Kor. J. Appl. Microbiol. Biotechnol.
Vol. 20, No. 6, 655-662 (1992)

Purification and Properties of Pectate Lyase Produced by Recombinant Strain Containing *pelK* Gene from Alkalitolerant *Bacillus* sp. YA-14

Han, Hye-Jeong, Jin-Man Kim, Hee-Kyoung Park, Dong-Hoon Bai[1] and Ju-Hyun Yu*

Department of Food Engineering, Yonsei University, Seoul 120-749, Korea
[1]Department of Food Engineering, Dan-Kook University, Chunan 330-714, Korea

알칼리내성 *Bacillus* sp. YA-14 유래의 Pectate Lyase 유전자를 함유한 재조합균주로부터 효소의 정제 및 특성

한혜정 · 김진만 · 박희경 · 배동훈[1] · 유주현*
연세대학교 식품공학과, [1]단국대학교 식품공학과

Abstract — Pectate lyase produced by recombinant strain containing pectate lyase gene from alkalitolerant *Bacillus* sp. YA-14 was succesively purified with 257.6 purification folds and a 10.2% yields by the affinity method, CM-cellulose column chromatography followed by gel filtration on Sephadex G-100 column. The optimal pH and temperature for pectate lyase activity were 10.0 and 60℃, respectively. The enzyme was stable between pH 4.0 and 10.0, and up to 50℃. The molecular weight of this enzyme was estimated to be 43,000 daltons by SDS-PAGE. Amino acid analysis showed that the enzyme contained more polar and basic amino acids, especially serine, glycine and tyrosine, than that of various pectate lyase from other strains. The N-terminal amino acid sequence was Ala-Asp-Leu-Gly-His-Gln-Thr.

Pectin is an important structural component of the plant cell wall and heteropolysaccharide with a backbone consisting of partially methyl-esterified galacturonic acid. Pectic enzymes are classified into two main types : de-esterifying enzyme such as pectin methyl esterase, which removes methoxyl groups from pectin to yield polygalacturonate and methanol, and a range of depolymerizing enzymes. This latter class can be further divided into those which break the glycosidic linkages of the pectin backbone by hydrolysis, e.g. polygalacturonase, and those which cleave by β-elimination, the lyase (20).

These enzymes act in concert at the onset of pathogenesis with pectin methyl esterase being required for maximum degradation since the pectate lyase will not degrade highly methoxylated regions in pectin.

In addition to their role in pathogenesis these enzymes provide an excellent model for the investigation of protein secretion in Gram-negative bacteria (5, 7, 8, 13, 15, 19, 23, 24) because these enzymes may have common domains or structures required for interaction with the export machinery.

Pectinase is used in the food industry, particulary for the clarification of fruit and vegetable juices. They are commonly used in combination (3).

In our laboratory, a pectate lyase gene from alkali-tolerant Bacillus sp. YA-14, which was isolated from soil to have produce the xylanase (25), xylosidase (16, 26), cyclodextrin glycosyl transferase (4, 9, 27), β-galactosidase (28), endoglucanase (11) and pectate lyase (18, 29) was cloned into *E. coli*, and

Key words: Pectate lyase, N-terminal amino acid sequencing, alkalitolerant *Bacillus* sp. signal peptide
*Corresponding author

invesgated its chemical or physical properties of the purified pectate lyase from donor and recombinant strain (29). In this report, we describe the characterization of the pectate lyase exported extracellularly from *B. subtilis* harboring recombinant plasmid and analysis of its N-terminal amino acid by the amino acid sequencing.

We also discuss the whole physical and chemical characterization of the purified pectate lyase.

Materials and Methods

Chemicals

DABITC (4-NN′-dimethylaminoazobenzene 4′-isothiocyanate), PITC (phenyl isothiocyanate), TFA (trifluoroacetic acid), polygalacturonic acid (sodium salt), Sephadex G-100, CM-cellulose were purchased from Sigma Co., LTD.

Polygram polyamide-6 TLC (thin layer chromatography) sheets were purchased from Macherey-Nagel Co., LTD.

Bacterial strains and media used

Descriptions of bacterial strains and plasmid used are given in Table 1.

For purifying the pectate lyase, *Bacillus* cells were grown in LB medium, or YC broth (0.2% $(NH_4)_2SO_4$, 0.3% casamino acid, 0.2% yeast extract, 0.02% $MgSO_4 \cdot 7H_2O$, 0.7% sodium polygalacturonic acid, 0.04% $CaCl_2 \cdot 2H_2O$) for enrichment of the enzyme, and for seed culture, we used PAB medium (Antibiotic 3 Medium). Antibiotic levels used to select and maintain plasmids were 10 μg/m*l* for kanamycin, 0.1 μg/m*l* for chloramphenicol.

Enzyme purification

Each 5 m*l* of the 13 hr-seed culture of the organism was inoculated into the main culture flask (1%), and incubated at 37℃ for 16 hrs on a rotary shaker. Crude enzyme solution was prepared by affinity method modifying the Ward's method (14). Bacterial cells were removed by centrifugation and the pH of the supernatant fluid was adjusted to 8.5. Sodium polygalacturonic acids as a substrate (5 mg/m*l* in medium) were slowly added in a stepwise manners. Next, 1.0 M $CaCl_2 \cdot 2H_2O$ was immediately added and the resulting calcium precipitate gel was filtered through Whatman No. 43 filter by vacuum with a Buchner funnel. And then it was washed with equal volume of 0.1 M Tris-HCl buffer (pH 8.5) containing 0.1 M $CaCl_2 \cdot 2H_2O$. Precipitates were then eluted with 50 mM Tris-HCl buffer (pH 7.5) containing 0.1 M $CaCl_2 \cdot 2H_2O$ and 0.5 M NaCl. The solution concentrated by an ultrafiltrater (Advance Model UHP-43, Toyo) was applied to a column (3.7×22 cm) of CM-cellulose, which was previously equilibrated with 50 mM Tris-HCl buffer (pH 7.5). The enzyme was eluted with a linear gradient of zero to 0.7 M NaCl in the same buffer.

Pectate lyase rich fractions were pooled, concentrated with ultrafiltrater, and then subjected to gel filtration using a Sephadex G-100 column (1.8×90 cm), previously equilibrated with the same buffer.

SDS-polyacrylamide (12.5%) electrophoresis was performed for homogeneity determination according to the method of Laemmli (12).

Table 1. Bacterial strains and plasmids used in experiment

Strains and plasmid	Properties	Source reference
Bacillus sp. YA-14	alkali-tolerant	Yu *et al.* (30)
Bacillus subtilis 207-25	rk^-, mk^-, amyEO7 arol906, hsrM lys21, leuA8, recE4	Shirozo *et al.*
p12BS-△f1-PL	p12BS-△f1+1.5 Kb pelk gene Km^r, CRS	Kim, J.M.

Assay method

Pectate lyase activity was assayed as described in the previously paper (21, 22). The initial reaction rate of the enzymatic action was determined by measuring absorbancy changes at 235 nm during the initial one minute in the reaction mixture (0.5 m*l* of 0.2 M Tris-HCl (pH 8.5), 0.2 m*l* of 3 mM $CaCl_2 \cdot 2H_2O$, 1 m*l* of 1% polygalacturonic acid, and 1.27 m*l* of H_2O). Under this condition, one unit of pectate lyase was defined as the increase in absorbancy at 235 nm for one minute per 1 m*l* of enzyme solution (△O.D. 235/min/m*l*). The amount of pro-

tein in the enzyme solution was estimated by the method of Bradford (1) using bovine serum albumin as a standard protein.

N-terminal amino acid sequence determination

To determine the sequence of amino acid near the N-terminal region of purified enzyme, DABITC/PITC double coupling method of Chang *et al.* was used (2).

Analysis of the amino acid composition

Amino acid analysis was performed on samples hydrolyzed with 6 N HCl at 105℃ for 24 hr by using a modification of the Pico-Tag system of Waters Associations, Inc.

Results and Discussion

Purification of enzyme

The overall purification procedures are summarized in Table 2. The results of the last two steps in the procedure are shown in Figs. 1 and 2. The enzyme was successively purified with 257.6 folds and a 10.2% yield. The purified enzyme was demonstrated to be homogeneous by polyacrylamide gel electrophoresis, as shown in Fig. 3A.

The molecular weight of the enzyme was estimated to be about 43,000 daltons by SDS-polyacrylamide gel electrophoresis as shown in Fig. 3B. Approximately the same value was also obtained by gel filtration with Sephadex G-100.

Properties of enzyme

Effect of pH on activity and stability: The effect of pH on the activity and stability of the enzyme is shown in Fig. 4. The activity was the greatest at pH 10.0. The enzyme was relatively stable within a pH range from 4.0 to 10.0 at 30℃.

Effect of temperature on activity and stability: The activity was greatest at 55℃ at pH 8.5. The enzyme was relatively stable below 50℃ and 60% of initial activity was lost at 65℃ (Fig. 6).

Effect of cations on activity: Highly purified sodium polygalacturonic acid was used to avoid the

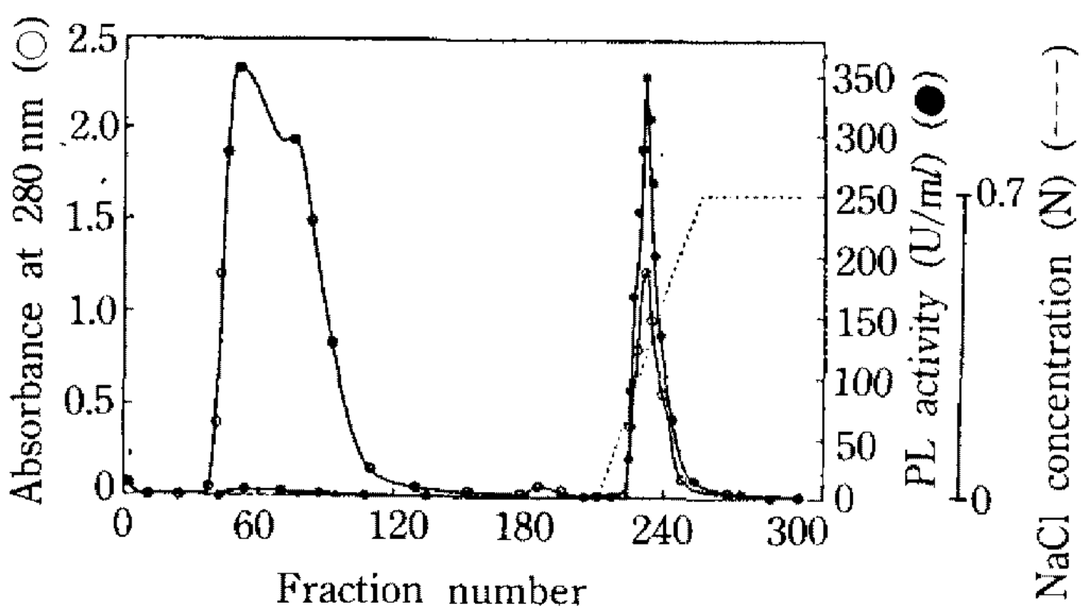


Fig. 1. Chromatogram of pectate lyase on a CM-cellulose column (3.7×22 cm).
Pectate lyase was pooled fractions which have pectate lyase activity and loaded directly to a column of CM-cellulose (pH 7.5) and eluted at 50 ml/h with 0~0.7 M NaCl. Collected fraction volume was 6 ml.

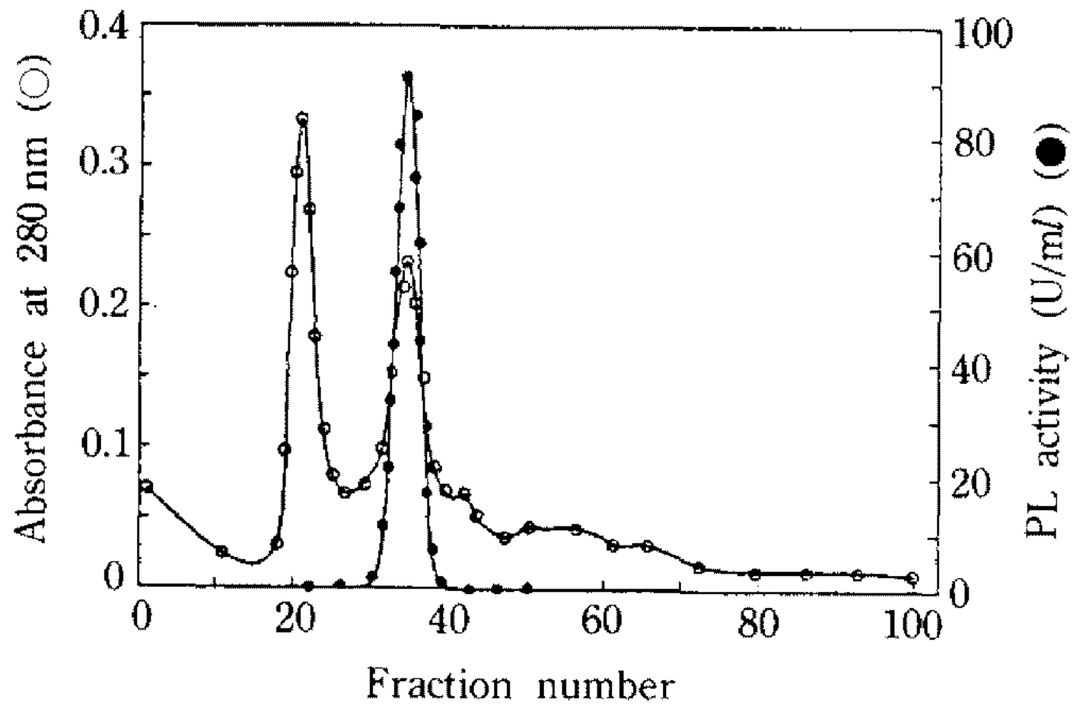
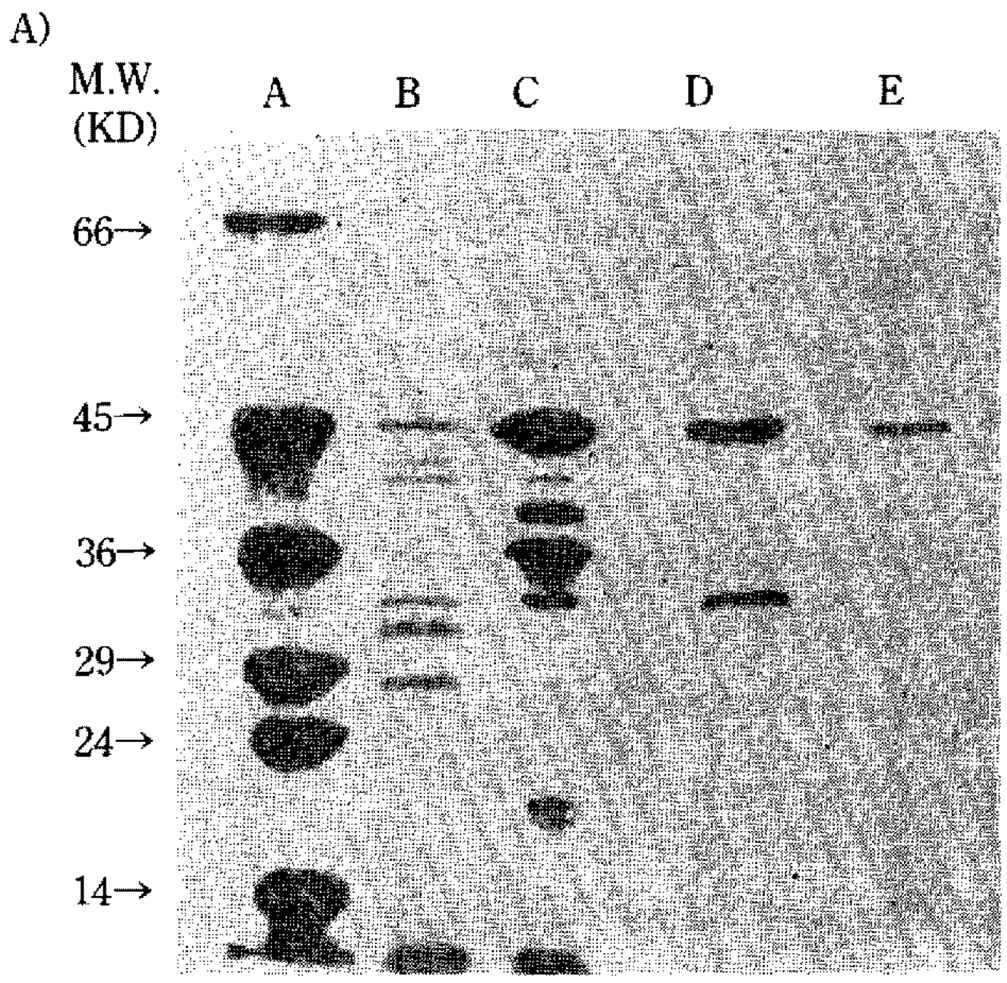


Fig. 2. Gel filtration chromatogram of pectate lyase on a Sephadex G-100 column (1.8×90 cm).
Flow rate: 10.2 ml/hr, Collected fraction: 3 ml

Table 2. Purification of pectate lyase from alkalitolerant *Bacillus* sp. YA-14

Step	Total activity ($U\times10^5$)	Total protein (mg)	Specific activity (U/mg)	Yield (%)	Purification fold
Culture supernatant	1.39	9672	14.34	100	1
Affinity in batch	1.04	66.1	1577.4	75	110
CM-cellulose	0.46	16.2	2824.9	32.8	197
Gel filtration	0.14	3.84	3694.4	10.2	257.6



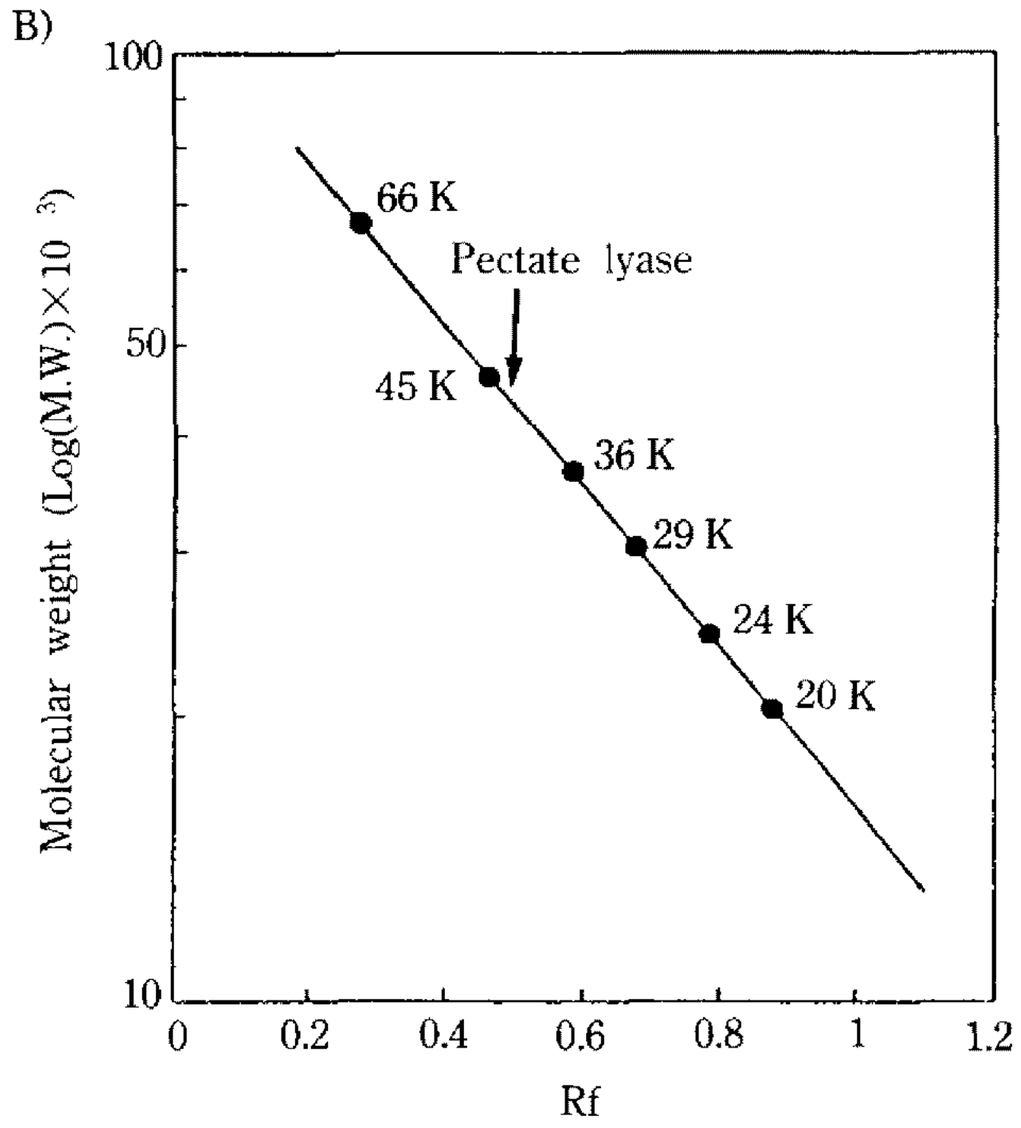


Fig. 3. A) SDS-polyacrylamide gel electrophoresis pattern of the pectate lyase in each purification step.
Electrophoresis on 12% acrylamide gel in the presence of SDS was performed. For each lane; A, standard protein molecular weight marker; B, culture supernatant of *B. subtilis* 207-25 (p12BS-△f1-PL); C, after the affinity method in batch; D, after CM-cellulose column chromatography; E, after Sephadex G-100 gel filtration column chromatography.
B) Molecular weight estimation of pectate lyase by SDS-PAGE.
Molecular weights of marker proteins are (from top to bottom); albumin bovine, 66,000; albumin egg, 45,000; glyceraldehyde-3-phosphate dehydrogenase, 36,000; carbonic anhydrase, 29,000; trypsinogen, 24,000 and trypsin inhibitor, 20,000

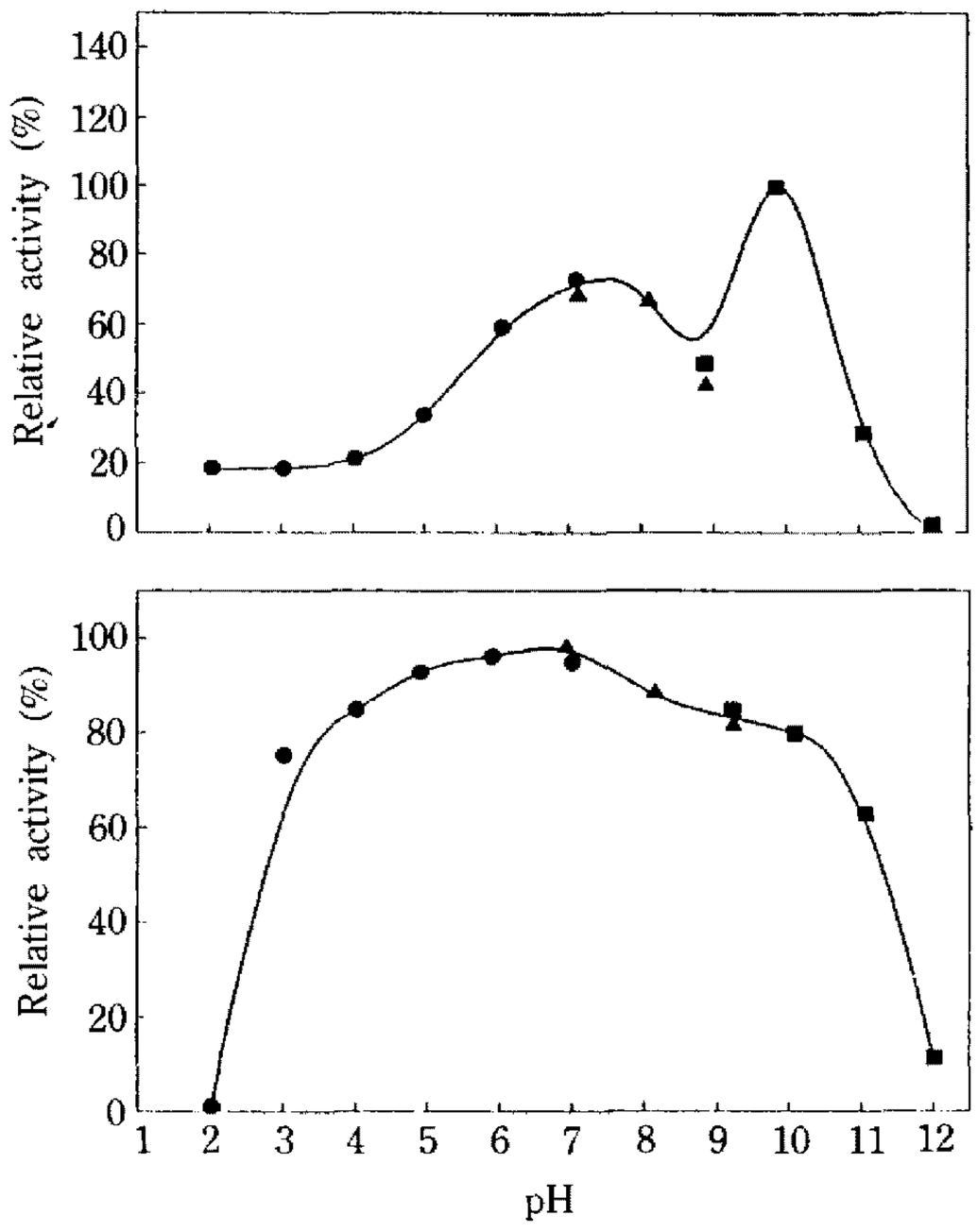


Fig. 4. Effect of pH on the activity (A) and stability (B) of pectate lyase.
These buffers were each used in the range; pH 2.0~7.0 (●), 50 mM citrate phosphate buffer, pH 7.0~9.0 (▲), 50 mM Tris-HCl buffer, pH 9.0~12.0 (■), 50 mM glycine-NaOH buffer. Reaction was performed at 30℃ for 30 min in each pH buffer.

masking effect due to contaminating cations in the substrate. The enzyme required cations for activity. Ca^{2-} was most effective in the activity. Sr^{2+}, Co^{2+}, K^{-}, and Na^{+} produced a third of the maximum activity, but Mg^{2+} had no effect on the activity. The optimum $CaCl_2 \cdot 2H_2O$ concentration was 0.4 mM on 0.1% sodium polygalacturonate, and 0.9 mM on 0.33 % sodium polygalacturonate, respectively (data not shown).

These results suggest the possibility that calcium combines with the substrate rather than the enzyme. Nagel and Wilson have discussed the possibility that the pectate lyase of *B. polymixa* may degrade the calcium-bridged substrate (6).

Amino acid composition analyses and N-terminal amino acid sequence determination

As shown in Table 3, this pectate lyase had relatively many Ser,Tyr and Gly. Additionaly, the com-

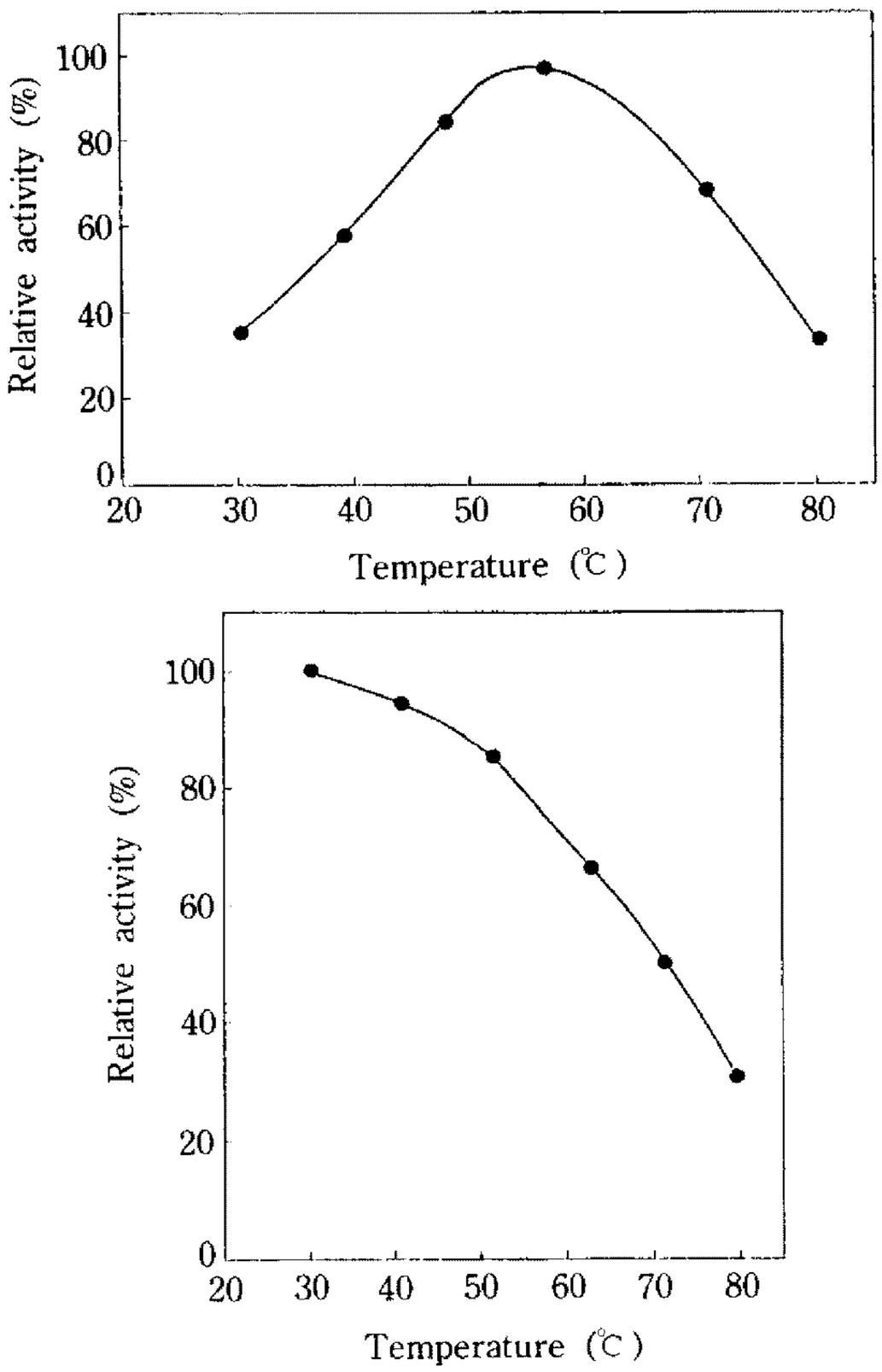


Fig. 5. Effect of temperature on the activity (A) and stability (B) of pectate lyase.
After the enzyme was treated at each temperature for 30 min, residual activity of the pectate lyase was measured.

Table 3. Amino acid composition analysis of pectate lyase from alkalitolerant *Bacillus* sp. YA-14

	Composition by nucleotide sequence	Composition by acid analysis
Non-polar		
Ala	26	27
Val	20	20
Leu	18	19
Ile	25	23
Pro	14	14
Met	3	2
Phe	8	9
Trp	7	UD
Polar		
Gly	40	40
Ser	47	46
Thr	29	29
Cys	1	1
Tyr	25	26
Asx	65	68
Glx	23	24
Basic		
Lys	26	25
Arg	11	10
His	11	11
Total	399	397
M.W.	43,890	43,670

*UD: not detected, **Asx: Asp+Asn, Glx: Glu+Gln.

position of basic amino acid such as Lys, Arg, and His was higher than acidic amino acid, and these compositions more high than pectate lyases from other strains such as *Yersinia pseudotuberculosis* (15), *Erwinia carotovora* (13), and *Erwinia chrysanthemi* (10).

To investigate the signal sequence required in exporting in *Bacillus* sp., the N-terminal amino acid sequence of pectate lyase was determined by DABITC/PITC double coupling method.

DABTH derivatives were identified by TLC on polygram polyamide-6. The sequence determined for the N-terminus of pectate lyase was Ala-Asp-Leu-Gly-His-Gln-Thr (Fig. 7), and each amino acid spot site was compared with its standard amino acid on TLC at the same condition.

This amino acid is identical to the amino acid sequence predicted from the neucleotide sequence if the protein starts at the 22 amino acid of the ORF. That is to say, the cleavage point of the signal peptide in pectate lyase is between two alanines (amino acids 21 and 22), and it has been confirmed by N-terminal amino acid sequence of the purified mature pectate lyase protein. Generally, signal peptide contains a positively charged N-terminal region, a 10-15 residue hydrophobic region, and a 5-6 residue polar carboxyl-terminal including a typical cleavage site for signal peptidase I (17). In the signal sequence deduced from nucleotide sequence it contains the two Lys-residues in the N-terminal region, a 12 residue hydrophobic region. These results agreed with above suggestions. Comparison of

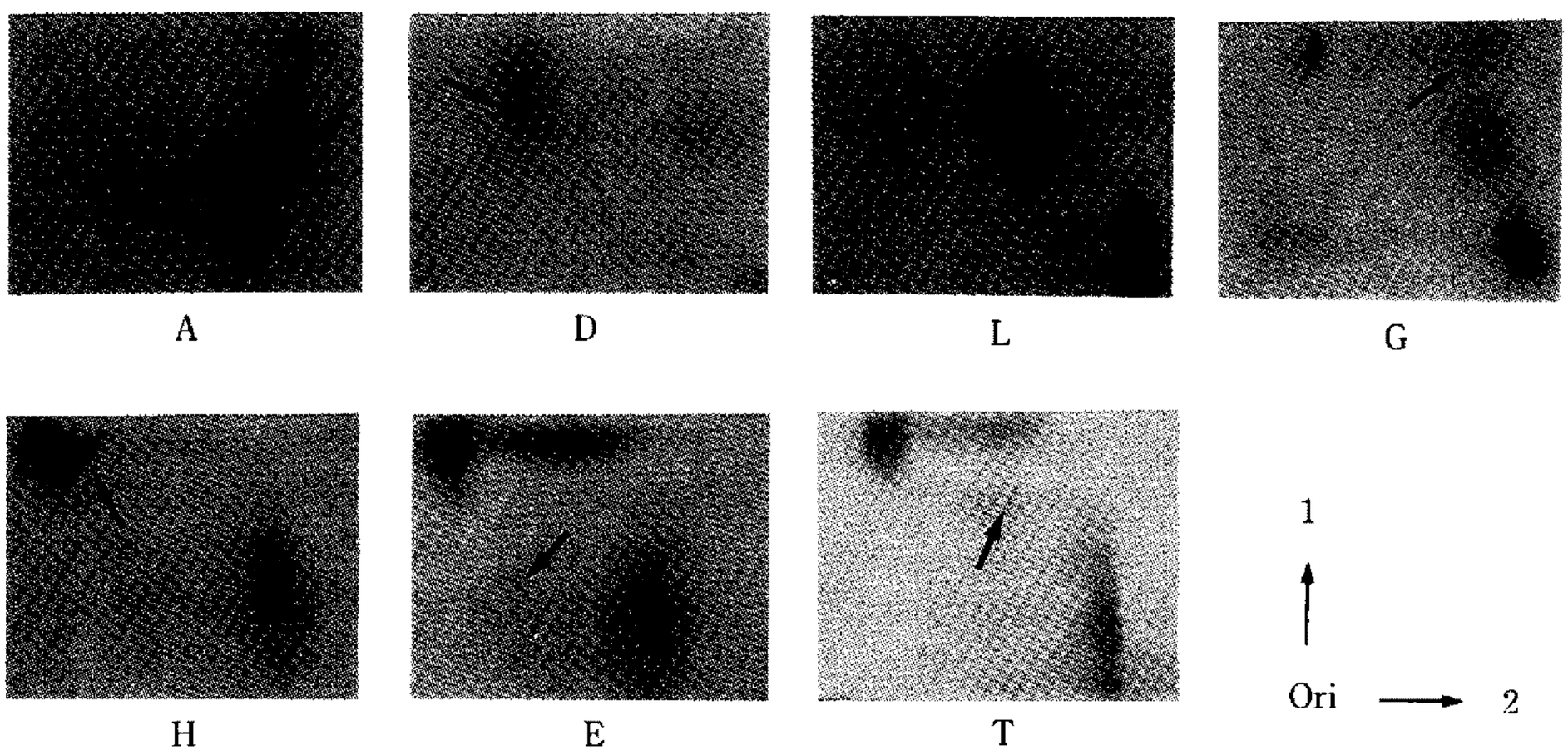


Fig. 6. Photograph of the polyamide TLC sheets resulting from sequence analysis of purified pectate lyase from *Bacillus* sp. YA-14.

Only DABTH-amino acids appeared red. Both the by-products and the added marker were blue. DABTH-derivatives are identified by the single letter amino acid code. 1; solvent 1, glacial acetic acid: water (1:2 by vol), 2; solvent 2, toluene: n-hexane: glacial acetic acid (2:1:1 by vol)

Table 4. Comparison of the signal peptide in pectolytic enzymes from other strains

Pectolytic enzymes	Signal peptide	Reference
Pectate lyase K (*Bacillus* sp. YA-14)	Met Lys Lys Val Met Leu Ala Thr Ala Leu Phe Leu Gly Leu Thr Pro Ala Gly Ala Asn Ala Ala Asp Leu Gly	This study
Pectate lyase I (*E. caroto*)	Met Lys Tyr Leu Leu Pro Ser Ala Ala Leu Gly Leu Leu Ala Ala Arg Gly Pro Thr Asp Asn Gly Ala Asn Thr Gly	Ito *et al.* (33)
Pectate lyase A (*E. caroto*)	Met Lys Tyr Leu Leu Pro Ser Ala Ala Ala Gly Leu Leu Leu Leu Ala Ala Gln Pro Thr Met Ala Ala Asn Thr Gly	Lei *et al.* (4)
Pectate lyase E (*E. chrysa*)	Met Lys Asn Thr Arg Val Arg Ser Ile Gly Thr Lys Ser Leu Leu Ala Ala Val Val Thr Ala Ala Leu Met Ala Thr Ser Ala Tyr Ala Ala Val Glu Thr	Keen *et al.* (31)
Pectate lyase B (*E. chrysa*)	Met Lys Ser Leu Ile Thr Pro Ile Ala Ala Gly Leu Leu Leu Ala Phe Ser Gln Tyr Ser Leu Ala Ala Asp Thr Gly	Keen *et al.* (31)
Pectate lyase (*Y. pseudo*)	Met Lys Lys Arg Ala Leu Leu Leu Ser Met Ser Val Leu Ala Met Leu Tyr Ile Pro Ala Gly Gln Ala Ala Glu Ile Asp	Manulis *et al.* (5)
Pectin esterase (*E. chrysa*)	Met Leu Lys Thr Ile Ser Gly Thr Leu Ala Leu Ser Leu Ile Leu Ala Ala Ser Val His Gln Ala Gln Ala Ala Thr Thr↓ Tyr	Plastow, G.S. (3)
Polygalacturonase (*P. solana*)	Met Asn His Arg Tyr Thr Leu Leu Ala Leu Ala Ala Ala Ala Leu Ser Ala Gly Ala His Ala Thr Gly Thr Ser	Huang *et al.* (8)

*Arrow indicates the cleavage site for the signal peptidase. *Bacillus* sp YA-14: alkalitolerant *Bacillus* sp. YA-14, *E. caroto*: *Erwinia carotovora*, *E. chrysa*: *Erwinia chrysanthemi*, *Y. pseudo*: *Yersinia pseudotuberculosis*, *P. solana*: *Pseudomonas solanacearum*.

signal peptide with various pectolytic enzyme produced by other strains is shown in Table 4. In most cases, basic amino acid such as Lys is commonly contained in N-terminal region, and the Ala-Ala bond is cleaved mostly by signal peptidase. The existence and its length of the signal peptide in

pectate lyase was also confirmed by amino acid composition analysis. The derived amino acid composition by DNA sequencing was agreed with composition by analysis to purified protein as shown in Table 3. In addition to this, the mass of matured protein was consisted with the one of precursor if the first 21 amino acids are removed.

From the above results, we can say that pectate lyase from alkalitolerant *Bacillus* sp. YA-14 is different from other pectolytic enzymes in physical properties and the part of protein structure containing N-termini region.

Acknowledgement

This study was supported by a research grant from the Korea Science and Engineering Foundation.

요 약

알칼리내성 *Bacillus* sp. YA-14 유래의 pectate lyase 유전자를 함유한 재조합균주로부터 affinity method, CM-cellose column chromatography와 gel filtration을 통해 효소를 정제하였으며 정제효소의 수율은 10.2%, 정제도는 258배였다. 효소의 최적활성 pH는 10.0이었고 pH 4.0~10.0까지의 범위에서 안정성이 있었으며,최적활성온도는 60℃ 이고 50℃ 까지 열안정성이 있으며 SDS-PAGE에 의해 추정된 분자량은 43 KDa이었다. 아미노산 조성 분석 결과 polar, basic 아미노산의 함량이 높고 특히 Ser, Gly, Tyr의 함량이 높았으며, 정제효소의 N-terminal은 Ala-Asp-Leu-Gly-His-Gln-Thr의 아미노산 서열이었다.

References

1. Bradford, M.M. 1976. A rapid and sensitive method for the quantititation of micro gram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**: 248-254.
2. Chang, J.Y., D. Brauer and B. Wittmann-Liebold. 1978. Micro-sequence of peptides and proteins using 4-NN'-dimethylaminoazobenzene 4'-isothiocyanate/phenylisothiocyanate double coupling method. *FEBS Letters* **93**: 205-214.
3. Chatterjee, A.K. and M.P. Starr. 1980. Genetics of *Erwinia* species. *Annu. Rev. Microbiol.* **34**: 645-676.
4. Chung, Y.J., I.S. Kong, Y.S. Kang and J.H. Yu. 1990. Purification and characterization of cyclodextrin glycosyltransferase from alkalophilic *Bacillus* sp. YC-335. *Kor. J. Appl. Microbiol. Biotech.* **18**: 44-48.
5. Gysler, C., J.A.M. Harmsen, H.C.M. Kester, J. Visser and J. Heim. 1990. Isolation and structure of the pectin lyase D-encoding gene from *Aspergillus niger*. *Gene* **89**: 101-108.
6. Hasegawa, S. and G.W. Nagel. 1966. A new pectic acid transeliminase produced exocellularly by a *Bacillus*. *J. Food Sci.* **31**: 838-845.
7. Huang, J. and M.A. Schell. 1990. DNA sequence analysis of pglA and mechanism of export of its polygalacturonase product from *Pseudomonas solanacearum*. *J. Bacteriol.* **172**: 3879-3887.
8. Ito, K., R. Kobayashi, N. Nikaido and K. Izaki. 1988. DNA structure of pectate lyase I gene cloned from *Erwinia carotovora*. *Agric. Biol. Chem.* **52**: 479-487.
9. Jung, Y.J., M.H. Jung and J.H. Yu. 1991. Enzymatic properties of cyclodextrin glycosyltransferase from alkalophilic *Bacillus* sp. YC-335. *Kor. J. Food. Sci. Technol.* **23**: 93-97.
10. Keen, N.T. and S. Tamaki. 1986. Structure of two pectate lyase gene from *Erwinia chrysanthemi* EC 16 and their high-level expression in *Escherichia coli*. *J.Bacteriol.* **168**: 595-606.
11. Kim, J.M., I.S. Kong, D.H. Bai and J.H. Yu. 1987. Molecular cloning of an endoglucanase gene from an alkalophilic *Bacillus* sp. and its expression in *Escherichia coli*. *Appl. Environ. Microbiol.* **53**: 2656-2659.
12. Laemmmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage. *Nature* **227**: 680-695.
13. Lei, S.P., H.C. Lin, S.S. Wang and G. Wicox. 1988. Characterization of the *Erwinia carotovora pel A gene* and its product pectate lyase A. *Gene* **62**: 159-164.
14. L. Ward and S.H. De Boer. 1987. A rapid procedure for purifying pectate lyase from *Erwinia carotovora* based on substrate affinity. *Appl. Environ. Microbiol.* **53**: 1189-1191.
15. Manulis, S., D.Y. Kobayashi and N.T. Keen. 1988. Molecular cloning and sequencing of a pectate lyase gene from *Yersinia pseudotuberculosis*. *J. Bacteriol.* **170**: 1825-1830.
16. Na, K.H., J.M. Kim, H.K. Park, D.H. Bai and J.H. Yu. 1990. Recombinant plasmid DNA containing xylanase and β-xylosidase gene of *Bacillus*

sp. YA-14. *Kor. J. Appl. Microbiol. Biotech.* **18**: 195-198.
17. Oliver, D. 1985. Protein secretion in *Escherichia coli. Annu. Rev. Microbiol.* **39**: 615-648.
18. Park, H.K., Y.S. Park, J.M. Kim and J.H. Yu. 1991. Studies on the properties of the promoter from alkali-tolerant *Bacillus* sp. *Kor. J. Appl. Microbiol. Biotech.* **19**: 21-24.
19. Plastow, G.S. 1988. Molecular cloning and nucleotide sequence of the pectin methyl esterase gene of *Erwinia chrysanthemi* B374. *Mol. Microbe* **2**(2): 247-254.
20. Rombouts, F.M. and W. Pilnik. 1980. Pectic enzymes pp. 227-282. *In* A.H. Rose (ed.) *Microbial Enzymes and Bioconversion.* (Economic Microbiology. Vol. 5) Academic Press, London.
21. Sato, M. and A. Kaji. 1977. Purification and properties of pectate lyase produced by *Streptomyces nitrosporeus. Agric. Biol. Chem.* **41**(11): 2193-2197.
22. Sato, M. and A. Kaji. 1979. Further properties of the new type of exopolygalacturonate lyase from *Streptomyces nitrosporeus. Agric. Biol. Chem.* **43**(7): 1547-1551.
23. Tamaki, S.J., S. Gold, M. Robeson, S. Manulis and N.T. Keen. 1988. Structure and organization of the pel genes from *Erwinia chrysanthemi* EC16. *J. Bacteriol.* **170**: 3468-3473.
24. Watson, M.E.E. 1984. Compilation of published signal sequences. *Nucleic Acids Res.* **12**: 5145.
25. Yu, J.H., D.C. Park, Y.J. Chung and I.S. Kong. 1989. Cloning and expression of a xylanase gene from alkali-tolerant *Bacillus* sp.YA-14 in *Escherichia coli. Kor. J. Appl. Microbiol. Bioeng.* **17**: 154-159.
26. Yu, J.H., D.C. Park, J.M. Kim, Y.J. Chung, I.S. Kong and D.H. Bai. 1989. Cloning and expression of β-xylosidase gene from alkali-tolerant *Bacillus* sp. YA-14 in *Escherichia coli. Kor. J. Appl. Microbiol. Bioeng.* **17**: 574-579.
27. Yu, J.H., Y.J. Chung and J.S. Lee. 1989. Isolation and characterization of cyclodextrin glycosyl transferase producing alkalophilic *Bacillus* sp. *Kor. J. Appl. Microbiol. Bioeng.* **17**: 148-153.
28. Yu, J.H. and S.S. Yoon. 1989. Production of β-galactosidase from alkalophilic *Bacillus* sp. (II). *Kor. J. Appl. Microbiol. Bioeng.* **17**: 524-528.
29. Yu, J.H., Y.S. Park, J.M. Kim, I.S. Kong and Y.J. Chung. 1988. Cloning of pectate lyase gene of alkali-tolerant *Bacillus* sp. YA-14 and its expression in *Escherichia coli. Kor. J. Appl. Microbiol Bioeng.* **16**: 316-319.
30. Yu, J.H., Y.J. Chung, K.S. Chung and D.H. Oh. 1986. Physiological properties and transformation of alkaline-tolerant bacteria. *Kor. J. Appl. Microbiol. Bioeng.* **14**: 239-244.

(Received November 24, 1992)