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Three Alginate Lyases from Marine Bacterium *Pseudomonas fluorescens* HZJ216: Purification and Characterization

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Abstract Three alginate lyases (A, B, and C) from an alginate-degrading marine bacterium strain HZJ216 isolated from brown seaweed in the Yellow Sea of China and identified preliminarily as *Pseudomonas fluorescens* are purified, and their biochemical properties are described. Molecular masses of the three enzymes are determined by SDS-PAGE to be 60.25, 36, and 23 kDa with isoelectric points of 4, 4.36, and 4.59, respectively. Investigations of these enzymes at different pH and temperatures show that they are most active at pH 7.0 and 35 °C. Alginate lyases A and B are stable in the pH range of 5.0–9.0, while alginate lyase C is stable in the pH range of 5.0–7.0. Among the metal ions tested, additions of Na^{+}, K^{+}, and Mg^{2+} ions can enhance the enzyme activities while Fe^{2+}, Fe^{3+}, Ba^{2+}, and Zn^{2+} ions show inhibitory effects. The substrate specificity results demonstrate that alginate lyase C has the specificity for G block while alginate lyases A and B have the activities for both M and G blocks. It is the first report about extracellular alginate lyases with high alginate-degrading activity from *P. fluorescens*.

Keywords *Pseudomonas fluorescens* · Alginate lyase · Purification · Characterization · Enzyme activity

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Introduction

Alginate is a linear polysaccharide consisting of α-L-guluronate and its C5 epimer, β-D-mannuronate, linked together with 1,4-*O*-glycoside bonds. There are three different possible arrangements for the polysaccharide: poly-α1,4-L-guluronate, poly-β1,4-D-mannuronate, and heteropolymeric regions. The polymers can be produced by brown seaweed and certain bacteria belonging to the genera *Azotobacter* and *Pseudomonas* [1, 2]. Alginate lyases, characterized as mannuronate lyase (EC4.2.2.3) and guluronate lyase (EC4.2.2.11), catalyze the degradation of alginate by the β-elimination reaction, leading to the products with an unsaturated uronic acid at the non-reducing terminus [3].

Alginate lyases have attracted considerable attention recently due to their ability to produce alginate oligosaccharides with special chemical properties and biological activities that are widely used in food industry, biotechnology, and medicine [4–7] and have been considered in the disposal and utilization of seaweed wastes for preservation of the marine environment and recycling of organic substances due to the increasing amount of seaweed wastes in recent years [8]. Alginate lyases can also be employed in the preparation of protoplasmic from brown algae and also be expected to become promising as biochemicals for treating cystic fibrosis patients [9]. Some alginate lyases have been identified and characterized from different sources, including marine algae, marine mollusks, and bacteria [10]. However, up to now, most strains reported previously have been found to produce only a single kind of alginate lyase except alginate lyase A I-VI from *Sphingomonas* sp. Al [11, 12]and alginate lyase AlyA1-3 from *Azotobacter vinelandii* [13], and these enzymes have not been used commercially [14–20].

In this paper, we report three alginate lyases from a marine bacterium strain HZJ216, as described below, which was isolated from rotted brown seaweed in the Yellow Sea of China. After optimization of culture conditions and medium composition, the enzyme activity of the cell-free fermenting fluid is about 1,123 Umg^{-1} by ultraviolet absorption method at 235 nm. Three alginate lyases from the strain are purified, and their activities at different temperatures, pH, and substrates and in the presence of various metal ions are investigated to evaluate their ability as potential tool enzymes to degrade and utilize alginate for the purposes mentioned above.

Materials and Methods

Materials

Sodium alginate from brown seaweed (viscosity, 500 cps grade) was purchased from Qingdao Bright Moon Seaweed Group Co. Ltd. (Qingdao, China). The M and G blocks were supplied by the Institute of Food and Medicine at the Ocean University, Qingdao, China. Q-Sepharose Fast Flow and Sephacryl S-100HR were purchased from Amersham Pharmacia Biotech (Uppsala, Sweden). Polyacrylamide and molecular mass marker were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The other chemicals used in this study were of analytical grade and commercially available.

Bacterial Culture

Marine bacterium (strain HZJ216) was isolated from brown seaweed in the Yellow Sea, China. The bacterium was maintained on a slant culture. The bacteria from the slant culture were inoculated in 250-mL flasks, containing 100 mL of artificial seawater (1.5% NaCl,

0.5% $MgSO_4 \cdot 7H_2O$, 1% KCl, 0.02% $CaCl_2$, 0.01% K_2HPO_4, and 0.002% $FeSO_4 \cdot 7H_2O$, *w*/*v*) supplemented with 0.5% sodium alginate (*w*/*v*), at 26 °C with shaking at 160 rpm for 18 h. Then, the seed culture was inoculated into 200 mL of liquid medium in 500-mL flasks by 5% (*v*/*v*) inoculum size for fermenting at 26 °C with shaking at 160 rpm for 20 h. After being cultured, the bacterial cells were separated from the culture medium by centrifugation at 7,000 × *g* (4 °C) for 10 min, and the supernatant was used for purification of alginate lyases.

Identification of an Alginate-Degrading Strain HZJ216

A 16S rRNA analysis was carried out to identify the species of the isolated marine bacteria. Genomic DNA of strain HZJ216 was extracted and purified by genomic DNA purification kit (Tiangen Biotech Co., Ltd., Beijing, China). The primers used to amplify the 16S rRNA fragment were 27 F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492R (5′-GGT TAC CTT GTT ACG ACT T-3′). Polymerase chain reaction (PCR) was carried out by using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) with the following conditions for amplification: initial denaturation at 94 °C for 5 min, 35 cycles consisting of denaturation at 94 °C for 30 s, annealing at 62 °C for 1 min, and extension at 72 °C for 1 min and then a final extension at 72 °C for 5 min. The amplification products were purified and sequenced by Shanghai Sangon Biological Engineering Technology Services Co., Ltd. The 16S rRNA gene sequence of strain HZJ216 was compared with those available in the GenBank database using the BLAST program, and the nucleotide sequence was deposited to GenBank with the accession number of HQ153102. Then, the nucleotide sequences of 16S rRNA were used for phylogenetic analysis. The sequences of closely related strains were retrieved and aligned using the CLUSTAL X program. Phylogenetic tree was generated using MEGA 4.0 software package and through bootstrap neighbor-joining algorithms.

The identification of physiological and biochemical characteristics on strain HZJ216 was carried out by the methods described in *Bergey's Manual of Systematic Bacteriology*, 2nd edn., according to the results from 16S rRNA gene sequence blast and phylogenetic analysis. The test items are listed in Table 1.

Table 1 Physiological and biochemical characterization of strain HZJ216

Test items	Results
Moveability	+
Arginine dihydrolase	+
Oxidase reaction	+
Nitrate reduction	+
Denitrification	+
Fluorescent pigments	+
Trehalose use	+
Levan formation from sucrose	+
2-Ketogluconate use	+
Gelatin hydrolysis	+
L-Valine use	+
L-Proline use	+
L-Phenylalanine use	+

+ positive, − negative

Enzyme Activity Assay

Alginate lyase was spectrophotometrically assayed by an increase in absorbance at 235 nm of the reaction products (unsaturated urinates) using a 1-cm cuvette [18]. The reaction mixture was composed of 2.0 mL of 0.5% sodium alginate in 1/15 M Na_2HPO_4-KH_2PO_4 buffer (pH 7.0) and 50 μL of an appropriate amount of the enzyme. The reaction was done at 30 °C for 30 min. One unit of the enzyme activity was defined as an increase of 0.1 in absorbance of the reaction mixture at 235 nm min^{-1}. The specific activity was represented by a unit per milligram of the protein.

Enzymatic Purification

All the purification procedures of alginate lyases were carried out at below 4 °C. The culture fluid was centrifuged at 7,000 × *g* for 10 min, and 200 mL of the cell-free supernatant was obtained. The cell-free supernatant was concentrated by acetone and precipitated for 30 min. The precipitate collected by centrifugation (7,000 × *g* for 10 min) was dissolved in deionization water, and then the clear supernatant was obtained after being centrifuged for getting rid of the insoluble part.

The clear supernatant was applied onto Q Sepharose Fast Flow column (1.7 cm×35 cm, Pharmacia) equilibrated with 1/15 M Na_2HPO_4-KH_2PO_4 buffer (pH 7.0, buffer A). The protein was eluted with 100 mL of buffer A containing a linear gradient of 0 to 1 M NaCl, and the eluting fractions were collected. The flow rate was adjusted to 1.3 mL min^{-1}. The eluting process was monitored continuously by the absorbance at 280 nm, and fractions were assayed for activity against alginate. The alginate lyases were eluted at about 0.5 M NaCl. Then, the fractions containing alginate lyase activity were applied onto gel filtration chromatography Sephacryl S100-HR column (1 cm×60 cm, Pharmacia) which was equilibrated with buffer A and eluted with the same buffer. The flow rate was adjusted to 0.4 mL min^{-1}. Finally, the fractions containing alginate lyase activity were applied onto Sephadex 75 (1 cm×60 cm, Pharmacia) which was equilibrated and eluted with buffer A with the flow rate of 0.3 mL min^{-1}. Fractions were assayed for activity against alginate. Active fractions were stored at 4 °C.

Characterization of the Purified Enzymes

Determination of MW and pI

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method was employed to assess the purification of the active fractions and to determine the molecular weight (MW) of the fractions. SDS-PAGE was performed in a 0.75-mm slab gel consisting of a stacking gel (5% polyacrylamide) and a separating gel (12% polyacrylamide). Precision Protein Standards (MBI Fermentas, Hanover, USA) were used as molecular marker standards. Proteins were stained with Coomassie brilliant blue R-250. Isoelectric focusing for identifying the isoelectric point (p*I*) of the enzymes was performed with Model 111 Mini IEF Cell system (Bio-Rad, America), as described in the instruction manual.

Optimum pH and Temperature

The optimum pH values of the activity for the purified enzymes were determined using alginate solution with the following buffers before addition of 50 μL enzyme solution:

0.1 M citrate buffer (pH 3.0–6.0), 0.1 M phosphate buffer (pH 5.0–8.0), 0.1 M Tris–HCl (pH 8.0–9.0), and 0.1 M glycine–NaOH buffer (pH 9.0–11.0). Enzyme activities at various pH values were determined by the assay method as mentioned at 32 °C in the presence of 1.5% NaCl.

The effects of temperature on the activity of the purified alginate lyases were examined under the standard assay condition except that the temperature was varied from 25 to 60 °C.

Thermal Stability of Alginate Lyases

The thermal stability of the purified alginate lyases was determined under the standard assay condition after incubating purified enzyme solutions at 25 to 60 °C for 30 min, respectively.

Effects of Metal Ions on the Enzyme Activity

Fifty-microliter enzyme solution was mixed with 50-μL metal ions solutions (50 mM) in 30 min at 4 °C, and then the mixture was assayed for activity against alginate under the standard assay condition in the presence of 1.5% sodium alginate. The mixture without any metal ion was used as the control with the corresponding enzyme activity designated as 100%.

Determination of Substrate Specificities

The purified enzymes were incubated with sodium alginate and two types of homopolymeric substrate, M and G blocks, to elucidate the substrate specificities by ultraviolet absorption method. M and G blocks were supplied by the Institute of Food and Medicine at the Ocean University, Qingdao, China, which were prepared from brown seaweed alginate as described by Haug et al. [21]. In the two latter cases, the reaction was carried out as described previously by replacing alginate with M or G block of 0.1% concentration.

Results and Discussion

Identification of an Alginate-Degrading Strain HJZ216

16S rRNA of the strain HZJ216 was amplified by PCR, and 1,430 bp of 16S rRNA was sequenced. The subsequent BLAST analysis on sequence similarity revealed that the closest relatives on the strain HZJ216 were *Pseudomonas* sp. (max identity is 99.9%). The nucleotide sequence of the 16S rRNA of *Pseudomonas* sp.HZJ216 was deposited to GenBank (accession number, HQ153102). Phylogenetic analysis based on the 16S rRNA gene sequencing indicates that the bacterium belongs to *Pseudomonas* sp. and shows a high similarity with *Pseudomonas fluorescens* and *Pseudomonas syringae* (see Fig. 1) Further identification of physiological and biochemical characteristics on strain HZJ216 was performed between *P. fluorescens* and *P. syringae* (see Table 1). The identification method is the same as that reported by D. Choi et al. [22]. The result demonstrated preliminarily that the strain HJZ216 belongs to *P. fluorescens*. Compared with other *P. fluorescens* strains reported previously, *P. fluorescens* HZJ216 is the only bacterium producing extracellular alginate lyases and contributing significantly to alginate degradation except *P. fluorescens* NCIMB10525 [23] which produced intracellular alginate lyase, while other strains all contributed to the process of alginate biosynthesis [24–26].

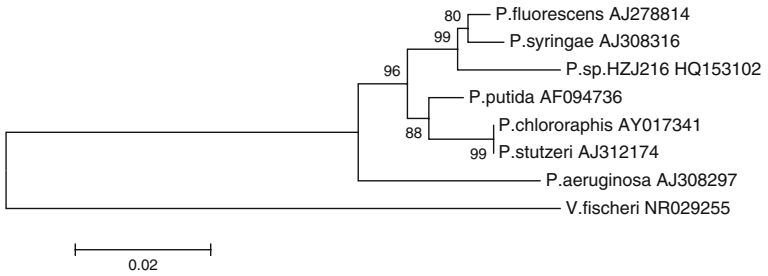

Fig. 1 Neighbor-joining phylogenetic tree based on 16S rRNA gene sequence. It showed the relationships among strain HZJ216 (GenBank accession no. 153102) and closely related members of the genus *Pseudomonas*. *Numbers* at nodes are levels of bootstrap support (%) based on analyses of 500 resample datasets. *Vibrio fischeri* (GenBank accession no. NR029255) was used as outgroup. *Bar*, 0.02 nucleotide substitutions per position

Enzyme Activity Assay

The enzyme activity of cell-free fermenting fluid after being centrifuged at 7,000 × *g* for 10 min is about 1,123 Umg^{-1} by ultraviolet absorption method at 235 nm, which is much higher than previously reported alginate lyases from *Vibrio* sp. YWA (0.017 Umg^{-1} after being converted to the same assay condition) [14], *Pseudomonas* sp. QD03 (197.7 Umg^{-1}) [15], and *Vibrio* sp. YKW-34 (148 Umg^{-1}, which is calculated according to the results) [16] measured by the ultraviolet absorption method. The cell-free fermenting fluid of alginate lyase in this study showed a high enzyme activity, and to develop it as a potential enzyme preparation, which has never been reported till now, to apply in the process of industry can be considered.

Enzymatic Purification

Three alginate lyases from *P. fluorescens* HZJ216 were purified preliminarily by applying ion-exchange chromatography Q-Sepharose Fast Flow, and active fractions were collected in peak I (see Fig. 2a). Peak I was applied on gel filtration chromatography Sephacryl S-100HR, and active fractions were collected in peaks II and III (see Fig. 2b). Peak III was applied on Sephadex 75, and active fractions were collected in peaks IV and V (see Fig. 2c). The purity of each enzyme activity peak was verified by SDS-PAGE. Three electrophoretic homogeneity enzyme peaks were shown in the Fig. 2a–c. Peaks II, IV, and V were in accordance with alginate lyase A, B, and C, respectively. Q-Sepharose Fast Flow is a kind of strong anion exchange chromatography and is usually used to purify alginate lyase with lower p*I* values [10], which is coincident with the p*I*s of alginate lyases A, B, and C determined later in this study.

Characterization of the Purified Enzymes

Determination of the MW and pI

Alginate lyases A, B, and C are monomeric enzymes with a molecular mass of 60.25, 36.3, and 23 kDa, respectively, determined by SDS-PAGE (see Fig. 3). Previous studies have not identified alginate lyases in *Pseudomonas* with the identical molecular weights as reported here. The only exception is a 36 kDa enzyme identified by M. Miyazaki et al. [27], which is

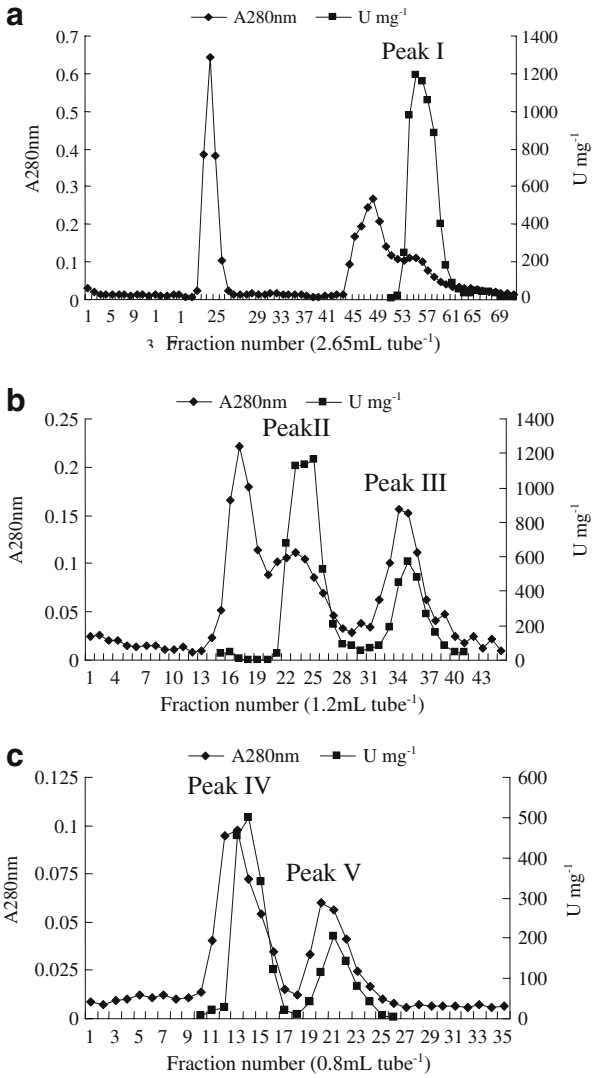

Fig. 2 Purification of alginate lyases from *P. fluorescens* HZJ216. During the procedure, enzyme activity (*squares*) and absorption value of protein (*triangles*) were measured. **a** Preliminary purification of alginate lyases by Q Sepharose Fast Flow. The flow rate was adjusted to 1.3 mL min^{-1}.The enzymes were eluted by 1/15 M Na_2HPO_4-KH_2PO_4 buffer (pH 7.0) using a linear NaCl gradient (0–1 M), and the elute fractions were collected. **b** Purification of alginate lyase A by Sephacryl S-100HR.The flow rate was adjusted to 0.4 mL min^{-1}.The enzymes were eluted by 1/15 M Na_2HPO_4-KH_2PO_4 buffer (pH 7.0), and the elute fractions were collected. **c** Purification of alginate lyases B and C by Sephadex 75. The flow rate was adjusted to 0.3 mL min^{-1}.The enzymes were eluted by 1/15 M Na_2HPO_4-KH_2PO_4 buffer (pH 7.0), and the elute fractions were collected

similar to the MW of alginate lyase B (36.3 kDa). However, alginate lyase B identified in this study could degrade both M and G blocks, while the 36-kDa enzyme in the earlier study was found to degrade G block only. Thus, the alginate lyases from *P. fluorescens* HZJ216 reported here seem to represent three novel alginate lyases.

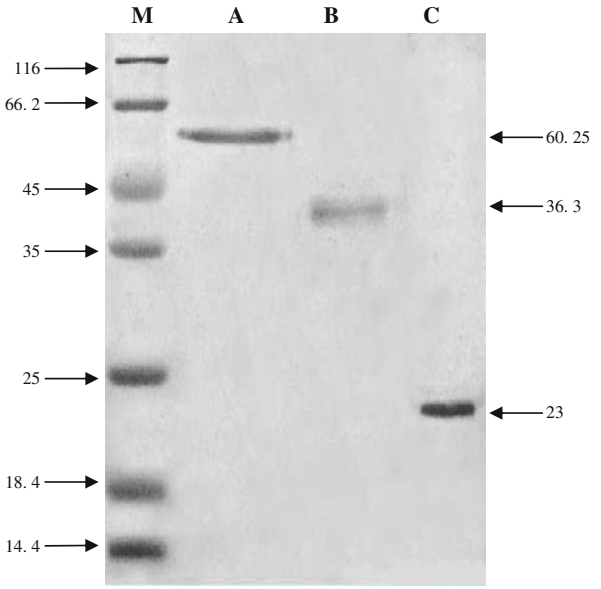

Fig. 3 SDS-PAGE of purified alginate lyases from *P. fluorescens* HZJ216. *Lane M*, the low molecular weight marker; *lane A*, alginate lyase A-containing fraction after Sephacryl S-100HR; *lane B*, alginate lyase B-containing fraction after Sephacryl S-100 HR and Sephadex 75; *lane C*, alginate lyase C-containing fraction after Sephacryl S-100HR and Sephadex 75. The standard protein markers for SDS-PAGE are: β-galactosidase, 116 kDa; bovine serum albumin, 66.2 kDa; ovalbumin, 45 kDa; lactate dehydrogenase, 35 kDa; REase Bsp981, 25 kDa; β-lactoglobulin, 18.4 kDa; lysozyme,14.4 kDa

The purified alginate lyases A, B, and C turned out to be negative charged protein with p*I*s of 4, 4.36, and 4.59, respectively, which is coincident to the investigation of Wong et al. [10] who reported that p*I*s for the majority of the enzymes from marine bacteria are quite low, such as alginate lyases from *Alteromonas* sp. strain H-4 (p*I* 4.7) [28] and from *Alteromonas* sp. strain no. 272 (p*I* 3.8) [18], but different from those from *Corynebacterium* sp. (p*I* 7.3) [29] and *Enterobacter* sp.(p*I* 8.9) [30]. This property could be used to direct the implementation of the enzymes as acidic enzymes.

Optimum pH and Temperature

The pH profiles of the alginate lyases are shown in Fig. 4. The maximum enzyme activities of the three lyases were observed at pH 7.0 commonly. Alginate lyases A and B showed relatively high enzyme activities around pH 5.0–9.0. The alginate lyase C was stable in the range of pH 5.0–7.0, followed by an abrupt decrease.

The temperature profile of three alginate lyases is shown in Fig. 5. The optimum temperatures of the three alginate lyases were 35 °C commonly, and the lyases possessed more than 60% activity in the range of 25 to 40 °C except alginate lyase C at 25 °C which was 53.89% activity, but the activities decreased above 40 °C. Compared with other alginate lyases reported, the optimal temperature of the lyases in this study was similar to those of alginate lyases [18, 31, 32] and lower than that of alginate lyases [16, 20, 33].

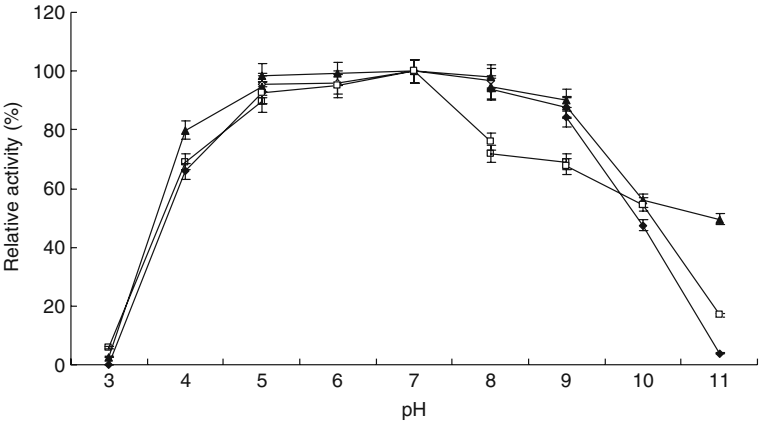

Fig. 4 The optimal pH of alginate lyases A (*diamonds*), B (*triangles*), and C (*squares*). The enzyme activities were measured at 32 °C in the presence of different buffers (0.1 M): citrate buffer pH 3.0–6.0, phosphate buffer pH 5.0–8.0, Tris–HCl pH 8.0–9.0, and glycine–NaOH pH 9.0–11.0. Results represent the means of triplicates, and *bars* indicate standard deviation

Thermal Stability of Alginate Lyases

The thermal stability profile of three alginate lyases is shown in Fig. 6. The alginate lyase A possessed more than 70% activity after incubation at 40 °C for 30 min and inactivated gradually as the temperature increased, while alginate lyases B and C only possessed about 59% and 55% activities after incubation at 35 °C for 30 min and inactivated gradually as the temperature increased. The result indicates that alginate lyase A is much more stable at higher temperature than alginate lyase B and alginate lyase C.

Effects of Metal Ions on Enzyme Activity

Effects of metal ions on the purified alginate lyases activities are shown in Table 2. Na^+ seems to be essential for the activity of all the three lyases, as no enzyme activity was

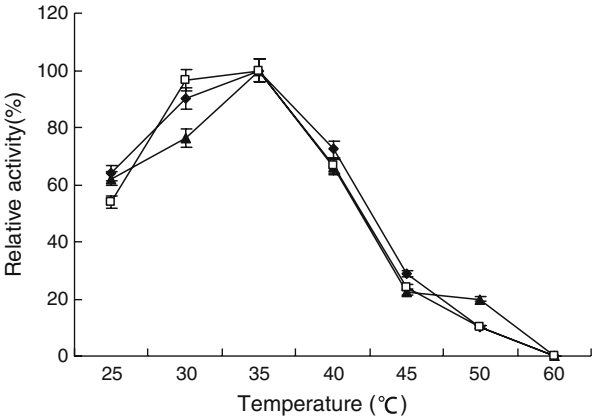

Fig. 5 The optimal temperature of alginate lyases A, B, and C. The enzyme activities for alginate lyase A (*diamonds*), alginate lyase B (*triangles*) and alginate lyase C (*squares*) were measured at 25, 30, 35, 40, 45, 50, and 60 °C in the Na_2HPO_4-KH_2PO_4 buffer (pH 7.0). The values are given as mean±SD, n=3

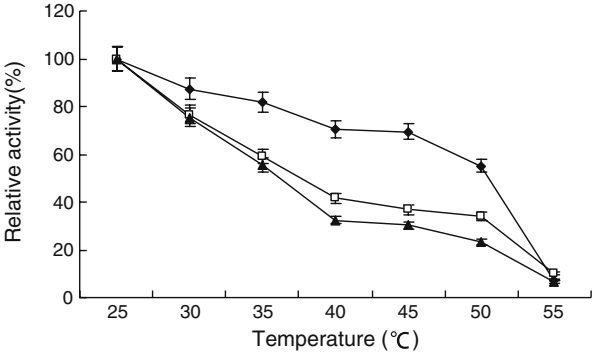

Fig. 6 The thermal stability of alginate lyases A, B, and C. The enzyme activities for alginate lyase A (*diamonds*), alginate lyase B (*triangles*), and alginate lyase C (*squares*) were measured under the standard assay condition after incubating 100 μL enzyme solutions at 25 to 60 °C for 30 min. The values are given as mean±SD, $n=3$

observed without the presence of Na^+ (data not shown). Metal ions such as Mg^{2+}, K^+, and additional Na^+ were found to enhance the enzyme activities for all the three lyases at different extents. The property of K^+ activating the purified three enzymes in the presence of Na^+ is similar to alginate lyase from *Vibrio* sp. YKW-34 [16], and that of Mg^{2+} was observed in alginate lyase from marine bacterium *Alteromonas* sp. H-4 [34]. The addition of calcium ions was reported to markedly enhance the reaction rate for the M block [34, 35] and enhance pG-degrading alginate lyase from *Pseudomonas* sp. strain F6 [27]. In our study, 50 mM Ca^{2+} could increase the activities of lyases A and B to 117% and 131%, respectively, but no obvious enzyme activity for alginate lyase C was observed; Alginate lyases A and B could degrade M and G blocks, but alginate lyase C only degraded G block.

Table 2 Effects of metal ions on relative activities of alginate lyases A, B, and C

Metal ions	Concentration (mM)	Relative activity (%)[a]		
		Alginate lyase A	Alginate lyase B	Alginate lyase C
$CaCl_2$	50	117±1.65	131.4±1.50	95.25±1.12
$CuSO_4$	50	124±1.60	130.9±1.89	92.49±1.47
$MgSO_4$	50	116±1.58	121.2±1.75	113.6±1.95
KCl	50	132±1.04	110.6±1.45	105.7±1.55
$FeSO_4{\cdot}7H_2O$	50	0±0.38	0±0.51	0±0.45
NaCl	50	109±0.58	106.3±0.92	103.15±0.73
$ZnCl_2$	50	10.28±0.92	14.16±1.20	12.50±1.25
$BaCl_2$	50	89.53±1.77	43.58±1.82	42.15±0.96
$CoCl_2$	50	66.73±1.83	65.93±1.65	28.92±1.23
LiCl	50	94±1.52	109.7±1.56	97.96±1.52
$FeCl_3$	50	55.14±1.38	24.78±1.24	0±0.21
Control	–	100±0.25	100±0.45	100±0.33

Metal ions: reactions were carried out for 30 min at 32 °C and H_2O in the presence or absence (control) of the above compounds. The activity of the control was relatively designated as 100%

[a] Values represent the mean±the standard deviation ($n=3$)

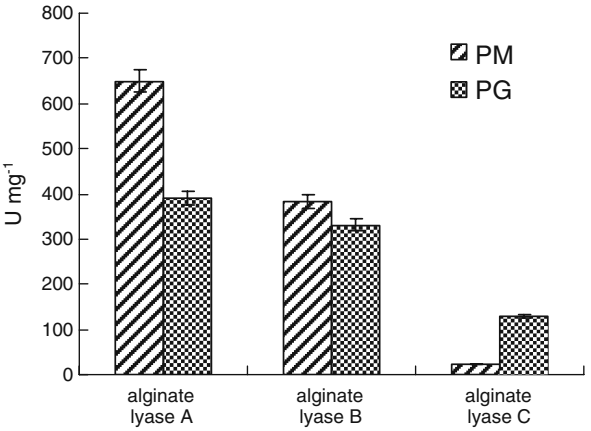

Fig. 7 Substrate specificities of alginate lyases A, B, and C. The enzyme activities for alginate lyase A, alginate lyase B, and alginate lyase C were evaluated in enzyme reaction system at 32 °C for 30 min with M block (*stripy*) or G block (*latticed*) of 0.1% concentration as substrate. The concentration of substrate was 1 mg mL^{-1}. The data represent the mean±the standard deviation (n=3)

Thus, it seems that Ca^{2+} might have the function of enhancing the reaction rate for the M block but had little effect on the reaction with G block, which is similar with the report from B. Larsen et al [34], while Fe^{2+} inhibited all lyases totally and Fe^{3+}, Ba^{2+}, and Zn^{2+} showed inhibitory effects on the three lyases in varying degrees.

The results indicate that Na^{+}, K^{+}, and Mg^{2+} can enhance the activities of alginate lyases A and B, and Ca^{2+} can enhance the activity of alginate lyase C though the details are still not known. Perhaps, the cations act as cofactors in the active sites of the enzyme or function through decreasing the surface density of the substrate charge and weakening the ionic interaction between alginate and enzyme [10].

Determination of Substrate Specificities

The substrate specificity of the lyases was analyzed by incubating each enzyme with equal molar amounts of homopolymeric substrate, M and G blocks of alginate, followed by measurements of DNS method. The results (see Fig. 7) showed that alginate lyase A could degrade M and G blocks, and the enzyme activity acting on M block (651 Umg^{-1}) was much more than that of G block (390 Umg^{-1}); for alginate lyase B, the enzyme activities on M block (382 Umg^{-1}) were slightly higher than that of G block (330 Umg^{-1}), and there was no obvious substrate specificity difference between them. This seems to be similar to the cases of alginate lyase A1-I from *Sphingomonas* sp. Al [11], AlyA3 from *A. vinelandii* [13], the alginate lyase from *Alteromonas* sp. strain H-4 [28], and the alginate lyase from *Bacillus circulans* 1351 [34], though the detailed degrading mechanism remains unknown. Alginate lyase C could degrade G block (129 Umg^{-1}), preferentially with no significant activity on M block (21.77 Umg^{-1}), which is similar to those found in *Klebsiella aerogenes* that are representatives of poly-G lyases [36, 37] and alginate lyase from *Vibrio* 510-64 [32]. All of the three lyases could act on sodium alginate, and the enzyme activities were about 653, 467, and 233 Umg^{-1} for alginate lyases A, B, and C, respectively. The different substrate specificities for the three enzymes can be utilized to produce special segment oligosaccharide with bioactivity from alginate.

Conclusions

An alginate-lyase-producing bacterial strain HJZ216 was identified preliminarily as *P. fluorescens*. Three novel purified alginate lyases from the strain showed maximal enzyme activity at pH 7.0 and 35 °C in the presence of Na^+, K^+, and Mg^{2+} ions and showed high stability in a wide range of pH and below 35 °C. This is the first report about extracellular alginate lyases with high alginate-degrading activity from *P. fluorescens*, and the lyases could be considered as potential tool enzyme or enzyme preparation to be implemented due to their high enzyme activity and different substrate specificity.

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