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Enhancing the Alginate Degrading Activity of *Streptomyces* sp. Strain M3 Alginate Lyase by Mutation

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A polyguluronate-specific lyase from *Streptomyces* sp. strain M3 has been previously cloned and characterized. In this study, the M3 alginate lyase gene in the pColdI vector was mutated by site-directed mutagenesis and random mutagenesis to enhance the alginate degrading activity. Six mutants were obtained: Ser25Arg, Phe99Leu, Asp142Asn, Val163Ala, Lys191Glu, and Gly194Cys. Phe99Leu and Lys191Glu mutants completely lost their alginate lyase activity, whereas the alginate degrading activity of Gly194Cys mutant increased by nearly 10 fold. The 3-D protein structure of M3 alginate lyase, which was constructed using the Swiss-Model automodeler, was also compared to the crystal structure of another alginate lyase. A mutated glycine residue was positioned between Gly193 and Tyr195 of the C-terminal conserved sequence, YFKAG**X**YXQ. A phenylalanine residue (at position 99) and a glycine residue (at position 194) mutated in this study were distant from the active site, but the degrading activity was strongly affected by their mutation.

Key words : Alginate lyase, polyguluronate-specific lyase, mutation

Introduction

Alginates are widely used in the food, cosmetics, pharmaceuticals and biotechnological purposes such as cell immobilization [23]. Alginate is linearly composed of (1-4)-linked β-D-mannuronate (M) and its C5-epimer α-L-guluronate (G). This acidic polysaccharide is produced by brown algae like *Laminaria* and *Undaria* [6] and some bacteria belonging to the genera *Azotobacter* and *Pseudomonas* [4]. Alginate from brown algae has three different blocks: poly-β-D-mannuronate block (polyM block), poly-α-L-guluronate block (polyG block) and hetero-polymeric block (polyMG block) (Fig. 1).

Various alginate lyases have been detected in algae, bacteria and marine organisms [13,18,20,23-25,]. These alginate lyases catalyze β-elimination reactions by releasing unsaturated oligosaccharides with 4-deoxy-α-L-erythro-hex-4-enopyranosyluronic acid at their non-reducing end [8,12]. Alginate lyases have their own substrate specificity, and they can be classified based on their preferential cleavage site on alginate substrate [26]. Many alginate lyases possessing a preference for polyG-block or polyM-block have been reported. PolyG-specific alginate lyase was cloned from *Corynebacterium* sp. strain ALY-1, *Sphingomonas* sp. strain A1 and *Streptomyces* sp. strain ALG-5. Some polyM-specific alginate lyases and polyMG-specific alginate lyase were also cloned and characterized from *Pseudomonas aeruginosa* and *Azotobacter* sp., respectively [5,23,26]. Those endotype alginate lyases depolymerize alginate to unsaturated di-, tri-, tetra-saccharides and so on. In addition to endotype alginate lyases, exotype alginate lyases were found from *Sphingomonas* sp. strain A1 and *Agrobacterium tumefaciens* C58 [14-16]. They are responsible for the complete degradation of alginate oligosaccharides into constituent unsaturated monosaccharides. Recently, we have cloned and characterized a polyG-specific alginate lyase from a marine bacterium, *Streptomyces* sp. strain M3 that degrades G-rich region of alginates [10,11]. It releases unsaturated di-, tri- and tetra-saccharides from alginate. We have also obtained polyM-specific lyase and oligoalginate lyase from *Pseudomonas* sp. strain KS-408 [9] and *Sphingomonas* sp. strain MJ-3, respectively [19]. Enrichment of the pools of the recombinant alginate lyases with different substrate specificities is valuable for expanding the utilization of alginate.

In this study, polyG-specific alginate lyase from *Streptomyces* sp. strain M3 was mutated site-specifically and randomly for enhancing the alginate degrading activity. The mutated amino acid positions of M3 alginate lyase mutants were analyzed by using homology model and also compared to crystal structures of other alginate lyases.

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Materials and Methods

Strains, plasmids, mutagenesis and PCR

The bacteria for cloning and expression of M3 alginate lyase were *E. coli* DH5α and BL21(DE3), respectively. The used vector were T-blunt vector (Solgent, Korea) and pColdI vector (Takara, Japan). The site directed mutagenesis of M3 alginate lyase gene was generated by using Quikchange II site-directed mutagenesis kit (Stratagene Co, USA) with various primers shown in Table 1. The recombinant pColdI/M3 lyase plasmid obtained previously was used as the template for site-directed mutagenesis [3]. The annealing temperature on mutagenic PCR operation conditions were as follows: 30 sec at 95°C, followed by 12-16 cycles of 30 sec at 95°C, 30 sec at 55°C, 1 min at 72°C, ending with 2 min on ice to cool the reaction to 37°C. Mutated DNA was treated by DpnI restriction enzyme for degradation of methlated template. Random mutagenesis of M3 alginate lyase gene was also performed by using GeneMorph II random mutagenesis Kit (Stratagene Co, USA). The primers were designed to contain M3 alginate lyase ending gene, restriction site plus 15 bases corresponding to pColdI vector sequence. The mixture of PCR product and restricted pColdI vecter was transformed to competent cell by using Cold Fusion cloning kit (System Biosciences, USA). The purified plasmid DNA was transformed into *E. coli* BL21(DE3) and cultured on the LB agar plate containing 50 μg/ml of ampicillin. To screen the mutant which had enhanced alginate lyase activity by random mutagenesis, colonies were inoculated to LB broth in 96 well plates and alginate lyase genes were overexpressed by induction of 1 mM IPTG. The cells were broken by freezing and thawing with 20 mg/ml lysozyme. The alginate lyase activity was assayed by TBA method [22].

Homology modelling and analysis of M3 alginate lyase protein

The conserved features of alginate lyase from *Streptomyces* sp. strain M3 were compared with other alginate lyases by multiple sequence alignment by using BioEdit program. A homology model for M3 alginate lyase was constructed

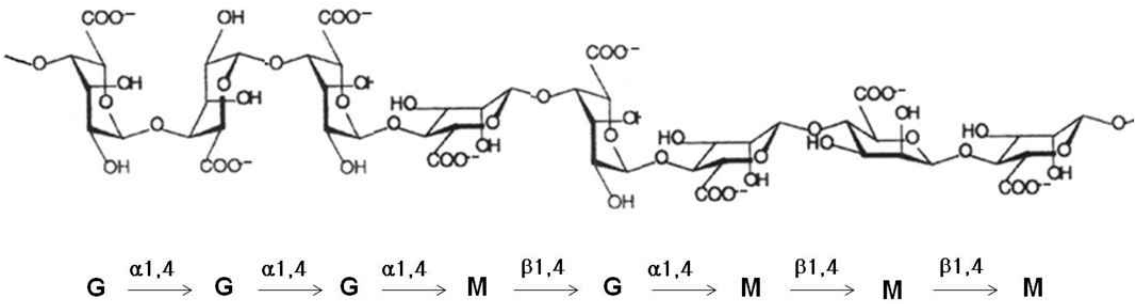

Fig. 1. Chemical structure of alginate. Alginate is a linear acidic polysaccharide in which β-D-mannuronate (M) and its C-5 epimer, α-L-guluronate (G), are covalently linked with glycosidic α(1-4) or β(1-4) bond.

Table 1. Primers used for mutagenesis

Primer name	Sequence
For site directed mutagenesis	
5M3F-V163A	CGAGGGCAAGTTCG**C**CGTCAGCGGCGGCC
3M3R-V163A	GGCCGCCGCTGACG**G**CGAACTTGCCCTCG
5M3F-K191E	CCGGCAACTACTTC**G**AGGCCGGCGGCTAC
3M3R-K191E	GTAGCCGCCGGCCT**C**GAAGTAGTTGCCGG
5M3F-F99L	CCACACCATGACCTT**A**CGGGAGGCGTTCAAC
3M3F-F99L	GTTGAACGCCTCCCG**T**AAGGTCATGGTGTGG
For random mutagenesis	
5Cold-M3MF-F	GAAGGTAGGCATATGGCCGCCCCGTGCGACTACCC
3Cold-M3-R	GCAGGTCGACAAGCTTCAGGAGTGGGTGACCAGCAG

-Bold letter means a substituted base for site-directed mutagenesis.
-Underlined sequence means restriction site.

based on X-ray crystallographic structure of alginate lyase (pdb code: 1UAI) from *Corynebacterium* sp. strain ALY1. Homology modelling was carried out using Swiss-Model, protein automodeling server [1]. The 3-D structure model of *Streptomyces* sp. strain M3 alginate lyase was visualized by using RasMol (http://www.umass.edu/microbio/rasmol/) and Python Molecule Viewer (PMV-1.5.4, http://mgltools.scripps.edu/).

Enzyme assay of alginate lyase

The enzyme solution was added into 1 ml of 20 mM phosphate buffer (pH 7.2) containing 0.2% (w/v) sodium alginate solution and incubated at 37°C for 10 min. The reaction mixture was heated to remove the enzyme activity for 10 min. The lyase activity was assayed by the TBA method [22].

Purification of M3 alginate lyase

For protein expression of M3 alginate lyase, the recombinant cells were cultured at 15℃ for 24 h with 1 mM IPTG [10]. The cells were harvested and sonicated in 50 mM potassium phosphate buffer (pH 7.2) containing 300 mM NaCl, 10 mM imidazole, 10% (v/v) glycerol, 0.5% (v/v) Triton X-100, 1 mg/ml lysozyme and 1 mM PMSF by using ultrasonicator Vibra Cell CX400 (Sonics & Materials Inc, USA). The cell homogenate containing the expressed $(His)_6$-tagged M3 alginate lyase was loaded on Ni-Sepharose column equilibrated with 20 mM phosphate buffer (pH 7.2) and 500 mM NaCl. The column was washed with the same buffer containing 20 mM imidazole, and the bound protein was eluted with the same buffer containing 300 mM imidazole. The protein fraction eluted with 300 mM imidazole was clarified to have alginate lyase activity by assaying with TBA method. Excess imidazole and NaCl were then removed using HiTrap desalting column by using 20 mM phosphate buffer and 150 mM NaCl. Protein concentration was calculated by measuring at 595 nm using Bradford method [2]. For evaluation of protein expression, the recombinant cells were cultured with and without IPTG induction, and the SDS-PAGE electrophoresis was carried out. The cells and lysates were boiled with Laemmli buffer at 95°C for 10 min, sonicated for DNA disruption and loaded into 12% SDS-polyacrylamide gel. The proteins on the gel were visualized by Coomassie staining.

Results and Discussion

Multiple sequence alignment analysis and crystal structure of PL-7 family

Previously obtained M3 alginate lyase protein (GenBank accession No.: ACN56743) was analyzed by multiple sequence alignment with other alginate lyase included in PL-7 family for homology modelling (Fig. 2). Protein sequence

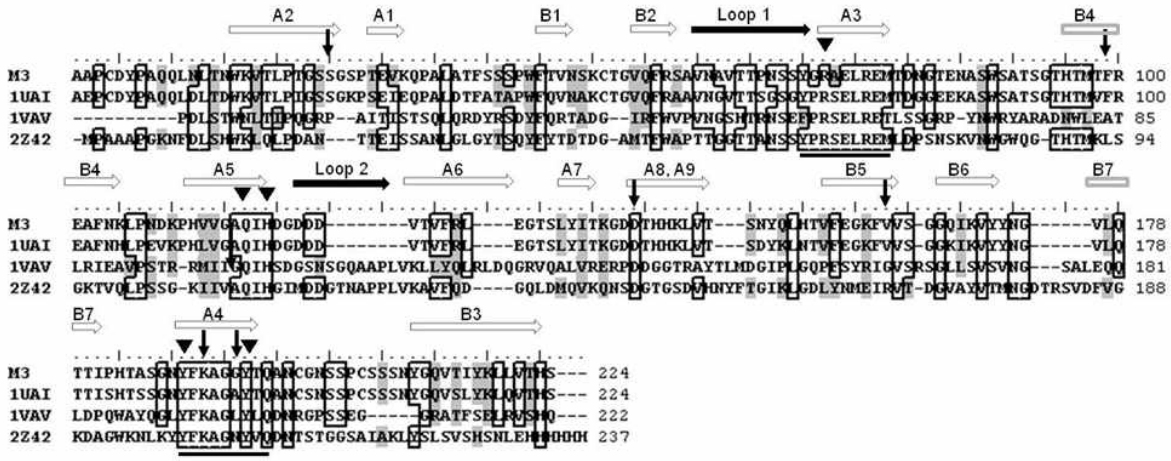

Fig. 2. Multiple sequence alignment of PL-7 alginate lyase for comparison of 3-D structure. 1UAI, 1VAV and 2Z42 are the PDB Codes of *Corynebacterium* sp. ALY-1 alginate lyase ALY-PG, *P. aeruginosa* protein PA1167 and *Sphingomonas* sp. A1 alginate lyase A1-II', respectively. The identical amino acid residues are outlined and similar amino acid residues are boxed in pale shaded. Triangles and downward arrows above the sequences indicate the residues that are the possible active sites and the mutated residues, respectively. Underlined sequences indicate conserved YXRSELREM sequence in N-terminal region and YFKAGXYXQ sequence in C-terminal region in PL-7 family. Opened and closed arrows on the sequence indicate β-strand secondary structure and loops.

without signal peptide of M3 alginate lyase was multiple-aligned with three proteins, alginate lyase ALY-PG (pdb code: 1UAI) of *Corynebacterium* sp. strain ALY-1, protein PA1167 (pdb code: 1VAV) of *P. aeruginosa* and alginate lyase and A1-II′ (pdb code: 2Z42) of *Sphingomonas* sp. strain A1, which their crystal structures were published [18,26]. M3 alginate lyase had high similarity with ALY-PG (81.9% identity) but low similarity with A1-II′ (31.9% identity] and P1167 (31.2% identity). There were very low sequence identities of 37.0%, 33.6% and 29.7% between A1-II′ and P1167, ALY-PG and A1-II′, and ALY-PG and PA1167, respectively. But the crystal structures of ALY-PG, A1-II′ and PA1167 had similar foldings as β-jelly sandwich (Fig. 3). Alginate lyases beloning to polysaccharide lyase group could be structurally classified to seven families, PL-5 ($(\alpha/\alpha)_6$ barrel) [26], PL-6 (parallel β-helix) [7], PL-7 (β-jelly roll) [18], PL-14 (glove-like β-jelly roll) [17], PL-15 (pocket-like structure involving an α/α barrel + anti-parallel β-sheet-fold) [15], PL-17 (another pocket-like structure involving an α/α barrel + anti-parallel β-sheet-fold) [19] and PL18 (another β-jelly roll) [20]. ALY-PG, A1-II′ and PA1167 involved in a PL-7 family but they showed different substrate specificities. ALY-PG and A1-II′ preferred to degrade poly-G block whereas PA1167 degraded poly-MG block preferably. As shown as Fig. 2, Arg72, Gln117, His119, Tyr189 and Tyr195 residues of M3 alginate lyase were strictly conserved in an active site like ALY-PG, A1-II′ and PA1167 (expressed by triangle). The overall structures of PL-7 family were composed of a few short helices and two large β-sheets. Sheet A consists of nine β-strands and sheet B is formed with seven β-strands, all of which use an antiparallel arrangement, except that a break occurs in the β-structure in the connecting loop between A8 and A9 (Fig. 3A-3C) [26]. The amino acid residues of an active site were positioned on A3 (Arg, R), A4 (two Tyr, Y) and A5 (Gln, Q and His, H) (Fig. 3D-3F). Yamasaki et al. [26] reported that two flexible loops (a lid and a tungue) in family PL-7 enzymes are necessary for a long linear substrate binding. They suggested that A1-II′ may be a cleft-type enzyme with two flexible loops that cover the active cleft and form a tunnel (Fig. 3B). ALY-PG (Fig. 3A) and PA1167 (Fig. 3C) are predicted to use open conformations.

Mutation and enzyme activity of M3 alginate lyase

Recombinant M3 alginate lyase from *Streptomyces* sp.

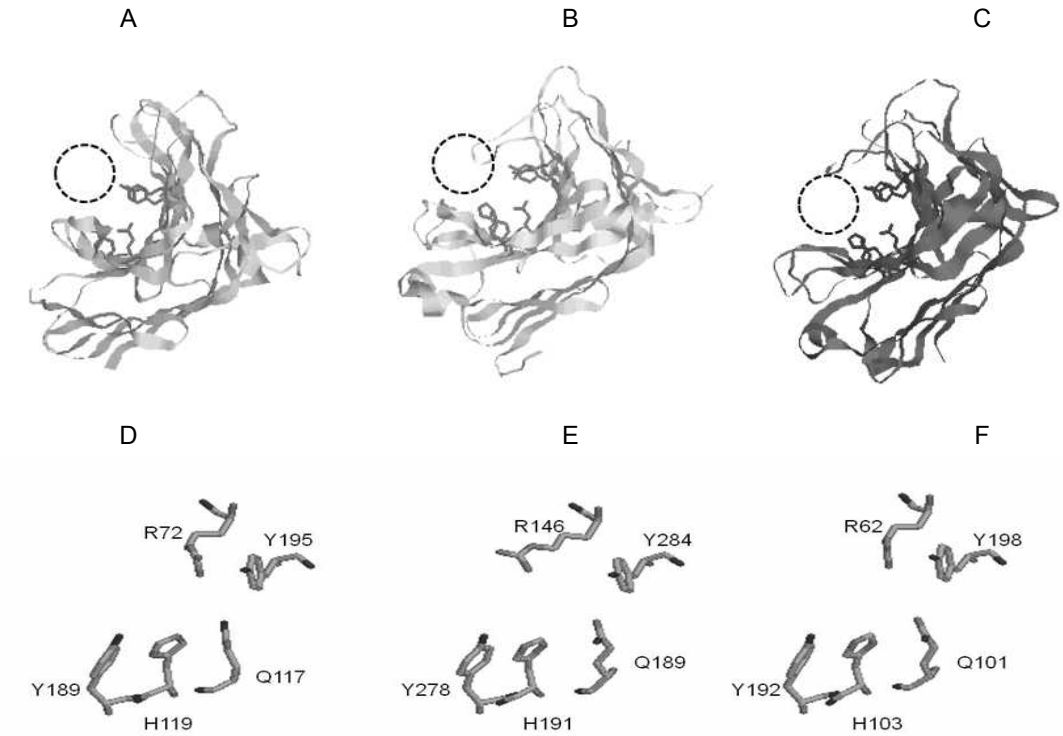

Fig. 3. Overall crystal structure of family PL-7 enzymes. A and D, ALY-PG of *Corynebacterium* sp. ALY-1 (PDB code; 1UAI). B and E, A1-II′ of *Sphingomonas* sp. A1 (PDB code; 2Z42). C and F, *P. aeruginosa* protein PA1167 (PDB code; 1VAV). Amino acid residues consisted of active site are represented with wireframe. Dotted circle indicates the position of alginate substrate. Structures are visualized by using a RasMol program.

strain M3 showed polyG-specific alginate lyase activity, previously [10,11]. When ALG-5 alginate lyase (GenBank accession No. ABS59291) was mutated Leu at 134 position to Phe, it lost alginate degrading activity (data not shown). Both ALG-5 and M3 alginate lyase from different strains of *Streptomyces* sp. showed similar substrate specificity preferable to cleave G-G and G-M linkage and second structure similarity. To enhance alginate degrading activity, M3 alginate lyase gene was mutated by site-specific or random mutagenesis. Six mutants, Ser25Arg, Phe99Leu, Asp142Asn, Val163Ala, Lys191Glu and Gly194Cys were obtained and their activities were compared to wild-type of M3 alginate lyase (Fig. 4). Mutants of Phe99Leu, Val163Ala and Lys191Glu lost their alginate degrading activities almost completely. Mutations of Asp142Asn showed slightly increase of activity. But, the catalytic acivity of Gly194Cys mutant increased 10 fold compared to wild-type alginate lyase. Fig. 5 showed that the low activities were not related to reduced expression levels, because the enzymes were expressed in amounts similar to the wild type enzyme.

Expression and partial purification of M3 alginate lyase

After the recombinant *E. coli* cultured with 1 mM IPTG induction, M3 alginate lyase wild-type and Gly194Cys mutant fused with $(His)_6$-tag were purified from cell lysates by Ni-Sepharose column. As shown as Fig. 6, both wild-type and Gly194Cys mutant overexpressed their recombinant alginate lyases very well (lane 1 and 6). When the protein profile of the lysates (lane 2 and 7) were compared, the lysate of Gly194Cys mutant (lane 7) showed to have alginate lyase protein higher proportion than that of wild-type (lane 2). This meant that alginate lyase of Gly194Cys mutant was produced to soluble form more than that of wild type. The reason why lane 8 (washing fraction) had alginate lyase protein was thought that the amounts of protein was higher than binding capacity of column. For the effects of pH and temperature on alginate degrading activity, there was no significant difference between wild-type and Gly194Cys mutant of M3 alginate lyase (data was not shown). As a result, Gly194Cys mutant was overexpressed more soluble and had higher activity than the wild type.

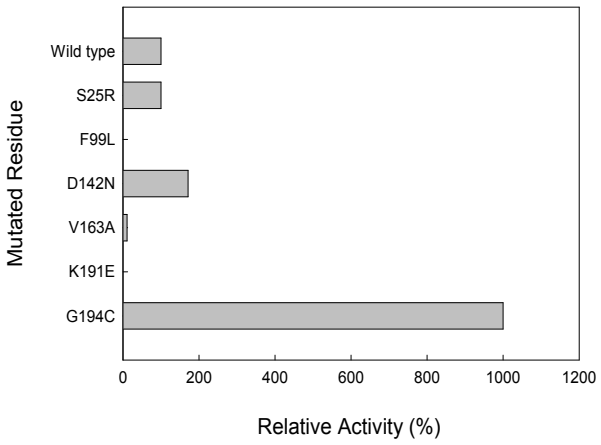

Fig. 4. Comparison of alginate degrading activity of M3 alginate lyase mutants on the wild type M3 alginate lyase. In this figure, the mutants of Ser25Arg, Phe99Leu, Asp142Asn, Val163Ala and Gly194Cys were demonstrated as S25R, F99L, D142N, V163A and G194C. The numbers indicate the positions of mutated residues.

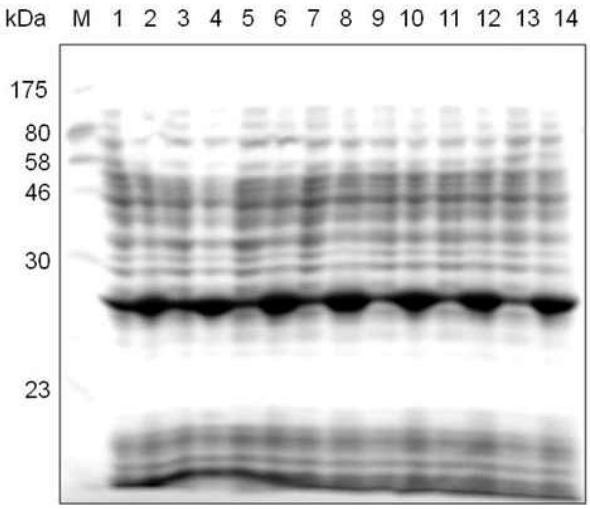

Fig. 5. SDS-PAGE analysis of recombinant cells with wild-type and mutant M3 alginate lyase. After recombinant cells were cultured until OD_{600}=0.6 and IPTG was added to overexpress the recombinant alginate lyase. 12% SDS-polyacrylamide gel was used for seperation of proteins. M: standard proteins. Lane 1 and 2: wild type. Lane 3 and 4: Ser25Arg mutant. Lane 5 and 6: Phe99Leu mutant. Lane 7 and 8: Asp142Asn mutant. Lane 9 and 10: Val163Ala mutant. Lane 11 and 12: Lys191Glu mutant. Lane 13 and 15: Gly194Cys mutant. Lane 1, 3, 5, 7, 9, 11 and 13: cells without induction. Lane: 2, 4, 6, 8, 10, 12 and 14: cells with induction.

Homology modelling and analysis of structure

Wild-type and three mutants of M3 alginate lyase were homology-modelled on the crystal structure of alginate lyase (ALG-PG, pdb code; 1UAI) from *Corynebacterium* sp. ALY-1 by using Swiss-model automodeller (Fig. 7). When the model of M3 alginate lyase was superimposed to the structures

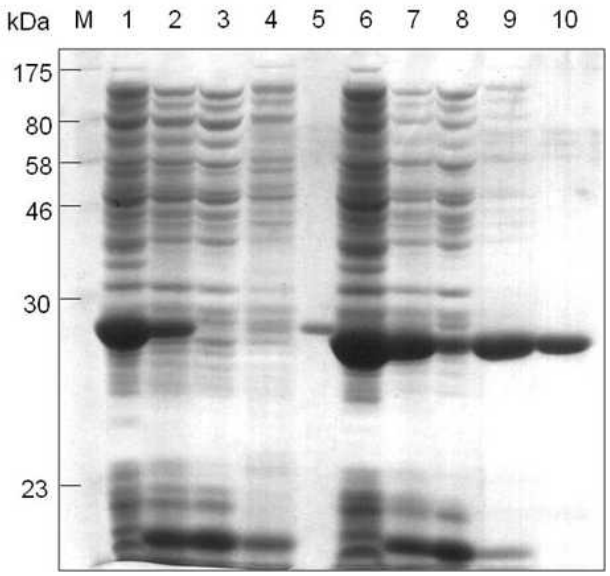

Fig. 6. Comparison on SDS-PAGE of recombinat wild type and Gly194Cys mutant. The recombinant cells for over-expression of protein by IPTG induction. The proteins were separated on 12% (w/v) SDS-PAGE. The proteins on SDS-PAGE gel were visualized by staining with Coomassie Blue R-250. Lane M; protein marker, lane 1-5; wild type, lane 6-10; Gly194Cys mutant, lane 1 and 6; recombinant cells induced with IPTG, lane 2 and 7; cell lysates, lane 3 and 8; unbound fractions of Ni-Sepharose column, lane 4 and 9; 20 mM imidazole fractions, lane 5 and 10; purified and desalted alginate lyases.

of family PL-7 alginate lyases, the amino acid residues of Arg72, Gln117, His119, Tyr189 and Tyr195 in an active site were superimosed exactly. When Lys191 was mutated to Glu (Fig. 7A and 7C), the alginate lyase activity was lost completely. Lys191 was located at strand A4 in C-terminal conserved sequence, YF**K**AGXYXQ (Fig. 2). All of substrate binding residues are located at strands A3, A4 and A5. Basic amino acid residue, Lys191 on strand A4 might involve in acidic substrate binding not participate directly in the catalytic reaction. Yamasaki et al [26] reported that Glu148, Gln189, and Lys280 of A1-II′ (2Z42 in Fig. 2) were located at strands A3, A4 and A5 and affected to the rigidity of the active cleft, the binding of the substrate, and the catalytic reaction.

Other mutant Phe99Leu also lost its activity (Fig. 4 and Fig. 7). Phe99 was located at strand B4 and its side chain was faced to the inside of the protein. In PA1167 protein (1VAV in Fig. 2), Phe34, Ser60, Leu62, Phe194, Ala196 and Leu218 which located within the protein was suggested to form hydrophobic blocks along the β-sheets together with the surrounding homologous residues like Try7, Ile11, Tyr29, Ile89, Ile103 and Phe215 for maintenance of structural stability of family PL-7 enzymes [26]. In Phe99Leu mutant, both Phe residue in wild type and Leu residue in Phe99Leu mutant are hydrophobic amino acids but the replacement by Leu caused the loss of activity. Although M3 alginate lyase and ALY-PG (1UAI in Fig. 2) had Phe residues, but PA1167 and A1-II′ had Ala and Leu on strand B4, respectively. When wild type and Phe99Leu mutant were homology-modelled and analyzed, the distance between the sidechains of Phe128 on strand A6 and Phe99 on strand B4 was 4.00 Å (Fig. 8A). But in the case of mutant, the distance between the sidechains of Phe128 on strand A6 and Leu99 on strand B4 was 9.0 Å (Fig. 8B). If Phe99 was replaced by Leu, the hydrophobic interaction inside protein tertiary structure maybe was unstabilized to cause the loss of its activity.

In the case of Gly194Cys mutant, Gly residue located at strand A4 was faced to B sheets not to substrate. Family PL-7 alginate lyase had the conserved domain YFKAGXYXQ

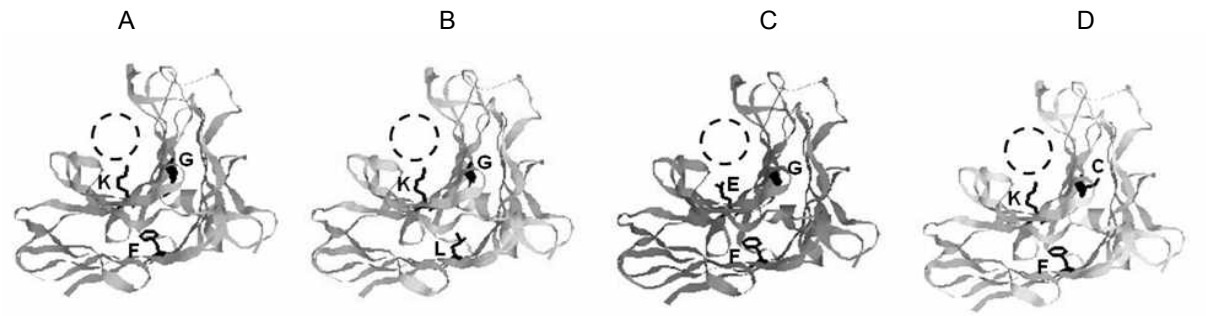

Fig. 7. Homology model of M3 alginate lyase constructed on the crystal structure of *Corynebacterium* sp. alginate lyase (PDB code: 1UAI). A, wild type. B, Phe99Leu mutant, C, Lys191Glu mutant. D, Gly194Cys mutant. Dotted circle indicates the position of alginate substrate. Replaced amino acid residue was displayed by black wireframe with one letter symbol. Structures were visualized by using a RasMol program.

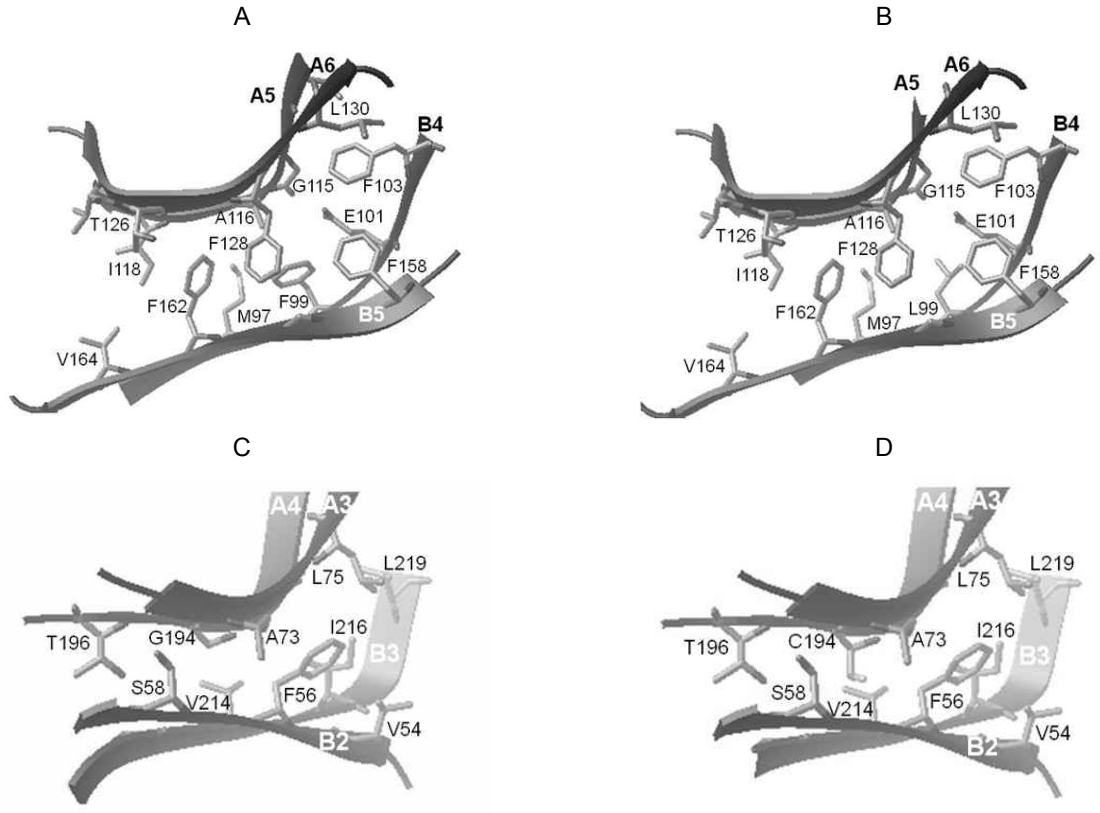

Fig. 8. Comparison of spatial arrangements between A sheets and B sheets. A and B; amino acid sidechains located on strands A5, A6, B4 and B5 of wild type and Phe99Leu (F99L) mutant. C and D; amino acid sidechains located on strands A3, A4, B2 and B3 of wild type and Gly194Cys (G194C) mutant. Amino acid residue was expressed one letter symbol. Structures were visualized by using a Python Molecule Viewer.

in C-terminal region. Gly194 was replaced to Ala, Leu and Asn residue in ALY-PG, PA1167 and A1-II′, respectively. There were polar amino acid residues like Thr196 and Ser58 in the proximity of Gly194 residue. The reason why Gly194Cys mutant had higher activity than wild type could be explained that the polar sidechain of Cys residue formed the stable interactions with adjacent residues as Ser58 and Thr196 (Fig. 8C and 8D). Tøndervik et al reported that AlyA from *Klebsiella pneumoniae* which cleaved both G-G and G-M linkage was mutated and improved G-G specificity by increased affinity for nonproductive binding of the alternating G-M structure [21]. In *Klebsiella* alginate lyase, when Ser37 and Pro39 on A2 strand and Ser86 on loop 1 were mutated to Leu37, His39 and Ile86, respectively, the substrate specificity was strongly affected.

Acknowledgement

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References

1. Arnold, K., L. Bordoli, J. Kopp, and T. Schwede. 2006. The SWISS-MODEL workspace, A web-based environment for protein structure homology modelling. *Bioinformatics* **22**, 195-201.
2. Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248-254.
3. Choi, S. H., H. S. Kim, and E. Y. Lee. 2009. Comparative homology modeling-inspired protein engineering for improvement of catalytic activity of *Mugil cephalus* epoxide hydrolase. *Biotechnol. Letters* **31**, 1617-1624.
4. Gacesa, P. 1998. Bacterial alginate biosynthesis-recent progress and future prospects. *Microbiol.* **144**, 1133-1143.
5. Gimmestad, M., H. Ertesvåg, T. M. B. Heggeset, O. Aarstad, B. I. G. Svanem, and S. Valla. 2009. Characterization of three new *Azotobacter vinelandii* alginate lyases, one of which is involved in cyst germination. *J. Bacteriol.* **191**, 4845-4853.
6. Haug, A., B. Larsen, and O. Smidsrot. 1966. A study of the

constitution of alginic acid by partial acid hydrolysis. *Acta. Chem. Scand.* **20**, 183-190.
7. Huang, W., A. Matte, Y. Li, Y. S. Kim, R. J. Linhardt, H. Su, and M. Cygler. 1999. Crystal structure of chondroitinase B from *Flavobacterium heparinum* and its complex with a disaccharide product at 1.7 Å resolution. *J. Mol. Biol.* **294,** 1257-1269.
8. Heyraud, A., C. Gey, C. Leonard, C. Rochas, S. Girond, and B. Kloareg. 1996. NMR spectroscopy analysis of oligoguluronates and oligomannuronates prepared by acid or enzymatic hydrolysis of homopolymeric blocks of alginic acid. application to the determination of the substrate specificity of *Haliotis tuberculata* alginate lyase. *Carbohydr. Res.* **289**, 11-23.
9. Kam, N., Y. J. Park, E. Y. Lee, and H. S. Kim. 2011. Molecular identification of a polyM-specific alginate lyase from *Pseudomonas* sp. strain KS-408 for degradation of glycosidic linkages between two mannuronates or mannuronate and guluronate in alginate. *Can. J. Microbiol.* **57,** 1032-1041.
10. Kim, H. S. 2010. Characterization of recombinant polyG-specific lyase from a marine bacterium, *Streptomyces* sp. M3. *J. Life Sci.* **20**, 1582-1588.
11. Kim, H. S. 2009. Cloning and expression of alginate lyase from a marine bacterium, *Streptomyces* sp. M3. *J. Life Sci.* **19**, 1522-1528.
12. Kim, H. S., C. G. Lee, and E. Y. Lee. 2011. Alginate lyase; structure, property and application. *Biotechnol. Bioproc. Eng.* **16**, 843-851.
13. Kim, D. E., E. Y. Lee, and H. S. Kim. 2009. Cloning and characterization of alginate lyase from a marine bacterium *Streptomyces* sp. ALG-5. *Marine Biotechnol.* **11**, 10-16.
14. Miyake, O., W. Hashimoto, and K. Murata. 2003. An exotype alginate lyase in *Sphingomonas* sp. A1, Overexpression in *Escherichia coli*, purification, and characterization of alginate lyase IV (A1-IV). *Protein Expres. Purif.* **29**, 33-41.
15. Ochiai, A., W. Hashimoto, and K. Murata. 2006. A biosystem for alginate metabolism in *Agrobacterium tumefaciens* strain C58, Molecular identification of Atu3025 as an exotype family PL-15 alginate lyase. *Res. Microbiol.* **157**, 642-649.
16. Ochiai, A., M. Yamasaki, B. Mikami, W. Hashimoto, and K. Murata. 2010. Crystal structure of exotype alginate lyase Atu3025 from *Agrobacterium tumefaciens*. *J. Biol. Chem.* **285**, 24519-24528.
17. Ogura, K., M. Yamasaki, T. Yamada, B. Mikami, W. Hashimoto, and K. Murata. 2009. Crystal structure of family 14 polysaccharide lyase with pH-dependent modes of action. *J. Biol. Chem.* **284**, 35572-35579.
18. Osawa, T., Y. Matsubara, T. Muramatsu, M. Kimura, and Y. Kakuta. 2005. Crystal structure of the alginate (poly α -L-guluronate) lyase from *Corynebacterium* sp. at 1.2 Å resolution. *J. Mol. Biol.* **345**, 1111-1118.
19. Park, H. H., N. Kam, E. Y. Lee, and H. S. Kim. 2011. Cloning and characterization of a novel oligoalginate lyase from a newly isolated bacterium *Sphingomonas* sp. MJ-3. *Mar. Biotechnol.* Online (DOI 10.1007/s10126-011-9402-7)
20. Sawabe, T., H. Takahashi, Y. Ezura, and P. Gacesa. 2001. Cloning, sequence analysis and expression of *Pseudoalteromonas elyakovii* IAM 14594 gene (alyPEEC) encoding the extracellular alginate lyase. *Carbohydr. Res.* **335,** 11-21.
21. Tøndervik, A., G. Klinkenberg, O. A. Aarstad, F. Drabløs, H. Ertesvåg, T. E. Ellingsen, G. Skjåk-Bræk, S. Valla, and H. Sletta. 2010. Isolation of mutant alginate lyases with cleavage specificity for di-guluronic acid linkages. *J. Biol. Chem.* **285,** 35284-35292.
22. Weissbach, A. and J. Hurwitz. 1959. The formation of 2-keto-3-deoxyheptonic acid in extracts of *Escherichia*. *J. Biol. Chem.* **234**, 705-709.
23. Wong, T. Y., L. A. Preston, and N. L. Schiller. 2000. Alginate lyase; Review of major sources and enzyme characteristics, structure-function analysis, biological roles, and applications. *Annu. Rev. Microbiol.* **54**, 289-340.
24. Xiao, L., F. Han, Z. Yang, X. Z. Lu, and W. G. Yu. 2006. A novel alginate lyase with high activity on acetylated alginate of *Pseudomonas aeruginosa* FRD1 from *Pseudomonas* sp. QD03. *World J. Microbiol. Biotechnol.* **22**, 81-88.
25. Yamamoto, S., T. Sahara, D. Sato, K. Kawasaki, S. Ohgiya, A. Inoue, and T. Ojima. 2008. Catalytically important amino-acid residues of abalone alginate lyase HdAly assessed by site-directed mutagenesis. *Enz. Microb. Technol.* **43**, 396-402.
26. Yamasaki, M., S. Moriwaki, O. Miyake, W. Hashimoto, K. Murata, and B. Mikami. 2004. Structure and function of a hypothetical *Pseudomonas aeruginosa* protein PA1167 classified into family PL-7, a novel alginate lyase with a β -sandwich fold. *J. Biol. Chem.* **279**, 31863-31872.
27. Yoon, H. J., W. Hashimoto, O. Miyake, K. Murata, and B. Mikami. 2001. Crystal structure of alginate lyase A1-III complexed with trisaccharide product at 2.0 Å resolution. *J. Mol. Biol.* **307**, 9-16.

초록 : *Streptomyces* sp. M3 알긴산분해효소의 돌연변이에 의한 활성증대

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이전 연구에서 *Streptomyces* sp. M3 균주로부터 polyguluronate에 기질특이성을 가지는 알긴산분해효소를 cloning하고 활성을 연구하였다. 이번 연구에서는 pColdI vector에 들어있는 M3 알긴산분해효소 유전자를 돌연변이시켜 알긴산분해효소의 활성을 증진시키고자 하였으며, 점-돌연변이 또는 무작위-돌연변이 방법을 사용하여 돌연변이를 실시하였다. Ser25Arg, Phe99Leu, Asp142Asn, Val163Ala, Lys191Glu 및 Gly194Cys 등 6 종류의 돌연변이 단백질을 얻을 수 있었다. Phe99Leu 및 Lys191Glu 돌연변이 단백질은 알긴산을 분해하는 능력을 완전히 잃었으나 Gly194Cys 돌연변이 단백질의 활성은 원래 단백질에 비하여 10배 증가하였다. 또한 돌연변이된 M3 알긴산분해효소 단백질의 3차 구조는 Swiss-Model 자동모델러를 이용하여 생성하였으며 다른 알긴산분해효소의 결정구조와 비교하였다. 194 번째 아미노산인 글리신은 알긴산의 C-말단 보존서열인 YFKAGXYXQ의 Gly193과 Tyr195 사이에 위치한다. 이 연구에서 돌연변이된 글리신과 페닐알라닌 잔기들은 활성자리로부터 많이 떨어져있음에도 불구하고 돌연변이에 의하여 알긴산 분해활성이 강하게 영향을 받는 것으로 나타났다.