megaterium* PCW; 3, *Bacillus* sp. YC-335 PCW plus purified lytic enzyme; 4, *Bacillus megaterium* PCW plus purified lytic enzyme; 5, DNP-lysine; 6, DNP-DAP; 7, DNP-glutamic acid; 8, DNP-alanine; 9, DNP-serine; 10, DNP-glycine; 11, purified lytic enzyme.

ATCC 14945 (data not shown). This indicated that the lytic enzyme of *Bacillus* sp. YJ-451 hydrolyzes the peptide bond between L-alanine and D-glutamic acid so as to liberate amino group of glutamic acid.

DISCUSSION

A alkalophilic *Bacillus* sp. YJ-451, which produces a novel type of bacteriolytic enzyme, was isolated from soil by spotting the culture supernatant on the plate containing autoclaved, lyophilized *Bacillus* sp. as substrate. The enzymatically active cell wall hydrolase activity could be detected directly as clear zone by SDS-PAGE containing 0.2% (w/v) cell wall of *Bacillus* sp. as substrate. The microbiological characteristic of *Bacillus* sp. YJ-451 was different from that reported as microorganisms producing bacteriolytic enzyme (7, 8, 9, 16, 25, 28) at the point of favorite pH of culture media. The optimum pH for growth was pH 10.2. The lytic enzyme of *Bacillus* sp. YJ-451 was purified 66 fold from the culture supernatant by ammonium sulfate precipitation, CM-cellulose, Sephadex G-100 and hydroxylapatite and the recovery of total activity was 3.2%. The molecular weight of purified lytic enzyme was determined to be 27,000 by gel filtration and SDS-PAGE. The molecular weight of lytic enzymes from *Streptomyces erythraeus* (16), *Streptomyces rutgersensis* (9), *Streptomyces orientalis* (25), were reported to be 18,500, 22,000, 33,000 respectively. The most marked difference between the lytic enzyme of *Bacillus* sp. YJ-451 and other lytic enzymes seemed to be optimum pH for the lytic activity. The optimum pH of the lytic enzyme from *Bacillus* sp. YJ-451 was pH 10.0, while those of lytic enzyme from *Streptomyses rutgersensis* (9), *Myxococcus xantus* (7), *Pseudomonas aeroginosa* (8) *Streptomyces griseus* (28) were pH 6.0, 7.5, 6.4, 7.0 respectively. Various test microorganisms were subjected to examining their susceptibility to lytic enzyme. The lytic enzyme of *Bacillus* sp. YJ-451 was mainly active against gram negative bacteria and the genus *Bacillus* such as *B. megaterium, B. licheniformis, B. circulans, B. pumilus, B. macerans, B. polymyxa.* Cell walls of gram negative bacteria are complex both in morphology and in chemical composition (21). Many reports have been published on bacteriolytic enzymes but the vegetative cells of gram negative bacteria are normally resistant to these enzymes. Egg white lysozyme fails to lyse gram negative bacteria without artificial detergents or chelating reagents as EDTA. But the lytic enzyme of *Bacillus* sp. YJ-451 could be able to lyse gram negative bacteria without artificial detergent. Especially the *Pseudomonas* were lysed well by the lytic enzyme of *Bacillus* sp. YJ-451. Substrate specificity studies showed that the lytic enzyme of *Bacillus* sp. YJ-451 did not release reducing sugars but produced free amino groups. This suggests that the lytic enzyme was either an amidase or an endopeptidase. The exposed aminoterminal amino acid was identified as glutamic acid by analysis of terminal amino acid by dinitrophenylation.

This result strongly suggests that the lytic enzyme of *Bacillus* sp. YJ-451 hydrolyzes the peptide bond between L-alanine and D-glutamic acid in the cell wall peptidoglycan. It is known that the SA endopeptidase (17), the AL-1 protease of *Myxobacter* (6), the KM endopeptidase of *Streptomyces* (3), the peptidase of *Myxococcus xanthus* (23) are able to hydrolze the peptide bond associated with D-alanine, and only bacteriophage 95-induced lytic enzyme LE95 of *Pseudomonas aeruginosa* (27) and bacteriophage lytic enzyme of *Bacillus stearothermophilus* (26) hydrolyze the peptide bond between L-alanine and D-glutamic acid.

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