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# Overexpression of Thermal and pH Stable Alginate Lyase of *P. aeruginosa* 293 and *in silico* Study of *algL* Gene

Maryam Zali Benekohal[1], Parinaz Ghadam[*1], Sara Gharavi[1], Ahya Abdi Ali[2] and Firoozeh Piroozmand[3]

[1] Department of Biotechnology, Faculty of Biological Sciences, Alzahra University, Tehran, Iran
[2] Department of Microbiology, Faculty of Biological Sciences, Alzahra University, Tehran, Iran
[3] Department of Microbiology, Faculty of Biological Sciences, University of Tehran, Tehran, Iran

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****Corresponding authors:***
✉ P. Ghadam
pghadam@alzahra.ac.ir

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**ABSTRACT**

*P. aeruginosa* is an opportunistic bacterium that produces a capsule-like polysaccharide called alginate in response to various stimuli. The mucoid strain of *Pseudomonas aeruginosa* produces alginate which is an exopolysaccharide and is involved in the pathogenicity and persistence of these bacteria in infections. The alginate lyase gene is required for alginate synthesis. The enzyme can also degrade this polymer. This enzyme has a polymorphism in different bacteria even in one species and finding an enzyme with tremendous characters is very important. In this study, alginate lyase from *P. aeruginosa* strain 293 which was previously isolated from the sputum, and the encoding gene was characterized, and thermal and pH stability, as well as the substrate specificity of the partially purified alginate lyase, were determined. The amount of 70% activity of the enzyme was maintained after incubation at 80 ˚C for 6 hrs and 50% activity retained after incubation in alkaline and acidic pH. Moreover, it showed activity towards guluronic acid blocks, mannuronic acid blocks, and alginate blocks with both of them. Due to the unique properties of the alginate lyase that are useful in medicinal, and industrial applications, the gene encoding the enzyme was expressed in pET-28a (+)/*E. coli* BL21 (DE3) system to produce 371 -amino acid alginate lyase protein, the molecular weight of which was estimated by Sodium Dodecyl Sulfate- Polyacrylamide gel electrophoresis to be about 40 kDa. Bioinformatic analysis of *P. aeruginosa* strain 293 *algL* gene revealed that G225A point mutation can improve its thermostability. Therefore, the *P. aeruginosa* 293 alginate lyase is proposed as an appropriate candidate for the evaluation of potential therapeutic and industrial applications.

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## Introduction

*P. aeruginosa* is a Gram-negative opportunistic bacterial pathogen that secretes a capsule-like polysaccharide called alginate in response to various environmental stimuli. The mucoid phenotype showed by bacteria when they infect the lungs of Cystic Fibrosis (CF) patients is due to the presence of this natural polymer (Gol Fakhrabadi *et al.*, 2015; Moradali *et al.*, 2015).

Two different uronic acids, β-D-mannuronic acid (M) and α-L-guluronic acid (G) are present in alginate as polymannuronic acid blocks (PolyM), polyguluronic acid blocks (PolyG) and Poly MG polymer (Li *et al.*, 2011, Zhu *et al.*, 2019). Alginate is a key component of the mucoid biofilm matrix. This biofilm is important for the evasion of host defenses and antibacterial therapies, especially during the chronic pulmonary disease of patients with CF.

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Most proteins involved in the alginate biosynthesis are encoded by 12-gene operon. Interestingly, this operon also encodes Alginate Lyase (EC 4.2.2.3) (AlgL) (Franklin *et al*., 2011; Ahmed, 2007), a lyase responsible for the degradation of alginate by a β-elimination reaction. This reaction involves the breakage of the 1–4 O-linkage between the uronic acids in the linear polymer and results in generating oligomers with 4-deoxy-L-erythro-hex-4-enepyranosyluronate at the nonreducing end. Due to their high efficiency, specificity, and mild degradation function, alginate lyases have attracted widespread attention in industrial applications, especially in the preparation of alginate oligosaccharides. Thus far, hundreds of alginate lyases have been purified, cloned, and characterized from marine microorganisms, brown seaweeds, and mollusks.

Therefore, there is an urgency to obtain an alginate lyase with the optimal characteristics (e.g., pH-stability, thermo-tolerance, and single product distribution) needed for industrial applications (Zhu *et al*., 2018, Huang *et al*., 2018).

AlgL is classified into three groups: Alginate lyase (E.C. 4.2.2.3 and E.C. 4.2.2.11) is a kind of polysaccharide lyase that degrades alginate by β-eliminating the glycoside 1-4 O-bonds between C4 and C5 at the non-reducing end, thus producing unsaturated alginate oligosaccharides (UAOS) as main products (Wang *et al*., 2019) and are distributed over various organisms including marine organisms, bacteria including *Bacillus circulans* and *P. aeruginosa*, viruses, fungi, brown seaweeds, and herbivorous mollusks (Chen *et al*., 2018; Inoue *et al*., 2014; Wong *et al*., 2000). In particular, this enzyme which promotes phagocytosis and killing of *P. aeruginosa* by human immune cells has been elucidated to enhance the efficacy of antibiotics against *P. aeruginosa* in biofilms and elimination of biofilms on medical devices (Sharma *et al*., 2013).

In this research, a thermal and pH stable alginate lyase from *P. aeruginosa* 293 (previously isolated from the sputum of burned patients), was partially purified and the gene encoding Alginate Lyase, *algL*, was cloned and expressed, and characterized by bioinformatics tools.

## Materials and Methods

### Bacteria and growth conditions

Mucoid *P. aeruginosa* strain 293 isolated from the sputum (Rajaee Hospital, Tehran, Iran), was grown in YTG (0.5% yeast extract, 1% tryptone, and 0.2% glucose) overnight at 37 ˚C with shaking. *Escherichia coli* DH5a and lysogenic *Escherichia coli* BL21 (DE3) were used as hosts for recombinant plasmids. *E. coli* was grown in Luria-Bertani (LB) broth at 37 ˚C (Sambrook and Russel, 2001) or on LB agar supplemented with kanamycin (50 $\mu g.ml^{-1}$) (Farrell and Tipton, 2012). Plasmid pET-28a (+), which was used as a cloning vector and expression of recombinant protein, was from Novagen.

### AlgL extraction

Bacteria were collected by centrifugation at 6500xg for 25 min at 4 ˚C (Eftekhar and Schiller, 1994), washed in 0.03 *M* Tris-HCl (pH 7.5) containing 0.2 M $MgCl_2$, then centrifuged at 6500xg for 10 min at 4 ˚C and repeated twice (Schiller *et al*., 1993). The enzyme was extracted with the heat shock method (Eftekhar and Schiller, 1994; Hoshino and Kageyama, 1980). Briefly, heat shock buffer ($MgCl_2$ 0.2 M and Tris-HCl 0.05 M, pH 7.3) was added to bacterial suspensions and incubated at 37 ˚C for 10 min followed by 15 min at 0 ˚C (four repeats) then centrifuged at 6500xg for 15 min. The supernatant containing AlgL activity was stored at 4 ˚C.

### Measurement of AlgL activity

AlgL activity was measured by the thiobarbituric acid (TBA) method. The enzyme extract, assay buffer (Tris-HCl 30mM, NaCl 500mM, and $MgCl_2$ 9mM) pH 8.5, and sodium alginate were mixed with a ratio of 2:2:1 in a 100 µl volume. The 125 µl periodic acid solution in 0.125 N $H_2SO_4$ was added to the mixture and placed at 37 ˚C for 20 min. Then, 250 µl of the 2% sodium arsenite solution in 50mM HCl was added to the mixture, and after 2-min incubation at room temperature, 1 ml of the 0.3% thiobarbituric acid solution in the assay buffer pH 2.0 was added with gentle shaking and the final mixture was placed in boiling water for 10 min. Finally, immediately after the solution became chilled, its absorbance was measured at

548 nm and reported as the enzyme unit (Weissbach and Hurwitz, 1958).
One unit of enzyme activity was defined as the amount of enzyme required to produce the equivalent of 1 nmol of β-formylpyruvic acid per *min* per *ml* at 37 ˚C (10 nM of β-formylpyruvic acid gives an OD reading of 0.290 at 548 nm) (Schiller *et al*., 1993)).

**Enzyme characterization**

Determination of enzyme-substrate exposure time: The extracted enzyme, sodium alginate as substrate, and assay buffer were incubated for different periods (0, 15, 30, 45, 60 min) at 37 ˚C (Shimokawa *et al*., 1997), then the rest of the TBA assay was carried out. The extracted enzyme and assay buffer were incubated with each PolyM, PolyG (the blocks were separated from sodium alginate (Haug *et al*., 1996)) and sodium alginate to elucidate the substrate specificity (Nguyen and Schiller, 1989).

**Thermal stability of AlgL**

The thermal stability of AlgL was determined by measuring the residual activity of the enzyme after incubation at 10 °C, 37 ˚C, 60 ˚C, and 80 ˚C for 1 hr and the measured enzyme activity after incubation at 80 ˚C for 2-6 hr. Enzyme activity without incubation was considered 100%.

**Optimum pH and pH stability**

To determine the optimal pH, AlgL activity was measured at 37 ˚C in the range of pH 2.0–11.0 in a Britton –Robinson buffer as assay buffer including phosphoric acid 0.04 M, acetic acid 0.04 M, and boric acid 0.04 M (Britton and Robinson, 1993). Also, the enzyme activity was considered 100% in assay buffer. The pH stability of AlgL was determined by measuring the residual activity of the enzyme after incubating the enzyme in Britton–Robinson buffer from pH 2 to pH 11 at 4 ˚C for 1-6 hrs (Wang *et al*., 2013). The enzyme activity in pH 7 without incubation was considered 100%.

**Plasmid extraction**

Total DNA from *P. aeruginosa* 293 was isolated by successive extraction with a modification of the SET buffer method of Del Mar Lleò (Del Mar Lleò *et al*., 2000). An overnight culture (100 ml) was harvested by centrifugation and the bacterial pellets were suspended in 1 ml of SET buffer (20% sucrose, 50 mM Ethylene Diamine Tetra Acetic Acid (EDTA), 50 mM Tris-HCl (pH 7.6)) following which 9 ml of 25% SDS was added, and samples were incubated for 60 min at room temperature. Subsequently, 3 µl of proteinase K (25 $mg.ml^{-1}$) was added, and the suspensions were incubated at room temperature for an additional 4 hr. The samples were treated with 500 µl ammonium acetate (7.5 M) for 15 min and the supernatant was collected by centrifugation at 22,000xg for 5 min at 4 ˚C. Two volumes of isopropanol were added and stored at -20 ˚C for 30 *min* then the supernatant was discarded by centrifugation and washed in 70% ($v.v^{-1}$) ethanol and finally redissolved in TE buffer (10 mM Tris. HCl, pH 8.0, contains 1 mM EDTA). Minipreparations of plasmid pET-28a (+) were obtained by using the method of Sambrook *et al*, 1989.

**Cloning of *algL***

After the complete nucleotide sequence of the gene was determined, the primers with restriction sites were designed to clone the *algL* in the expression vector pET-28a(+) (Tavafi *et al*., 2016). The forward primer [ATATGAATTCATGAAAACGTCCCACCTGAT (EcoRI)] and the reverse primer [ATATAAGCTTTCAACTTCCCCCTTCGC (HindIII)] were used to amplify the translational region of *algL*, with genomic DNA of *P. aeruginosa* 293 as the template. DNA fragments were purified from agarose gels by using the GF-1 Gel DNA Recovery Kit (Sinaclon, Iran). The PCR (Polymerase Chain Reaction) fragment was digested with *Eco*RI and *Hind*III (Fermentas, Canada), purified with EZ-Spin Colum PCR Purification Kit (Bio Basic, Canada), and ligated into a similarly digested pET-28a (+) vector. *E. coli* DH5a was transformed using the ligation mixture, and transformants were grown on LB agar plates containing 50 $µg.ml^{-1}$ kanamycin. Transformants were initially screened for the presence of *algL* using colony PCR.

**DNA sequencing**

DNA fragments containing the *algL* were sequenced by the dideoxy chain termination

method (Sanger *et al.*, 1977) with synthetic oligonucleotides as primers. Both strands of DNA were sequenced and analyzed by the BioEdit program. Examination of similarities and differences in gene sequences compared to other related genes was performed by BLASTn of the NCBI database.

**AlgL expression**

The pET28-*algL* plasmid was transformed into *E. coli* BL21 (DE3), which expressed a recombinant protein containing 6xHis-Tags at N-terminus. The recombinant *E. coli* BL21 (DE3) harboring the pET28-*algL* plasmid was cultured on an LB medium supplemented with 50 µg.ml$^{-1}$ kanamycin for 2–3 hr to obtain $OD_{600}$=0.4–0.6 in a shaking incubator at 37 ˚C and 200 rpm. The cells were cultured for 8 hr to express the *algL* by addition of 1 mM Isopropyl-β-D-Thiogalactopyranoside (IPTG) (Farrell and Tipton, 2012): After 2, 4, 6, 8 hrs of induction the cells were harvested and sonicated in Phosphate Buffer Saline (PBS) (1.8 *mM* KH2PO4 (pH 7.2), 137 mM NaCl, 2.7 mM KCl, 10 mM $Na_2HPO_4$). Sonication was set at a 30% duty cycle and 60% power output for 10 min (Swift *et al*., 2014). The lysate was cleared by centrifugation at 22,000xg for 10 min at 4 ˚C, and the supernatant was used for Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis (Laemmeli, 1970). Fractions containing activity were combined and used as purified AlgL.

**AlgL purification and activity**

The supernatant obtained from sonication containing a maximum amount of expressed (His)6-tagged AlgL was loaded on an Immobilized Metal Affinity Chromatography (IMAC) column (Bio-Rad), equilibrated with 5 *mM* phosphate buffer (pH 8), 300 *mM* KCl and 50 mM $KH_2PO_4$ (wash buffer 1). The column was rinsed twice with wash buffer 1 and containing 10 and 50 mM imidazole, respectively; tagged AlgLs were then eluted by the same buffer containing 250 *mM* imidazole, and protein was eluted with a flow rate of 2 ml.min$^{-1}$. The purified recombinant protein was stored at 4 ˚C (Bio-Rad protocol of IMAC). Protein concentrations were determined by Bradford assay, using BSA as the standard (Bradford, 1976). Protein aliquots were run on a 15% SDS-PAGE gel to assess solubility and purity and the gels were stained with Coomassie Brilliant Blue (Laemmeli, 1970). AlgL activity was quantitatively measured by the TBA method as described before and the thermal stability of the recombinant enzyme was assayed at 37 ˚C and 80 ˚C for 6 hr.

***In silico* studies**

The nucleotide sequence of AlgL produced by *P. aeruginosa* 293 was virtually translated to the corresponding amino acid sequence using the ExPASy Translate Tool. The 3D structure of the enzyme was built using Modeller 9.17. Alginate lyase with PDB Code 4ozv was used as a template for homology modeling. The values of the MODELLER objective function of all the built models were screened and the model with the least value was chosen as the best model for further studies. Examining the accuracy and the refining of the model was performed using web servers Qmean and 3Drefine, respectively. It has been reported that the alginate lyase undergoes cleavage to remove the signal peptide after the translation process.

Therefore, the signal peptide of the enzyme was specified using Signal-BLAST and Phobius tools and was deleted from the structure of the built model. CASTp server was used to predict the active site of the enzyme. ConTEXT, UCSF Chimera 1.12, and Avogadro tools were used for the preparation of the enzyme and ligand before docking simulation. AutoDock 1.5.6 software package was applied to determine the key residues of the alginate lyase involving in the interaction with its corresponding ligand, alginic acid. One hundred conformers of the ligand were assessed as possible conformers interacting with the residues in the active site of the enzyme. Among these conformers, one with the least value of binding energy and the most built hydrogen bonds with the enzyme was selected as the best conformer.

The PDB file of the enzyme-ligand complex was written. The residues contributing to the hydrogen bindings and hydrophobic interactions with alginate ligand in the written complex were analyzed using Ligplot software version v.1.4.5. Other important residues in alginate lyases are cysteine residues involved in disulfide bridges.

Cysteine residues forming disulfide bonds were recognized using DISULFIDE, TargetDisulfide, and DIANNA tools. HotSpot Wizard 2.0 was used to specify the residues with the most mutability score among the residues without the importance in the enzyme activity located in the non-conserved regions on the surface of the enzyme.

To increase the heat stability, a mutation of Gly to Ala was proposed for Gly225 which is located in a loop of *P. aeruginosa* 293 AlgL without contributing to the enzyme activity, disulfide bridges, or the signal peptide. Thereupon, G225A mutation was introduced to the enzyme structure using Chimera (version 1.12) and the stability of the mutant enzyme was analyzed using SDM and MCSM web servers.

## Results

As shown in Fig. 1A, the extracted enzyme initially showed maximum activity and its activity slightly decreased over time. AlgL had activities with both PolyG and PolyM as substrate, indicating that it is a bifunctional AlgL (Fig. 1B). It had almost the same activity towards sodium alginate and PolyM, but lower reactivity with PolyG.

Other hands, as shown in Fig. 2A, the enzyme was active after treated at various temperatures (10, 37, 60, and 80 ˚C) and pH 7.3 (Tris HCl buffer) for 1 hr. The enzyme retained 88% of activity after 1 hr at 10 ˚C, as well as the enzyme retained 70% of activity after incubating for 6 hr at 80 ˚C, the activity measurement without incubation was considered 100% (Fig. 2B).

Maximum enzyme activity was observed at pH 7 but 84%, 94%, and 90% enzyme activity observed at pH 2, 4, and 11, respectively (Fig. 3A). After incubation of enzyme at various pH, 55%, 60%, 68%, and 50% of the activity was retained at pH 2, 4, 7, and 11, respectively (Fig. 3B).

*E. coli* DH5a cells were transformed with pET28-*algL* plasmid and cultured on LB agar plates including kanamycin. To determine the accuracy of cloning, colony PCR, and screening of colonies containing recombinant vector was performed randomly on colonies. PCR was carried out with primers designed by Tavafi *et al*., 2016 for the *algL* from *P. aeruginosa* 293 (Bradford, 1976). AlgL-positive clones were identified (Fig. 4A). Results show that the cloned sequences had homology to some AlgLs indicating, for example, 97% identity with AlgL from *P. aeruginosa* TAG48. Based on DNA sequence information, the full-length gene with 1,117 nucleotides was cloned. AlgL protein consists of 371 amino acids.

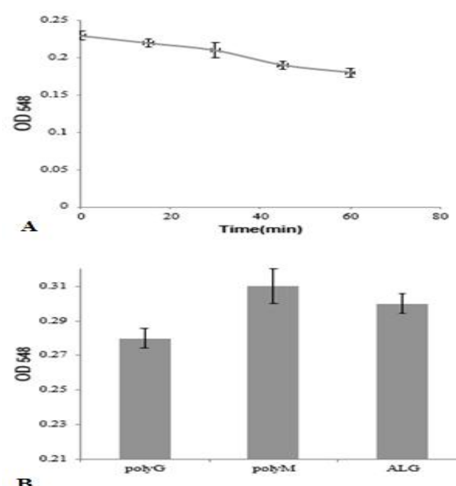


**Fig. 1.** Characterization of the enzyme-substrate relationship between alginate lyase **(**AlgL) from *P. aeruginosa* strain 293 and its substrates. A) The effect of enzyme-substrate exposure time on enzyme activity. B) Substrate specificity of the AlgL. Guluronic acid blocks (PolyG), mannuronic acid blocks (PolyM) and compared with that of sodium alginate (ALG).

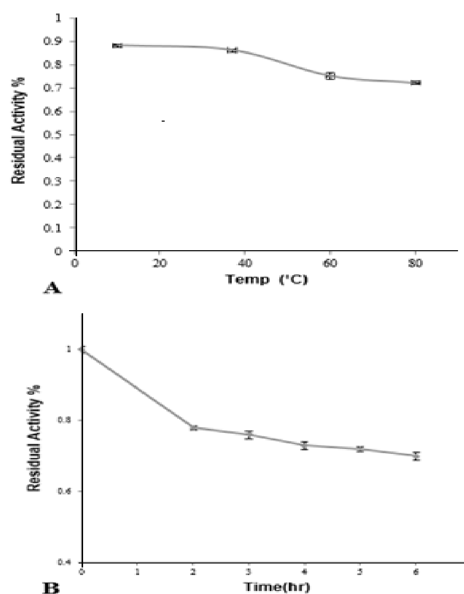


**Fig. 2.** Thermal stability of alginate lyase from *P. aeruginosa* strain 293. A) The enzyme was incubated at various temperatures for 1 hr. B) The enzyme was incubated at 80 ˚C up to 6 hr.

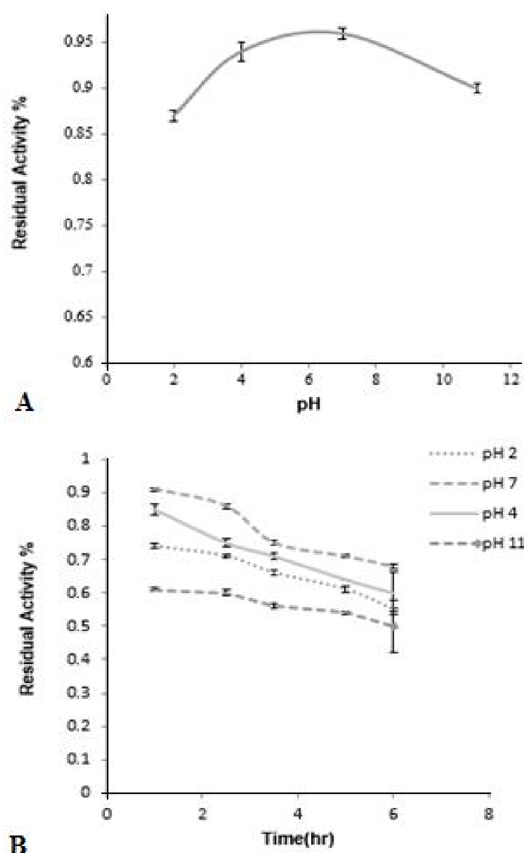


**Fig. 3.** Effect of pH on the activity and stability of alginate lyase (AlgL) from *P. aeruginosa* strain 293. A) Effect of pH on AlgL activity. Determination of the optimum pH for use in preparing Britton–Robinson buffer (pH 2, 4, 7, 11). B) Enzyme stability at various pH. The enzyme solution was incubated for various times at pH 2, 4, 7, and 11, using Britton–Robinson buffer at 4 ˚C, and residual activities were assayed.

The nucleotide sequence of AlgL was submitted to GenBank (accession number: BankIt1985231 Seq1 [P. MF615385]. As shown in Fig. 4B, the *algL* from *P. aeruginosa* 293 was expressed in *E. coli* BL21 (DE3) containing plasmid pET28-*algL* with the induction of T7 polymerase by 1 mM IPTG (Farrell and Tipton, 2012). Expression of the cloned *algL* in *E. coli* verified the location of the structural gene for AlgL as well as providing a source of the enzyme, which could be used for protein purification and characterization studies (Schiller *et al*., 1993). The resulting recombinant proteins at different times (2, 4, 6, 8 hr) were analyzed by SDS-PAGE, and the highest expression was related to 8 hr after the induction that was used to load the column.

The enzyme was purified by the IMAC column, an excellent chromatography technique for purification of His-tagged proteins. The final preparation of recombinant protein gave a single band on SDS-PAGE with a molecular mass of approximately 40 kDa (Fig. 4B).

Thermal stability characteristics of the recombinant enzyme were investigated. The recombinant AlgL was active after 6 hrs of incubation at 37 ˚C up to 80 ˚C. The enzyme retained 56% and 26% of its activity at 37 ˚C and 80 ˚C, respectively.

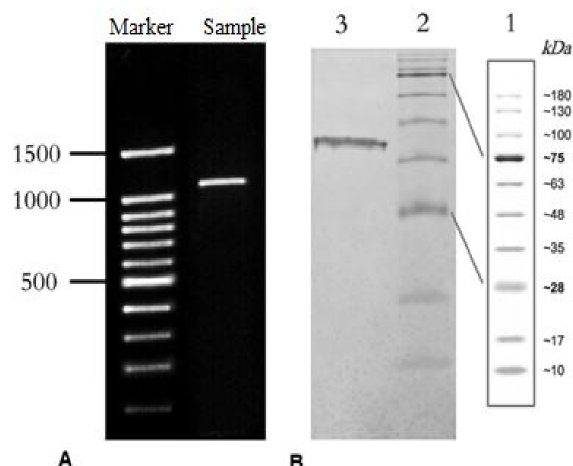


**Fig. 4.** Colony PCR (Polymerase Chain Reaction) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of the recombinant *P. aeruginosa* AlgL protein. A) PCR product of recombinant plasmid. B) Purified recombinant enzyme; 1 and 2 are molecular mass markers; 3 is the eluted protein from immobilized metal affinity chromatography (IMAC) column.

As shown in Fig. 5, analysis of docking results using Ligplot software indicated that Ser58, Lys59, Tyr60, Met136 and Phe78, Ser135, Trp139, His195 of *P. aeruginosa* 293 are involved in H-bonds and hydrophobic interactions with alginate, respectively. Also, AlgL amino acid sequence analysis showed that it contains the conserved region of NNHSYW in its active site which is related to the PolyM activity and is observed in alginate lyases with an average molecular weight of 40 kDa (Wong *et al*., 2000). The cleavage site of the signal peptide was predicted to be located between residues 20 and 21 in the protein sequence. It

was predicted that the alginate lyase of *P. aeruginosa* 293 contains 5 Cysteines, but they do not contribute to any disulfide bridges. Analysis of the computationally mutant G225A AlgL showed increased stability of the enzyme structure.

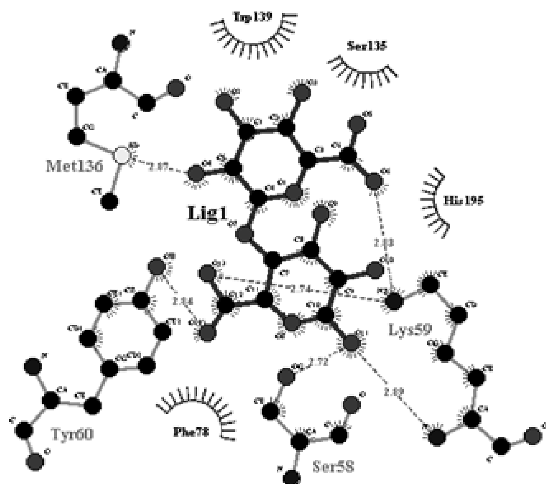


**Fig. 5.** 2D representation of interactions between *P. aeruginosa* Alginate Lyase (AlgL) protein and alginic acid ligand using Ligplot. Amino acids Ser58, Lys59, Tyr60, and Met136 participate in hydrogen bonding with the ligand molecule. Phe78, Ser135, Trp139, and His195 of the AlgL are involved in hydrophobic interactions.

## Discussion

A wide range of microorganisms such as *P. aeruginosa*, produce AlgL. Substrate specificity of AlgLs differ in various strains and are classified into three groups: PolyM lyase, PolyG lyase, and PolyMG lyase (Wong *et al*., 2000). Researchers have focused their efforts to find AlgL enzymes that have thermal and pH stability for use in therapeutic applications, food industries (Zhu *et al*., 2018; Wang *et al*., 2019; Li *et al*., 2020), and bioethanol production (Park *et al*., 2012). Moreover, PolyM lyase activity is required for the treatment of alginate polysaccharide build-up in the lungs of CF patients (Kim *et al*., 2011). Previous studies have shown that treated mucoid *P. aeruginosa* by alginate lyase is more susceptible to antibiotics both *in vitro* (Tavafi *et al*., 2019; Bayer *et al*., 1991) and *in vivo* (Bayer *et al*., 1992).

In this study, an AlgL from *P. aeruginosa* 293 with PolyM lyase activity was isolated. Characterization of the AlgL showed that the enzyme initially had maximum activity but the activity decreased over time. However, Shimokawa revealed that the enzyme from *Enterobacter cloacae* M-1 showed an increase in activity during the time (Shimokawa *et al*., 1997). Previous studies indicated that the AlgL of *P. aeruginosa* WcM # 2 degraded PolyM significantly better than PolyG (Eftekhar and Schiller, 1994), and bifunctional AlgLs have been purified from some bacterial strains, such as two types of *P. aeruginosa* (Farrell and Tipton, 2012; Jain and Ohman, 2005) and *Pseudoalteromonas atlantica* AR06 (Li *et al*., 2011) while AlgL from *P. aeruginosa* 293 was capable of breaking all three of its substrates including PolyM, PolyG, and sodium alginate, the ability of the enzyme to break down of PolyM was greater. Moreover, AlgL in this research had high thermotolerance at 10-80 ˚C with 6 hrs incubation which would facilitate its application in bioethanol production and other industries. AlgL from *P. aeruginosa* 48 and AlyV5 from *Vibrio sp.,* however, kept 35% and 40% of their activity after incubation at 80 ˚C for 4 hr and 90 ˚C for 10 min, respectively (Ghadam *et al*., 2017; Wang *et al*., 2013). The optimal pH of AlgL of *P. aeruginosa* 293 was more extended compared with other strains such as the optimal pH of aly-SJ02 from *Pseudoalteromonas* sp. SM0524 and FlAlyA from Flavobacterium sp. Strain UMI-01 were, respectively, 8 and 7.8 (Inoue *et al*., 2014; Li *et al*., 2011). This enzyme exhibited high pH stability and retained 50% of its activity after incubation in alkaline and acidic pH, another good feature of the enzyme. These studies revealed that there is a wide diversity in molecular weight, pI, substrate specificity, pH, and thermostability of the enzyme even in various strains of one species and there is a poor similarity in protein and gene sequences in these enzymes of different strains (Piroozmand *et al*., 2020).

Due to these unique properties, the *algL* gene was cloned and expressed in *E. coli* DH5α and expressed in *E. coli* BL21 (DE3) (Peciña *et al*., 1999) using pET-28a (+) which is a suitable vector for inducible expression of recombinant proteins in *E.coli* (Farrell and Tipton, 2012). The purification of the recombinant enzyme was carried out by IMAC column and was shown as

a single band on SDS-PAGE with a molecular mass of approximately 40 kDa. As reported previously, the molecular mass of the AlgL from *P. aeruginosa* FRD1 and *Pseudomonas sp.* Eo3, determined to be about 42 and 40 kDa, respectively, by activity staining after SDS-PAGE (Xiao *et al*., 2006; Zhu *et al*., 2015). The thermostability of the recombinant protein was assayed. AlgL expressed from *Azotobacter chroococcum* completely lost its heat resistance (Peciña *et al*., 1999), while in this study, the recombinant protein activity was retained after 6 hrs at 37 ˚C and 80 ˚C. These results show that the recombinant AlgL is thermostable similar to the native protein. Likewise, applying the computationally proposed mutation of G225A in an experimental procedure will, with a great probability, increase the heat stability of the enzyme. In conclusion, this research revealed that the alginate lyase of *P. aeruginosa* 293 had unique features such as thermal and pH stability and affected PolyM, PolyG, and alginate as a substrate. A source for enzyme production was provided by overexpression in *E. coli*, which can be used in studies to evaluate potential therapeutic applications and facilitate its application in medicine and industries. It was suggested that the induction of single point mutations to the alginate lyase structure could help in increasing the stability and specifically the thermostability of the enzyme making it more suitable for industrial applications.

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## Conflicts of interest

The authors declare that there is no conflict of interest.

## References

Ahmed N. 2007. Genetics of bacterial alginate: alginate genes distribution, organization and biosynthesis in bacteria. *Curr Genomics* 8:191-202.

Bayer AS, Park S, Ramos MC, Nast CC, Eftekhar F, and Schiller NL. 1992. Effects of alginase on the natural history and antibiotic therapy of experimental endocarditis caused by mucoid *Pseudomonas aeruginosa*. *Infect Immun* 60: 3979-3985.

Bayer AS, Speert DP, Park S, Tu J, Witt M, Nast CC, *et al.* 1991. Functional role of mucoid exopolysaccharide (alginate) in antibiotic-induced and polymorphonuclear leukocyte-mediated killing of *Pseudomonas aeruginosa*. *Infect Immun* 59: 302-308.

Bradford MM. 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.

Britton HTS, Robinson RA. 1993. CXCVIII. Universal buffer solutions and the dissociation constant of veronal. *J Chem Soc* 1931: 1456-1462.

Chen P, Zhu Y, Men Y, Zeng Y, Sun Y. 2018. Purification and characterization of a novel alginate lyase from the marine bacterium Bacillus sp. Alg07. *Mar Drugs* 16: 86-99.

Del Mar Lleò M, Pierobon S, Tafi MC, Signoretto C, Canepari P. 2000. mRNA detection by reverse transcription-PCR for monitoring viability over time in an Enterococcus faecalis viable but nonculturable population maintained in a laboratory microcosm. Applied and Environmental Microbiology. *Appl Environ Microbiol* 66: 4564-4567.

Eftekhar F, Schiller NL. 1994. Partial purification and characterization of a mannuronan-specific alginate lyase from *Pseudomonas aeruginosa. Curr Microbiol* 29: 37-42.

Farrell EK, Tipton PA. 2012. Functional characterization of AlgL, an alginate lyase from *Pseudomonas aeruginosa*. *Biochemistry* 51:10259-10266.

Franklin MJ, Nivens, DE, Weadge JT, Howell PL. 2011. Biosynthesis of the *Pseudomonas aeruginosa* Extracellular Polysaccharides, Alginate, Pel, and Psl. *Front Microbiol* 2: 167-182.

Gol Fakhrabadi H, Ghadam P, Mohammadi, P, Abdi-Ali A. 2015. Purification and Characterization of Alginate Lyase from

Mucoid *Pseudomonas aeruginosa* Strain 214. *J Genet Resour* 1: 55-60.

Ghadam P, Akhlaghi F, Abdi Ali A. 2017. One-step purification and characterization of alginate lyase from *a* clinical *Pseudomonas aeruginosa* with destructive activity on bacterial biofilm. *Iran J Basic Med Sci* 20: 467-473.

Haug A, Larsen B, Smidsrød O. 1966. A study of the constitution of alginic acid by partial acid hydrolysis. *acta chem Sc* 20: 183-190.

Hoshino T, Kageyama M. 1980. Purification and properties of a binding protein for branched-chain amino acids in *Pseudomonas aeruginosa*. *J Bacteriol* 141: 1055-1063.

Huang G, Wang Q, Lu M, Xu C, Li F, Huang S. 2018. AlgM4: A new salt-activated alginate lyase of the PL7 family with endolytic activity. *Mar Drugs* 16: 120- 133.

Inoue A, Takadono K, Nishiyama R, Tajima K, Kobayashi T, Ojima T. 2014. Characterization of an alginate lyase, FlAlyA, from *Flavobacterium* sp. strain UMI-01 and its expression in *Escherichia coli*. *Mar Drugs* 12: 4693-4712.

Jain S, Ohman DE. 2005. Role of an alginate lyase for alginate transport in mucoid *Pseudomonas aeruginosa. Infect Immun* 73: 6429-6436.

Kim HS, Lee CG, Lee EY. 2011. Alginate lyase: structure, property, and application. *Biotechnol Bioprocess Eng* 16: 843-851.

Laemmeli UK. 1970. Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. *Nature* 227:680-685.

Li JW, Dong S, Song J, Li CB., Chen XL, Zhang YZ. 2011. Purification and characterization of a bifunctional alginate lyase from *Pseudoalteromonas* sp. SM0524. *Mar Drugs* 9: 109-123.

Li S, Wang L, Jung S, Lee B, He N, Lee M. 2020. Biochemical Characterization of a New Oligoalginate Lyase and Its Biotechnological Application in *Laminaria japonica* Degradation. *Front Microbiol* 11: 1-12.

Moradali MF, Donati I, Sims IM, Ghods S, Rehm BH. 2015. Alginate polymerization and modification are linked in *Pseudomonas aeruginosa*. *MBio* 6: e00453-15.

Nguyen LK, Schiller NL. 1989. Identification of a slime exopolysaccharide depolymerase in mucoid strains of *Pseudomonas aeruginosa*. *Curr Microbiol* 18: 323-329.

Park HH, Kam N, Lee EY, Kim HS. 2012. Cloning and characterization of a novel oligoalginate lyase from a newly isolated bacterium *Sphingomonas* sp. MJ-3. *Mar Biotechnol* 14:189-202.

Peciña A, Pascual A, Paneque A. 1999. Cloning and expression of the *algL* gene, encoding the *Azotobacter chroococcum* alginate lyase: purification and characterization of the enzyme. *J Bacteriol* 181:1409-1414.

Piroozmand F, Ghadam P, Zarrabi M, Abdi Ali A. 2020. Biochemical and computational study of an alginate lyase produced by *Pseudomonas aeruginosa* strain S21. *Iran J Basic Med Sci* 23: 454-460.

Sambrook J, Fritsche F, Maniatis T. 1989. Molecular Cloning: A Laboratory Manual. 2nd ed. New York: Cold Spring Harbor Laboratory Press.

Sambrook J, Russel DW. 2001. Molecular Cloning: A Laboratory Manual. 3rd ed. New York: Cold Spring Harbor Laboratory Press.

Sanger F, Nicklen S, Coulson AR. 1977. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* 74: 5463-5467.

Schiller NL, Monday SR, Boyd CM, Keen NT, Ohman DE. 1993. Characterization of the Pseudomonas aeruginosa alginate lyase gene (*algL*): cloning, sequencing, and expression in *Escherichia coli. J Bacteriol* 175: 4780-4789.

Sharma G, Rao S, Bansal A, Dang S, Gupta S, Gabrani R. 2013. *Pseudomonas aeruginosa* biofilm: potential therapeutic targets. *Biologicals* 42: 1-7.

Shimokawa T, Yoshida S, Kusakabe I, Takeuchi T, Murata K, Kobayashi H. 1997. Some Properties and Action Mode of (1→4)-α-L-Guluronan Lyase from *Enterobacter cloacae* M-1. *Carbohydr Res* 304: 125-132.

Swift SM, Hudgens JW, Heselpoth RD, Bales PM, Nelson DC. 2014. Characterization of AlgMsp, an alginate lyase from *Microbulbifer* sp. 6532A. *PLoS One* 9: e112939-e112949.

Tavafi H, Abdi-Ali A, Ghadam P, Gharavi S. 2016. Screening of alginate lyase-producing bacteria and optimization of media compositions for extracellular alginate lyase production. *Iran Biomed J* 21: 48-56.

Tavafi H, Abdi-Ali A, Ghadam P, Gharavi S. 2019. Evaluation of the synergistic effect of bacterial recombinant alginate lyase and therapeutic antibiotics on the growth of planktonic *Pseudomonas aeruginosa*. *JMW* 12: 160-171

Wang Y, Chen X, Bi X, Ren y, Han Q, Li S. 2019. Characterization of an Alkaline Alginate Lyase with pH-Stable and Thermo-Tolerance Property. *Mar Drugs* 17: 308-322.

Wang Y, Guo EW, Yu WG, Han F. 2013. Purification and characterization of a new alginate lyase from marine bacterium Vibrio sp. *Biotechnol Lett* 35: 703-708.

Weissbach A, Hurwitz J. 1958. The formation of 2-keto-3-deoxyheptonic acid in extracts of *Escherichia coli. J Biol Chem* 234: 705-709.

Wong TY, Preston LA, Schiller NL. 2000. Alginate lyase: review of major sources and enzyme characteristics, structure-function analysis, biological roles, and applications. *Annu Rev Microbiol* 54: 289-340.

Xiao L, Han F, Yang Z, Lu XZ, Yu WG. 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.

Zhu BW, Huang LSX, Tan HD, Qin YQ, Du YG, Yin H. 2015. Characterization of a new endo-type polyM-specific alginate lyase from *Pseudomonas* sp. *Biotechnol Lett* 37: 409-415.

Zhu B, Ni F, Ning L, Sun Y, Yao Z. 2018. Cloning and characterization of a new pH-stable alginate lyase with high salt tolerance from marine *Vibrio* sp. NJ-04. *Int J Biol Macromol* 115: 1063–1070.

Zhu B, Ni F, Sun Y, Ning L, Yao Z. 2019. Elucidation of degrading pattern and substrate recognition of a novel bifunctional alginate lyase from *Flammeovirga* sp. NJ-04 and its use for preparation alginate oligosaccharides. *Biotechnol Biofuels* 12:1-3.