

## Purification and characterization of the extracellular alginate lyase from *Streptomyces* sp. MET 0515

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We isolated a new extracellular alginate lyase-producing microorganism, which displayed alginate-depolymerizing activity in plate assays, from coastal soils in Wando, Jeollanam-do, Korea. This alginate-depolymerizing bacterium belonged to the genus *Streptomyces* and it was named *Streptomyces* sp. MET 0515. An extracellular alginate lyase (ALY1) secreted by *Streptomyces* sp. MET 0515, was purified to homogeneity by a combination of acetone precipitation, anion-exchange chromatography (Q-Sepharose and DEAE-Sepharose) and Sephacryl S-200 HR gel filtration chromatography. Its molecular mass was 26 kDa as determined by SDS-PAGE analysis. The enzyme had an optimal temperature of 70°C for its activity, and was most active at pH 7.5. The thermal and pH stability were 0-50°C, and pH 6.0-9.0, respectively. The enzyme activity was stimulated by 1mM Mn<sup>2+</sup>, and inhibited by 1mM Fe<sup>3+</sup>, 1mM EDTA and 1mM Zn<sup>2+</sup>. Preliminary analysis of substrate specificity showed that this alginate lyase had activity on both poly- $\alpha$ -1,4-L-guluronate and poly- $\beta$ -1,4-D-mannuronate in the alginate molecule.

**Key words** – *Streptomyces* sp. MET 0515, alginate lyase, thiobarbituric acid (TBA), mannuronate, guluronate

### Introduction

Alginate is a linear polysaccharide composed of  $\beta$ -D-mannuronate and  $\alpha$ -L-guluronate. The residues are organized in blocks of poly-mannuronate (poly M), poly-guluronate (poly G), and hetero-polymeric sequences (poly MG) [9]. Alginate is widely used in food additives, medicals, cosmetics, and other applications, and oligosaccharides derived from alginate have been investigated for new functional materials. Alginates are enzymatically depolymerized by alginate lyases which cleave the  $\beta$ -1,4-glycosidic linkage by  $\beta$ -elimination, resulting in an 4,5-unsaturated, nonreducing terminus.

Alginate lyases have been isolated from a variety of marine molluscs, soil bacteria, marine brown algae and fungi and some of these lyases have been characterized. Alginate lyases have been tentatively classified into two types based on their substrate specificity, defined as the preference for either poly M block or poly G block. The alginate lyases found in *Haliotis tuberculata* [10,11], *Photobacterium* sp. [20] and *Pseudomonas aeruginosa* [18] are representative of poly

M lyase, and those found in *Klebsiella aerogenes* [17] and *P. alginovora* [2] are representative of poly G lyase.

Although many alginate lyases has been reported, the definition of enzyme specificity have not been fully understood and most of enzymes were purified from bacteria. We tried to obtain different types of alginate lyases and to examine further the relationship between these enzymes and their substrates. We recently isolated *Streptomyces* species which produces extracellular alginate lyase, purified this enzyme from a culture filtrates of the isolate, and characterized it in order to study the above issues. This paper presents the results on this alginate lyase. To our knowledge, this is the first report on the extracellular alginate lyase (ALY1) from *Streptomyces* species.

### Materials and Methods

#### Materials

Sodium alginate was obtained from Junsei Co. Ltd (Japan). Oligomers enriched with sodium mannuronate (M-rich fraction) and sodium guluronate (G-rich fraction) were prepared from the commercial alginate after partial acid hydrolysis by the method of Haug et al [9]. Q-Sepharose and DEAE-Sepharose were purchased from

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Sigma (St. Louis, MO, USA). Silica gel 60/Kieselguhr F254 thin-layer chromatography (TLC) plates were obtained from E. Merck (Darmstadt, Germany). Highprep Sephacryl S-200 was purchased from Pharmacia Biotech (Uppsala, Sweden).

#### Culture conditions for alginate lyase production

A strain of *Streptomyces* sp. MET 0515 was used as the source of enzyme production. Media for extracellular alginate lyases production was composed to 0.1% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.2% KH<sub>2</sub>PO<sub>4</sub>, 0.02% Urea, 0.03% CaCl<sub>2</sub>, 0.03% MgSO<sub>4</sub> · 7H<sub>2</sub>O, 0.1% Bacto™ Proteose Peptone No.3, 0.5% NaCl, 0.02% Tween 80, and 0.5% alginate (pH 5.0). The cell culture was performed at 25°C for 72 hr in a liquid alginate medium on 120 rpm rotary shaker. The clarified culture fluid was obtained by centrifugation of the cell suspension at 10,000 × g for 30 min.

#### Assay for enzymes and protein

The thiobarbituric acid (TBA) reaction was carried out according to the Preiss et al. [25]. One unit of ALY1 enzyme activity was defined as the amount of enzyme required to liberate the equivalent of 1 μmol of β-formylpyruvic acid per 1 min; 0.01 μmol of β-formylpyruvic acid produces an A<sub>549</sub> of 0.298 in the TBA reaction. The protein concentration in the purification process of enzyme was measured by the Lowery method [19] with bovine serum albumin as a standard or by measuring absorbance at 280 nm, assuming that E<sub>280</sub> = 1.0 corresponds to 1 mg/ml.

#### Thin-layer chromatography (TLC)

TLC was done as described previously and modified [5]. Oligosaccharides degraded by alginate lyase was analyzed by TLC with a solvent system of 1-butanol-acetic acid-water (2 : 1 : 1, vol/vol). The reaction products were visualized by heating the TLC plate at 150°C for 5min after spraying with 10% sulfuric acid in ethanol (vol/vol).

#### Purification of extracellular alginate lyase

The culture fluid was precipitated by 2 volume acetone and allowed to stand overnight. The precipitate was collected by centrifugation and dried with air for 1 day at room temperature. The precipitate dissolved in 20 ml of 10 mM Tris-HCl buffer (pH 7.0) and dialyzed against 10 mM Tris-HCl buffer (pH 7.5). Insoluble materials were removed by centrifugation. The dialyzed enzyme obtained above was put on a Q-Sepharose column (1.5 by 25 cm) equili-

brated with 10 mM Tris-HCl buffer (pH 7.5) at the flow rate of 24 ml/hr. After the column was washed enough with the same buffer, were eluted with a linear gradient of 0 to 1.5 M NaCl in the same buffer (total volume, 100 ml). The active fraction was dialyzed against 10 mM Tris-HCl buffer (pH 7.5) and then the enzyme solution was applied to a DEAE-Sepharose column (1.5 by 25 cm) previously equilibrated with the 10 mM Tris-HCl buffer (pH 7.5). The enzyme was eluted with an increasing NaCl gradient (0 to 1.5 M) at the flow rate of 24 ml/hr. The active fraction was dialyzed against 10 mM Tris-HCl buffer (pH 7.0) and concentrated by ultrafiltration with an Amicon model 8200 (Amicon Co, Beverly, Mass, USA). The enzyme solution was put on a Highprep Sephacryl S-200 (16×600 mm), equilibrated with 10 mM Tris-HCl buffer (pH 7.5) containing 0.15 M NaCl, at the flow rate of 12 ml/hr. The active fraction was dialyzed against 10 mM Tris-HCl buffer (pH 7.5) and concentrated by ultrafiltration. Purified alginate lyase was stored at -20°C.

#### Measurement of molecular weight

The molecular weight of alginate lyase was calibrated by two methods: SDS-PAGE, and gel filtration. SDS-PAGE was carried out according to the method Laemmli [14], with a 12% running gel and 5% stacking gel, under denaturation conditions in the presence of SDS and β-mercaptoethanol. Sephacryl S-200 HR column chromatography was carried out at the flow rate of 12 ml/hr with 10 mM Tris-HCl buffer (pH 7.5). Molecular weight of the enzyme was determined with reference to calibrated proteins.

#### Effect of temperature and pH on the enzyme activity

The optimum pH of lyase activity was examined for 20 min at 70°C in a pH range 4.0 ~ 9.0 with 10 mM McIlvaine buffers (pH 4.0 ~ 8.0) and 10 mM Tris-HCl buffers (pH 7.0 ~ 9.0). The effect of temperature was determined in the range of 10 ~ 90°C for 20 min using the enzyme solution in 10 mM Tris-HCl buffer (pH 7.5). The stability of temperature of enzyme was determined for 20 min, 30 min and 1 hr in 10 mM Tris-HCl buffer, pH 7.5. After the enzyme was treated at various temperatures from 10 to 80°C and the remaining activity was assayed.

#### Effect of metal compounds on the enzyme activity

Most of the bacterial alginate lyases have required some

metal ions for maximal activity. To test their effect on the activity, the enzyme was treated with EDTA and metal compounds. The enzyme solution was treated with EDTA at 1mM of final concentration at pH 7.5 and 50°C for 20 min, and then the enzyme activity was assayed. Next, each of metal compounds was added to the EDTA-treated enzyme solution to bring 2 mM of final concentration and the mixture was left at 50°C for 20 min. Then the enzyme activity was assayed and expressed as the relative activity to the control.

#### N-terminal amino acid sequence of alginate lyase

Purified extracellular alginate lyase of *Streptomyces* sp. MET 0515 was applied to the SDS-PAGE, and then the protein was electroblotted to an ImmobilonTM-PSQSQ PVDF membrane (Millipore) using 48 mM Tris containing 20% methanol. Electroblotting occurred at 30 mA for 2 hr. The membrane was stained with CBB R-250 to visualize the protein. The protein band of alginate lyase was cut and air dried, and stored at -80°C until analysis. An Applied Biosystems Model 476A-01-120 protein sequencer was used for automated Edman degradation.

## Results

#### Culture conditions for alginate lyase production

We screened the alginate lyase-producing microorganisms from samples obtained at the coastal soils of Wando. During the course of screening of strains with alginate lyase activity, 33 strains producing alginate lyases were isolated using agar medium containing alginate as a carbon source. Among them, the strain MET 0515 showed the highest enzyme activity for extracellular alginate lyase. This strain belonged to the genus *Streptomyces* and it was named *Streptomyces* sp. MET 0515.

The time course for *Streptomyces* sp. MET 0515 lyase production and pH is shown in Fig. 1. The *Streptomyces* sp.

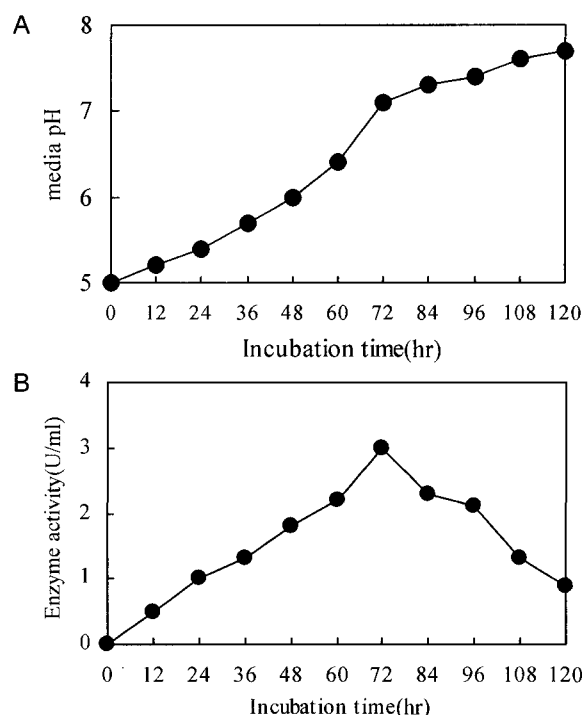


Fig. 1. Time course of alginate lyase producing strain, *Streptomyces* sp. MET 0515. (A) The change of media pH by incubation time. (B) The change of enzyme activity by incubation time. \* *Streptomyces* sp. MET 0515 represented the pellet-type growth. So we could not detect the cell growth with OD 600 - dependent turbidity. But, the cell growth was maintained for 96-hour point of culture, and decreased from 96-hour point of culture to end-point of culture (data not shown).

MET 0515 represented the highest enzyme activity when cultured in 0.5% alginate, pH 5.0 (media pH), at 25°C for 72 hr.

#### Purification of extracellular alginate lyase

The purification procedure for alginate lyase from the culture fluid is summarized in Table 1. In the first step of acetone precipitation, the specific activity was two times higher than that of the culture fluid. After further purifica-

Table 1. Purification of extracellular alginate lyase

Step <sup>a</sup>	Total amount (mg) of protein	Total activity (U)	Specific activity (U/mg)	Yield (%)	Purification (fold)
Culture-fluid	2560	178.28	0.07	100	1.00
Acetone precipitation	812.50	134.88	0.17	76	2.43
Q-sepharose	12.88	76.36	5.93	43	84.71
DEAE- sepharose	3.00	33.72	11.24	19	160.10
Sephacryl S-200HR	2.50	30.88	12.35	17	176.43

<sup>a</sup> Purification procedures are described in Materials and Methods.

tion by Q-Sepharose column chromatography with a linear gradient of 0-1.5 M of NaCl, the specific activity was 84 times higher than that in the fluid. The lyase activity fractions eluted from the subsequent chromatography by a DEAE-Sepharose column were obtained as a single symmetrical peak (Fig. 2). The specific activity was 160 times higher than that in the fluid. Highprep Sepharacryl S-200 column resulted in an increase in specific activity. The enzyme activity was approximately, 176 times higher than that in the culture fluid. The total yield of the purified sample was about 17% from the culture fluid.

#### Estimation of the molecular weight of alginate lyase

SDS-PAGE patterns for the purified alginate lyase are shown in Fig. 3. The preparations of the purified enzyme obtained by Sephacryl S-200 HR column chromatography in the final step (lane A), and the molecular mass standard (lane B), are shown. The purified lyase had a molecular weight close to 26 kDa. Comparison of the active peak of the enzyme with standard proteins used for calibration of Sephacryl S-200 HR column yielded a native molecular weight of 25 kDa, indicating that the active lyase consists of a single polypeptide chains.

#### Effect of temperature and pH on the enzyme activity

The effects of pH and temperature on lyase activity are shown in Fig. 4. The optimum activity of the enzyme was shown between pH 7.5 and pH 8.0 (Fig. 4A). About 77% of the activity still remained after treatment at pH 9.0. The enzyme seemed to be relatively stable in an alkaline pH

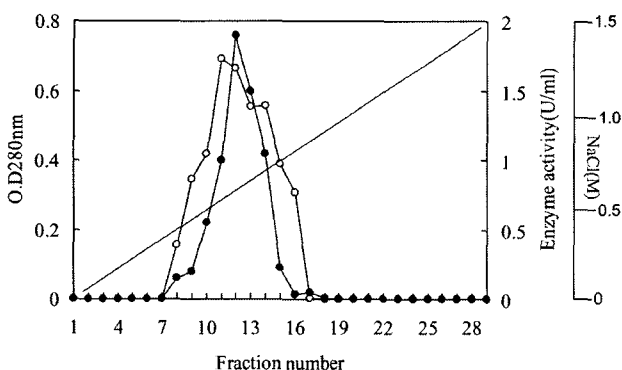


Fig. 2. DEAE-Sepharose anion exchange chromatography of alginate lyase from *Streptomyces* sp. MET 0515. (●), Enzyme activity ; (○), O.D 280 nm ; (---), NaCl gradient

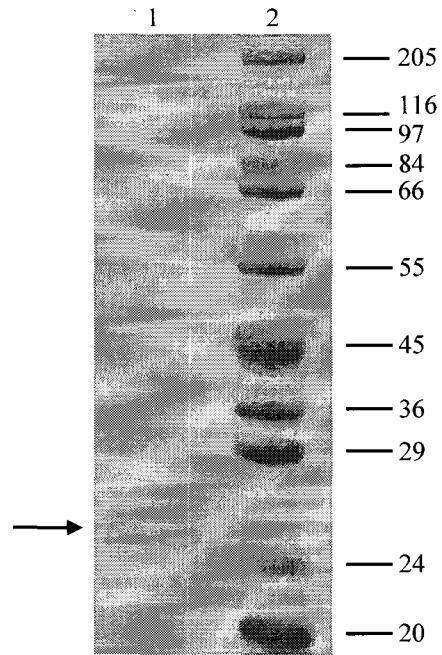


Fig. 3. SDS-PAGE of alginate lyase from *Streptomyces* sp. MET 0515. lane 1, molecular mass standards; lane 2, purified enzyme. The arrow indicates the position of purified alginate lyase.

and unstable in a range outside acidic pH 5.5. The optimum temperature was 70°C at pH 7.5 (Fig. 4B). Almost 80% of the activity still remained after treatment for 20 min at 60°C. The ALY1 (alginate lyase in this study) was stable at below 70°C when treated in 10 mM Tris-HCl buffer (pH 7.5) condition for 20min, 30min and 1 hr (Fig. 4C). But the activity of ALY1 at 70°C was declined when treated for 1hour condition.

#### Effect of metal ions on the enzyme activity

The effects of metal compounds on the purified enzyme, either native or EDTA-treated, in Tris-HCl buffer are shown in Fig. 5. The activity of the enzyme was a little enhanced in the presence of  $MnCl_2$ . Other compounds inhibited with enzyme activity. Especially, the original enzyme activity was almost completely inhibited with  $FeCl_3$  and  $ZnCl_2$ . The activity was not completely inhibited by treatment with 1 mM of EDTA. The addition of 2 mM of  $CaCl_2$  or  $MgCl_2$  to the enzyme treated with 1 mM of EDTA resulted in complete recovery of the activity. The activity of EDTA-treated enzyme was also restored 92% or more by the addition of 2 mM of  $CoCl_2$  or  $NiCl_2$ , suggesting that the enzyme conformation dependent on bivalent cations such as  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Co^{2+}$ ,  $Ni^{2+}$  was presumably

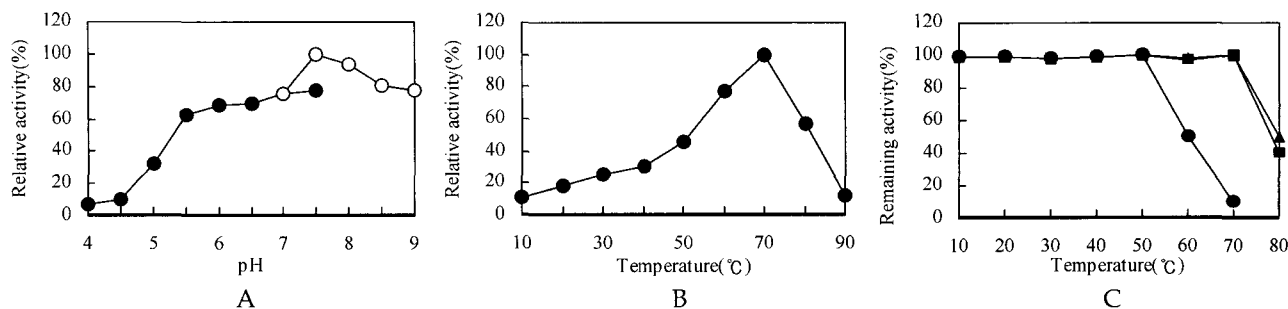


Fig. 4. Effects of pH and temperature on the activity of alginate lyase. Experiments were carried out at 70°C using alginate solution (500 µg) and purified enzyme (10 mU). (A) To determine the effect of pH, reactions were performed at 70°C for 20 min in the following 10 mM buffers; Mcllvaine buffer (●) and Tris-HCl buffer (○). Activity at pH 7.5 in Tris-HCl buffer was set at 100%. (B) To determine the optimal temperature, reactions were performed for 20 min at various temperature in 10 mM Tris-HCl buffer, pH 7.5. Activity at 70°C was set at 100%. (C) To determine the thermal stability, after preincubation of the enzyme at various temperatures for 20 min (▲), 30 min (■) and 1 hr (●), the remaining activity was measured under the conditions specified in Materials and methods. The activity of the enzyme preincubated at 0°C was relatively taken as 100%.

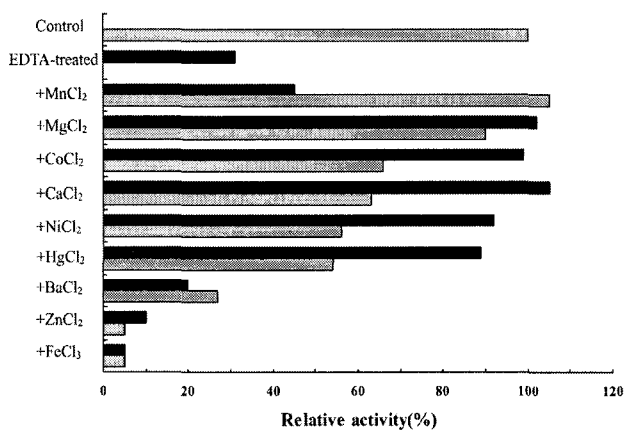


Fig. 5. Effects of various compounds on alginate lyase activity. Reactions were carried out for 20 min at 50°C and pH 7.5 (Tris-HCl) in the presence or absence(control) of the above compounds. The activity of the control was relatively taken as 100%. (▨), native enzyme + metal compounds. (■), EDTA-treated enzyme + metal compounds.

important for the appearance of lyase activity.

### Substrate specificity of alginate lyase

Fig. 6 shows the characterization of the degradation products. The enzyme degraded alginate, poly M and poly G (Fig. 6A, B). The major end-product of the endo-acting alginate lyases appear to be the unsaturated disaccharide and trisaccharide. Fig. 6C shows time course of the enzyme reaction on the alginate of alginate lyase. The degradation products appear to be disaccharide, trisaccharide, tetrasaccharide and oligomer to 12 hr. However, the products were mainly disaccharide and trisaccharide.

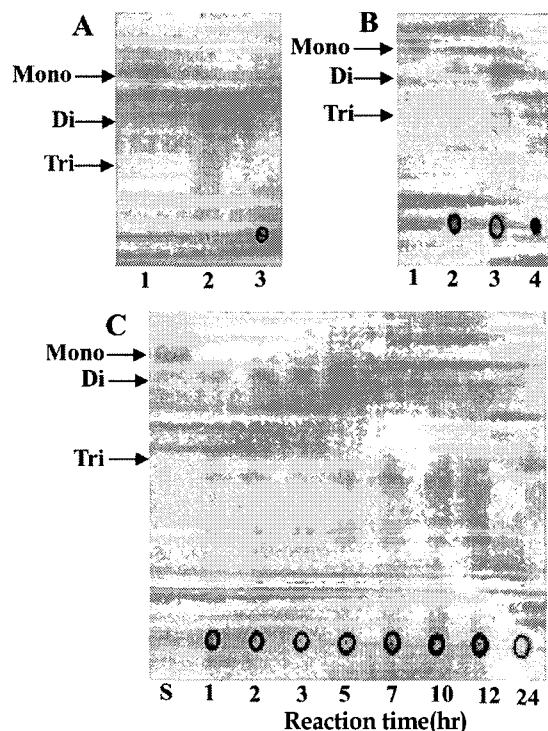


Fig. 6. TLC patterns of enzymatic degradation products from substrates. (A) Alginate (5 µg) was incubated with the enzyme (1 U) for 24 hr at 50°C (lane 2), and the products were analyzed by TLC. lane 1, alginate monosaccharide (5 µg); lane 3, alginate (5 µg). (B) Poly M and poly G (5 µg) was incubated with the enzyme (1 U) for 24 hr at 50°C (lane 2 and 3), and the products were analyzed by TLC. lane 1, alginate monosaccharide (5 µg); lane 4, poly M (5 µg). (C) Alginate (5 µg) was incubated with alginate lyase (1 U) at 50°C for several hours, followed by TLC analysis. Lane S, alginate monosaccharide (5 µg). Tri, Di, and Mono indicate the tri-, di-, and monosaccharides from alginate, respectively.

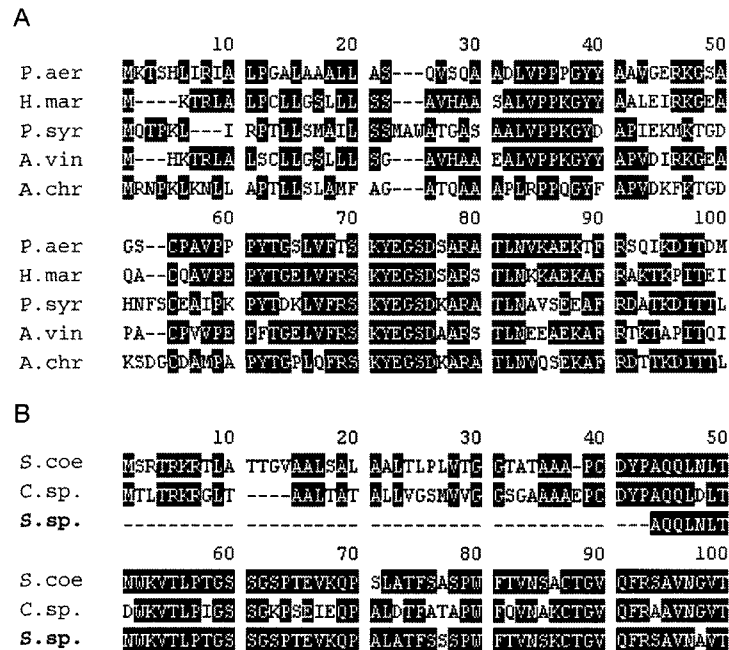


Fig. 7. Multiple sequence alignment of several alginate lyases. (A) Several alginate lyases that have not similarity with *S.sp.* (*Streptomyces sp.*) alginate lyase, in this study. P.aer, *Pseudomonas aeruginosa* (GenBank Acc. No. L09724); H.mar, *Halomonas marina* (GenBank Acc. No. AB018795); P.syr, *P. syringae* (GenBank Acc. No. AF222020); A.vin, *Azotobacter vinelandii* (GenBank Acc. No. AF037600); A.chr, *A. chroococcum* (GenBank Acc. No. AJ223605). (B) Alginate lyases that have some similarity with *S.sp.* alginate lyase, in this study. S.coe, *Streptomyces coelicolor* (GenBank Acc. No. NP\_627698); C.sp., *Corynebacterium sp.* (GenBank Acc. No. BAA83339); S.sp., *Streptomyces sp.* (GenBank Acc. No. AF513503 (ALY1): alginate lyase in this study).

### N-terminal sequence and alignment of several alginate lyases

The N-terminal sequence of ALY1 was determined to <sup>1</sup>AXPXDYPAQQLN<sup>12</sup>, X is not identified amino acid. Multiple sequence alignment of several alginate lyases was presented at Fig. 7. *P. aeruginosa*, *Halomonas marina*, *P. syringae*, *Azotobacter vinelandii* and *A. chroococcum* have no similarity with ALY1. But *S. coelicolor* and *Corynebacterium sp.* have some similarity with our enzyme.

## Discussion

As shown in Fig. 1, *Streptomyces sp.* MET 0515 represented the highest enzyme activity when cultured in 0.5% alginate, pH 5.0 (media pH), at 25°C for 72 hr. Initial pH for alginate lyase production was 5.0, which was low in comparison with that reported generally of medium pH 7.0 [5,13,23]. When the media pH was raised, cell growth nor enzyme activity was not detected.

The yield of purified lyase from *Streptomyces sp.* MET 0515 was 17%, which was to be like that reported enzyme. We purified the extracellular alginate lyase from *Streptomyces*

*sp.* MET 0515 to a single band on SDS-PAGE (Fig. 3). The molecular weight of the enzyme was 26 kDa by SDS-PAGE, and was 25 kDa by Native-PAGE, suggesting that the enzyme is probably composed of a single polypeptide. Most of alginate lyases are also composed of a single polypeptide [15] except bacterial alginate lyase which has two subunits [16]. The size of our enzyme was a smaller than other alginate lyase, *Alternomonas sp.* strain H-4 [26], *P. aeruginosa* [1], *Vibrio harveyi* AL-128 [27], *Vibrio sp.* mutant strain 510-64 [13], *Enterobacter cloacae* M-1 [24], *Bacillus sp.* ATB-1015 [23] and *Sphingomonas sp.* etc [5,6].

Almost all of alginate lyases have optimum pH around neutral (pH 7-8), and the optimum temperatures of many alginate lyases are 20-50°C [1,7,13,23,24,26]. As the shown Fig. 4, the enzyme was around pH 7.5 and 70°C, respectively. The optimum pH of this enzyme also corresponded to these values (Fig. 4A). However, the optimum temperature of the ALY1 enzyme activity (70°C) was higher than that of *Bacillus sp.* ATB-1015 [23], *Sphingomonas sp.* A1 [7], and *Vibrio sp.* mutant strain 510-64 [13] (Fig. 4B). Even if the optimum temperature of ALY1 enzyme activity was 70°C, the stability of ALY1 was fall down rapidly

when the incubation time was over the 30 min (Fig 4C). Because of instability of ALY1 at optimal temperature (70 °C), it is not suitable for industrial application at optimal temperature (70°C). But ALY1 was stable for long time at below 50°C. So, these temperature reaction conditions were suitable for industrial application. According to this reason, the temperature of enzyme reaction with long time was selected to 50°C. These value was higher than other alginate lyases. This enzyme has specific property that it was distinguishable to make of a present of other enzyme. Accordingly, our enzyme is clearly different to compared with other enzymes.

The activity of ALY1 was a little enhanced in the only presence of MnCl<sub>2</sub> (Fig. 5). However, no remarkable difference in activity was observed between presence and absence. The original enzyme activity was almost completely inhibited with FeCl<sub>3</sub>, ZnCl<sub>2</sub> (Fig. 5). Also, the original enzyme activity was completely inhibited with EDTA at reported enzyme [23,24], but the activity of our enzyme was not completely inhibited with EDTA. The activity was inhibited 69% by 1 mM of EDTA. The addition of 2 mM of CaCl<sub>2</sub> and MgCl<sub>2</sub> to the enzyme treated with 1 mM of EDTA resulted in complete recovery of the activity. The activity of EDTA-treated enzyme was also restored 88% or more by the addition of 2 mM of CoCl<sub>2</sub>, NiCl<sub>2</sub> and HgCl<sub>2</sub>, suggesting that the enzyme conformation depend on bivalent cations such as Ca<sup>2+</sup>, Mg<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Hg<sup>2+</sup> was presumably important for the appearance of lyase activity.

The activity of FeCl<sub>3</sub>-treated enzyme was little restored by treatment with metal compounds. We were to suppose that it was bind to site of enzyme activity. This result was completely different to compared with other enzymes.

In general, it appears to be difficult to assay the substrate specificity for poly-guluronate and poly-mannuronate separately prepared from alginate. Hisano et al [12] reported that the two types of lyase, poly-mannuronate-specific and poly-guluronate-specific enzymes, are distinguishable by the white halo and the white ring, respectively, that appeared on the agar plate containing alginate. Both a white ring and a white halo around the paper discs which contained of an appropriate amount of alginate lyase were clearly observed, indicating that the enzyme had specificity to both substrates.

Some studies demonstrated that endo poly-guluronate lyases also slightly attacked poly M. One reason may be that the poly M contains significant proportions of the G.

Another reason is perhaps that the enzyme has cleaved not only G-G linkage but also G-M linkage among the four possible glycosidic linkages in alginate (G-G, M-M, G-M and M-G). Most of reported enzyme has either cleaved at M-rich [3,15] or G-rich alginates [17,21]. As the shown Fig. 6, appearance of both phenomena indicated that our enzyme possessed substrate specificity for both units, poly-mannuronate and poly-guluronate, in the alginate molecule. These properties were clearly different to compare with reported enzyme. The major end-product of alginate lyases appear to be the unsaturated trisaccharide and disaccharide. Accordingly, our enzyme action endo-type but it was not action exo-type [22].

The N-terminal sequence of ALY1 (alginate lyase in this study) that determined to be <sup>1</sup>AXPXDYPAQQLN<sup>12</sup>, X is not identified, has no similarity with *P. aeruginosa*, *H. marina*, *P. syringae*, *A. vinelandii* and *A. chroococcum*, but with *S. coelicolor* and *Corynebacterium* sp. has some similarity with our enzyme.

Finally, the ALY1 from *Streptomyces* sp. MET 0515 was first reported alginate lyase (GenBank Acc. No AF513503, 02-Jun-2003) in *Streptomyces* sp. that has some relationship with *Corynebacterium* but has some novel feature compare with other alginate lyases reported elsewhere.

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**초록 : *Streptomyces* sp. MET 0515의 균체외 Alginate lyase의 정제 및 특성**김현경 · 이재창<sup>1</sup> · 강남현 · 김송희 · 김종국<sup>2</sup> · 정기철\*(전남대학교 생명과학기술학부, <sup>1</sup>나주대학 치위생과, <sup>2</sup>경북대학교 미생물학과)

알긴산분해효소(EC 4. 2. 2. 3)를 균체외로 생산하는 새로운 방선균을 전라남도 완도의 연안토양으로부터 분리하고 이 균주를 *Streptomyces* sp. MET 0515라 명명하였다. 이 균주가 생산하는 균체외 알긴산분해효소를 아세톤 침전, 음이온 교환 크로마토그래피(Q-Sepharose and DEAE-Sepharose), 겔 크로마토그래피(Sephacryl S-200 HR gel filtration chromatography)를 이용하여 정제하였다. 이 효소의 최적 활성 온도와 pH는 각각 70℃와 pH 7.5이고, 온도 안정성은 0-50℃, pH 안정성은 pH 6.0-9.0였다. 이 효소는  $Mn^{2+}$  이온 첨가시에는 활성이 증가하였으며 1mM  $Fe^{3+}$ , 1mM EDTA, 1mM  $Zn^{2+}$  이온 첨가시에는 활성이 억제되었다. 또한 이 효소는 poly-alpha 1,4-L-guluronate와 poly-beta 1,4-D-mannuronate 두 종류의 기질에 대하여 활성을 나타냈다. 따라서 본 효소는 다른 미생물 기원의 효소와 비교해 볼 때 *Streptomyces* sp.가 생산하는 첫번째 효소로 인정되었다.