Current Trends in Biotechnology and Pharmacy
Vol. 17(Supplementary issue - 3B) 1191-1207,September 2023, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2023.3s.56

Computational Analysis of Alginate Lyases Produced by Different Microorganisms

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Abstract

Alginates are considered as the major structural material present in the cell-wall of brown seaweeds and are produced by bacterial species like *Azotobacter* and *Pseudomonas*. Alginate lyase depolymerizes alginate into alginate oligosaccharides. Alginate lyase (AL) can be classified into two groups such as G block and M block-specific lyase as well as endolytic and exolytic enzymes based on their substrate specificities and mode of action respectively. Oligosaccharides derived from alginate have innumerable biological activities including immune regulation, anti-coagulation, anti-oxidant, anti-cancer and health-promoting activities. In the present study, *in silico* characterization of the different reported alginate lyases was performed. A total of 50 protein sequences of alginate lyase produced by different microorganisms were retrieved from UniProt database and characterized. Multiple sequence alignment was executed using Clustal Omega. ProtParam tool was used to evaluate physicochemical parameters analyses. Phylogenetic tree was constructed by Neighbor-Joining method using MEGA-X. Motif analysis of alginate lyase sequences was carried out by MEME server. 45 structures of alginate lyase were modelled using SWISS-MODEL. Multiple sequence alignment validates that sequences are highly variable among various alginate lyases reported in different species. Alginate lyase from different species possesses different molecular weights with amino acid residues ranging from 320 to 1059. The pI ranged between 4.42 and 9.59. The variation within these alginate lyases along with other physicochemical parameters was also examined. The active site for the different reported alginate lyases were predicted through CASTp server.

Keywords: Alginate Lyase, Multiple Sequence Alignment, Phylogenetic Analysis, Motif, Homology Modeling, CASTp

Introduction

Alginate is made up of monomeric units, β-D-mannuronate (M) and its C5 epimer α-L-guluronate (G) linked by 1, 4-O-glycosidic bonds. Alginate can be classified into poly- β -D-mannuronate (polyM), poly- α -L-guluronate (polyG) and heteropolymeric regions (1). Alginate lyases are enzymes that use the β-elimination reaction for the breakdown of alginate and generate unsaturated alginate oligosaccharides (2, 3), G block-specific lyase and M block-specific lyase are two classes of alginate lyases. Few enzymes have catalytic activity for both polyM and polyG (4). Alginate is a major structural material present in the cell-wall of brown seaweeds or macroalgae and is produced by bacterial species like *Pseudomonas* and *Azotobacter* (5). In recent years, much attention has been focused on alginate lyase due to biological activities and wide range of applications. Alginate lyases can be isolated from different sources, including marine algae (6), marine mollusks, marine and terrestrial bacteria. Some have been isolated from viruses and fungi (7,8). Based on the mode of action, alginate lyase can be classified into endolytic and exolytic alginate lyase. Endolytic alginate lyase can degrade alginate by cleaving glycosidic bonds and produce unsaturated oligosaccharides (disaccharides, trisaccharides, and tetrasaccharides) and exolytic alginate lyase can degrade oligosaccharides into monosaccharides (9). Alginate lyase has numerous applications in agriculture, pharmaceuticals and food industries. This enzyme plays a key role in the production of oligosaccharides from alginate and the structure determination of alginate (10). Alginate oligosaccharides (AOs) have been reported to exhibit prebiotic, antioxidant, antitumor, immune-stimulatory, antihypertensive, and antidiabetic activities. AOs functions as plant growth promoter and helps in enhancing the stress and drought tolerance (8). Alginate lyase is used in degradation of *Pseudomonas aeruginosa* biofilm. (11) and alginate-derived oligosaccharides are used as a therapy for cystic fibrosis by enhancing antibiotic effect on mucoid *Pseudomonas aeruginosa* (11). Further, these enzymes are employed biorefinery of brown seaweeds. Concerning the industrial and medical importance of this enzyme, the present study was focused on exploring the characteristic features and active site residues of different reported alginate lyase using computational tools.

Computational Tools and Methods

Retrieval of alginate lyase sequences

FASTA format of amino acid sequences of different 50 alginate lyases produced by different microorganisms was retrieved from the UniProt database.

Multiple sequence alignment of alginate lyases

FASTA sequences of different alginate lyases retrieved were aligned using the Clustal Omega.

Phylogenetic analysis of alginate lyases

By first aligning the sequences using ClustalW and then making further predictions using the Neighbour Joining method (NJ), the phylogenetic analysis of the recovered alginate lyase sequences was performed using the MEGA software (12).

Physicochemical parameters of alginate lyases

To estimate the physicochemical parameters of the retrieved alginate lyase sequences, ProtParam was employed (13). The physicochemical properties of the retrieved alginate lyase sequences such as theoretical pI, molecular weight, instability index, amino acid composition, Grand Average of Hydropathicity (GRAVY), and Aliphatic Index (14, 15)] were evaluated.

Motif analysis of alginate lyases

Distribution of the motif in alginate lyase sequences was analysed using MEME server. The sequences were compared and functional analysis was done using protein BLAST (BLASTp) (16, 17).

Homology modeling of alginate lyase structures

The annotated 3D protein structure models can be obtained in the SWISS-MODEL repository database. Crystal structures of five alginate lyase sequences (with accession number A0A2D0ADJ9, A0A084C6X8, A0A086C1H4, Q9KWU1 and O25075) were available in SWISS-MODEL repository. Structures were modelled for rest of the forty five alginate lyase sequences using homology modelling through SWISS-MODEL server (18). Ramachandran plot analysis was employed to assess the model's quality.

Prediction of active sites for reported alginate lyases

Computed Atlas of Surface Topography of Proteins (CASTp) web server, was used to predict the tertiary structure. It aids in identifying and measuring protein surface pockets and inner gaps (19). It was used to predict the active sites of reported alginate lyase (20, 21). The protein structures were analyzed using CASTp server. The surface area of pockets and amino acids present in the pocket were analyzed.

Results and Discussion

Different alginate lyases along with the accession number for which FASTA sequences were retrieved from the UniProt database are mentioned in Table 1. Multiple sequence alignment, phylogenetic tree construction, and motif analysis were performed. Further the physiochemical parameters of the fifty alginate lyase sequences were predicted using various bioinformatics tools.

Table 1: Active Site Prediction for Different Alginate Lyases

S. No	**Source Organism**	**Accession number**	**Rama chandran favoured region(%)**	**Number of Pockets**	**Surface Area of Pockets**	**Amino acids in Pockets**
1	*Pseudomonas nitroreducens*	A0A2D0ADJ9	-	45	504.799	SER68, LYS69, TYR70, ARG77, PHE88, ARG89, ILE92, THR96, GLU99, ARG100, THR142, SER145, MET146, TRP149, ASN204, HIS205, TRP208, ARG255, SER258, TYR259, ASN261, TYR262, PRO265, ASP315, ALA317, ASP318, LEU319, SER323, LYS324, ALA326, SER353, PHE354, ARG355, LEU356.
2	*Pseudomonas capeferrum*	A0A084C6X8	-	51	610.824	SER63, LYS64, TYR65, ARG72, LEU75, LEU77, GLU80, PHE83, ARG84, THR137, SER140, MET141, TRP144, ASN199, ILE92, HIS200, TRP203, ARG250, ALA253, TYR254, ASN256, TYR257, PRO260, ASP310, LYS312, ASP313, LEU314, SER318, LYS319, ALA321, SER348, PHE349, ARG350, LEU351.
3	*Pseudomonas aeruginosa*	A0A086C1H4	-	46	631.812	SER65, LYS66, TYR67, SER72, ARG74, PHE85, ARG86, ILE89, THR93, GLU96, ARG97, HIS138, THR139, SER142, SET143, TRP146, LYS197, ASN200, ASN201, HIS202, TRP205, ASN245, LYS248, ARG249, ARG252, ALA255, TYR256, ASN258, TYR259, PRO262, ASP312, THR314, ASP315, LEU316, ASN320, LYS321, ALA323, ASN349, SER350, PHE351, ARG352, LEU353.
4	*Sphingomonas* sp.	Q9KWU1	-	50	429.021	ARG, ASN, ASP, GLN, GLU, GLY, HIS, ILE, PRO, TRP, TYR, VAL.
5	*Helicobacter pylori*	O25075	-	59	2556.444	ARG26, ASP27, PHE28, LYS29, SER30, ASP32, LEU33, GLN35, LYS36, LEU37, HIS38, LEU53, ASN54, SER56, LYS57, HIS58, VAL64, ARG65, PRO67, ASP68, LYS69, THR71, PHE74, LYS75, ALA78, LEU79, TYR83, ASP83, ALA85, LEU86, GLY87, LEU89, VAL90, LYS92, TYR117, GLN118, GLU120, ASP121, ASN122, ILE123, ASN124, PHE125, TYR126, PRO128, TYR129, MET132, ALA133, TRP135, PHE136, MET153, ARG154, GLN155, TYR156, SER157, GLN158, SER159, ALA160, LEU161, THR163, ASN164, HIS165, GLY166, TRP168, GLY169, ILE170, LEU171, PHE172, ASP173, SER175, LEU178, LEU185, ASN188, ARG192, ARG213, GLY221, GLY222, PRO223, THR224, LYS225, GLY226, ILE227, LYS228, ALA231, TYR232, PHE235, LEU238, THR241, ILE242, GLU245, LEU246, GLU249, ASN293, ALA294, HIS302, TYR303, SER304, SER305, PRO306, SER307, ALA308, ASN309, GLU310, LEU311, LEU312, LYS313, GLY315, ASP316, LEU317, GLU319, ASP320, PHE322, LYS325, LEU326, SER328, PRO329.

6	*Pseudomonas trivialis*	A0A0R2ZU83	95.78	48	798.478	SER62, LYS63, TYR64, LYS69, ARG71, LEU74, GLU79, PHE82, ARG83, THR86, THR90, GLU93, LYS94, HIS135, THR136, SER139, MET140, TRP143, GLU194, LYS195, ASN197, ASN198, HIS199, TRP202, ASN242, LYS245, ARG249, SER252, TYR253, ASN255, TYR256, PRO259, ASP309, LYS311, ASP312, LEU313, LYS315, MET317, LYS318, ALA320, THR347, PHE348, ARG349, LEU350.
7	*Pseudomonas parafulva*	A0A147GDN5	97.07	51	600.166	SER63, LYS64, TYR65, ARG72, LEU75, GLU80, PHE83, ARG84, THR87, THR91, GLU94, LYS95, HIS136, THR137, SER140, MET141, TRP144, ASN199, HIS200, TRP203, ARG250, ALA253, TYR254, ASN256, TYR257, PRO260, ASP310, LYS312, ASP313, LEU314, THR318, LYS319, SER348, PHE349, ARG350, MET351.
8	*Azotobacter beijerinckii*	A0A1H6S8X7	97.30	41	552.917	SER60, LYS61, TYR62, ARG69, LEU72, GLU77, PHE80, ARG81, THR84, THR88, GLU91, ARG92, HIS133, THR134, SER137, MET138, TRP141, ASN196, HIS197, TRP200, ARG247, ALA250, TYR251, ASN253, TYR254, PRO257, ASP308, GLU310, ASP311, LEU312, LYS317, ASN346, PHE347, AR348.
9	*Azotobacter chroococcum*	A0A4U1KWH0	97.27	43	553.255	ALA, ARG, ASN, ASP, GLU, HIS, LEU, LYS, MET, PHE, PRO, SER, THR, TRP, TYR.
10	*Pseudomonas mosselii*	A0A290HDG3	97.31	53	600.737	SER63, SER63, LYS64, TYR65, ARG72, LEU75, GLU80, PHE83, ARG84, THR91, GLU94, LYS95, HIS136, THR137, SER140, MET141, TRP144, ASN199, HIS200, TRP203, ARG250, ALA253, TYR254, ASN256, TYR257, PRO310, ASP310, LYS312, ASP313, LEU314, SER318, LYS319, ALA321, SER348, PHE349, ARG350, LEU351.
11	*Armillaria ostoyae*	A0A284RBB7	90.31	25	204.063	ALA, ASN, ASP, GLN, GLU, GLY, LEU, LYS, PHE, PRO, SER, THR, VAL.
12	*Pseudomonas taiwanensis*	V7DIG1	97.01	48	596.830	SER63, LYS64, TYR65, ARG72, LEU75, GLU80, PHE83, ARG84, THR87, THR91, GLU94, LYS95, HIS136, THR137, SER140, MET141, TRP144, ASN199, HIS200, TRP203, ARG250, ALA253, TYR254, ASN256, TYR257, PRO260, ASP310, LYS312, ASP313, LEU314, SER318, LYS319, SER321, SER348, PHE349, SRG350, LEU351.
13	*Pseudomonas putida*	A0A177SQL8	97.31	39	527.374	SER60, LYS61, TYR62, ARG69, PHE80, ARG81, THR84, THR88, GLU91, ARG92, HIS133, THR13, SER137, MET138, TRP141, ASN196, HIS197, TRP200, ARG247, ALA250, TYR251, ASN253, TYR254, PRO257, ASP307, ASP310, LEU311, SER315, LYS316, ALA318, SER345, PHE346, ARG347, LEU348.
14	*Pseudomonas cannabina*	A0A0P9N1M2	97.01	51	711.112	SER67, LYS68, TYR69, LYS74, ARG76, PHE87, ARG88, THR91, THR95, GLU98, ARG99, HIS140, THR141, SER144, MET145, TRP148, GLU199, LYS200, ASN202, ASN203, HIS204, TRP207, ASN247, LYS250, ARG251, ARG254, SER257, TYR258, ASN260, TYR261, PRO264, ASP314, THR316, ASP317, LEU318, LYS320, PRO322, LYS323, THR352, PHE353, ARG354, LEU355.
15	*Gymnopilus dilepis*	A0A409YJI7	90.01	34	540.176	ALA, ASN, ASP, GLN, GLU, GLY, LEU, LYS, PHE, PRO, SER, THR, VAL.
16	*Cryptococcus wingfieldii*	A0A1E3IM26	91.07	8	119.683	ALA, ASN, GLN, HIS, ILE, LEU, MET, PRO, SER, TYR, VAL.
17	*Absidia glauca*	A0A163MJ41	82.93	96	1125.572	ALA, ARG, ASN, ASP, GLN, GLU, GLY, HIS, ILE, LEU, LYS, MET, PHE, PRO, SER, TYR, VAL.
18	*Pseudomonas guariconensis*	A0A1G7A6T7	97.31	50	629.616	SER64, LYS65, TYR66, ARG73, LEU76, LYS78, GLU81, PHE84, ARG85, THR88, THR92, GLU95, ARG96, HIS137, THR138, SER141, MET142, TRP145, ASN200, HIS201, TRP204, ARG251, ALA254, TYR255, ASN257, TYR258, PRO261, ASP311, HIS313, ASP314, LEU315, SER319, LYS320, SER349, PHE350, ARG351, LEU352.

19	*Parasitella parasitica*	A0A0B7NR67	87.63	72	614.125	ALA, ARG, ASP, GLN, GLU, HIS, ILE, LEU, LYS, MET, PHE, PRO, SER, THR, TYR, VAL.
20	*Streptomyces bingchenggensis*	D7C3V0	91.11	41	738.159	ALA, ARG, ASN, GLN, GLU, GLY, HIS, ILE, LEU, PHE, PRO, SER, THR, TRP, TYR, VAL.
21	*Streptomyces stelliscabiei*	A0A0L0L7D9	90.71	45	1318.046	ALA, ARG, ASN, ASP, CYS, GLN, GLU, GLY, HIS, LEU, MET, PHE, PRO, SER, THR, TRP, TYR, VAL.
22	*Pseudomonas chlororaphis*	A0A1Q8EW13	97.31	48	699.613	SER67, LYS68, TYR69, LYS74, ARG76, PHE87, ARG88, THR91, THR95, GLU98, ARG99, HIS140, THR141, SER144, MET145, TRP148, ASP199, LYS200, ASN202, ASN203, HIS204, TRP207, ASN247, LYS250, ARG251, ARG254, ALA257, TYR258, ASN260, TYR261, PRO264, ASP314, THR316, ASP317, LEU318, SER322, LYS323, ALA325, LYS351, THR352, PHE353, ARG354, LEU355.
23	*Catenulispora sp.*	A0A1Q7W2H9	91.75	52	1647.793	ALA, ARG, ASN, ASP, CYS, GLN, GLU, GLY, HIS, ILE, LEU, LYS, MET, PHE, PRO, SER, THR, TRP, TYR, VAL.
24	*Bifidobacterium primatium*	A0A2M9HAB6	94.32	9	520.846	ALA, ASP, GLY, ILE, LEU, LYS, MET, PHE, PRO, SER, THR, TRP, TYR, VAL.
25	*Panaeolus cyanescens*	A0A409VFV0	88.99	8	4497.720	ALA, ARG, ASN, ASP, GLN, GLU, GLY, ILE, LEU, LYS, MET, PHE, PRO, SER, THR, TRP, TYR, VAL.
26	*Pseudomonas hunanensis*	A0A2I0CJJ3	96.71	52	577.042	SER63, LYS64, TYR65, ARG72, LEU75, GLU80, PHE83, ARG84, THR87, THR91, GLU94, LYS95, HIS136, THR137, SER140, MET141, TRP144, ASN199, HIS200, TRP203, ARG250, ALA253, TYR254, ASN256, TYR257, PRO260, ASP310, THR312, ASP313, LEU314, SER318, LYS319, SER321, SER348, PHE349, ARG350, LEU351.
27	*Dentipellis fragilis*	A0A4Y9YUX5	94.13	16	107.586	ALA, ASN, ASP, GLY, ILE, LEU, LYS, PHE, PRO, SER, THR, TRP, TYR, VAL.
28	*Kwoniella heveanensis*	A0A1B9HL52	95.35	19	42.704	CYS, GLN, ILE, LYS, THR.
29	*Mucor ambiguus*	A0A0C9MAA2	95.35	6	92.491	ARG, ASN, ASP, ILE, LEU, LYS, MET, PHE, TRP.
30	*Rhizophagus irregularis*	A0A2I1G671	95.00	5	65.329	ALA, ILE, LEU, MET, PHE, TRP.
31	*Pseudomonas veronii*	A0A0R3BFA2	96.71	46	796.939	SER66, LYS67, TYR68, LYS73, ALA74, ARG75, LEU78, GLU83, PHE86, ARG87, THR90, THR94, GLU97, LYS98, HIS139, THR140, SER143, MET144, TRP147, GLU198, LYS199, ASN201, ASN202, HIS203, TRP206, ASN246, LYS249, ARG250, ARG253, SER256, TYR257, ASN259, TYR260, PRO263, ASP313, LYS315, ASP316, LEU317, GLU319, MET321, LYS322, ALA324, LYS350, THR351, PHE352, ARG353, LEU354.
32	*Pseudomonas antarctica*	A0A1H0DXW5	97.31	48	628.389	SER62, LYS63, TYR64, ARG71, LEU74, GLU79, PHE82, ARG83, THR86, THR90, GLU93, LYS94, HIS135, THR136, SER139, MET140, TRP143, ASN198, HIS199, TRP202, ARG249, SER252, TYR253, ASN255, TYR256, PRO259, ASP309, LYS311, ASP312, LEU313, GLU315, MET317, LYS318, ALA320, LYS346, THR347, PHE348, ARG349.
33	*Pythium brassicum*	A0A5D6Y6W5	91.19	7	36.044	ASN, ASP, ILE, LEU, PRO, TRP, VAL.
34	*Diversispora epigaea*	A0A397HXH2	94.87	7	43.346	ALA, ARG, ASP, ILE, LEU, PHE, PRO.
35	*Piloderma croceum*	A0A0C3G7M6	89.47	37	2228.268	ALA, ARG,ASN,ASP, CYS, GLN, GLU, GLY, HIS, ILE, LEU,LYS, MET, PHE, PRO, SER, THR, TRP, TYR, VAL.
36	*Aspergillus lentulus*	A0A0S7DJP4	79.07	4	1227.722	ALA, ARG, ASN, ASP, GLU, GLY, HIS, LEU, LYS, MET, PHE, PRO, SER, THR, TRP, TYR.
37	*Valsa mali*	A0A194W6T4	85.71	4	1494.096	LEU354, GLU355, ALA356, PRO357, TRP358, THR359, THR360, GLU361, TYR362, LEU363, ASN364, TYR365, THR366, VAL367, ASN368, SER369, TYR370, GLY371, PHE373, GLU374, PRO375, GLY376, ALA377, GLY378, SER379, LEU380, GLY381, GLU382, GLY383, SER384, GLY385, ARG386, TYR387, ASP388, GLY389, LEU390, GLY391, TRP392, GLY393, SER394, LEU395, LEU396, TYR397.

38	*Fomitopsis rosea*	A0A4Y9YDI1	93.99	18	221.309	VAL317, LEU318, SER321, ILE322, TYR324, VAL325, SER330, ALA336, ALA337, ILE340, GLN390, LYS393, ALA394, PRO398, TRP400, THR401, ARG404, ASP405, MET408.
39	*Streptomyces azureus*	A0A0K8PV81	92.65	49	469.517	LEU39, HIS40, THR41, ASP44, PHE45, MET48, ALA49, VAL52, PRO58, TRP59, GLY62, TRP63, ARG65, LEU66, SER69, TYR104, GLN105, LEU108, ILE112, ILE148, GLN149, TYR151, GLN152, ASN155, ALA208, MET269, VAL272, GLY273, TRP274, GLY276, ALA277, CYS279, GLU280, TYR290, PHE297, LEU309.
40	*Glycomyces buryatensis*	A0A4S8QGJ7	89.13	5	1311.623	GLY713, ALA714, VAL715, TYR716, THR717, THR718, ALA719, SER720, ASN721, GLU722, PRO723, TYR724, GLN725, ALA726, ALA727, LEU728, ALA729, ALA730, ALA731, LYS732, ASP733, ALA734, ALA735, GLU736, ASP737, GLY738, THR739, ASP740, GLU741, GLU742, PHE743, ARG744, THR745, ALA746, PHE747, GLU748, ALA749, LEU750, LEU751, ASP752, GLY753, ILE754, GLU755, ALA756, LEU757, GLU758, LEU759, LEU760.
41	*Aspergillus thermomutatus*	A0A397GRX2	88.10	15	20862.081	LEU23, LEU24, VAL25, SER26, ASP27, SER28, ASP29, ILE30, THR31, ARG32, ALA33, GLN34, GLN35, LYS36, ILE37, LYS38, SER39, ASN40, GLU41, ASP42, PRO43, TRP44, THR45, ALA46, SER47, TRP48, ASN49, VAL50, LEU51, THR52, SER53, LEU54, PRO55, PHE56, ASP58, PRO59, SER60, TYR61, VAL62, SER64, PRO65, VAL66, SER67, VAL68, VAL69, TYR70, ARG71, SER72, ALA73, TRP74, ASP75, ASP76, ASN77, ALA78, GLU79, ASN80, ALA81, GLU82, ASN83, LEU84, TRP85, HIS86, ASP87, VAL88, ALA89, ALA90, ALA91, PHE92, ASN93, LEU94, ALA95, LEU96, AARG97, TRP98, LYS99, ILE100, SER101, SER102, ASN103, THR104, SER105, PHE106, ALA107, ASP108, ALA109, ALA110, SER111, ASN112, ILE113, LEU114, ASP115, ALA116, TRP117, ALA118, THR119, THR120, LEU121, THR122, ALA123, LEU124, GLY125, GLY126, GLY127, ASP128, ASP129, LYS130, TYR131, LEU132, THR133, ALA134, GLY135, LEU136, GLN137, GLY138, TYR139, GLU140, LEU141, ALA142, ASN143, ALA144, ALA145, GLU146, LEU147, LEU148, ARG149, ASP150, TYR151, GLU152, PRO153, PHE154, ALA155, THR156, ASN157, VAL158, LEU159, PRO160, SER161, VAL162, ILE163, ASN164, MET165, ALA166, ASN168, THR168, ILE169, PHE170, ILE171, PRO172, MET173, HIS174, TYR175, LYS176, TRP177, LEU178, HIS179, HIS180, GLU181, GLU182, PRO183, SER184, GLU185, HIS186, ASN187, ILE188, LEU189, HIS190, PHE191, PHE192, ALA193, ASN194, TRP195 GLU196, LEU197, CYS198, ASN199, ILE200, ALA201, SER202, ALA203, MET204, ALA205, MET206, GLY207, VAL208, LEU209, THR210, GLU211, ASN212, GLN213, THR214, VAL215, TRP216, ASP217, PHE218, ALA219, VAL220, ASN221, TYR222, PHE223, LYS224, GLU225, GLY226, ASP227, GLY228, ASN229, GLY230, ALA231, ILE232, ASN233, ASN234, ALA235, ILE236, THR237, ASP238, ILE239, VAL240, ARG241, GLU242, PRO243, GLY244, THR245, GLY246, THR247, PRO248, LEU249, GLY250, GLN251, GLY252, GLN253, GLU254, SER255, GLY256, ARG257, ASP258, GLN259, GLY260, HIS261, SER262, ALA263, LEU264, ASP265, ILE266, GLN267, LEU268, LEU269, ALA270, ALA271, ILE272, GLY273, GLN274, GLN275, ALA276, TRP277, ASN278, GLN279, GLY280, GLU281, ASP282, LEU283, PHE284, GLY285, PHE286, ASN287, ASP288, SER289, ARG290, ILE291, LEU292, ARG293, GLY294, SER295, ALA296, GLU297, GLN298, SER299, ARG300, VAL301, PRO302, SER303, THR304, SER305, GLN306, THR307, ASP308, VAL309, SER310, TYR311, ARG312, ALA313, GLU314, TYR315, TRP316, ALA317, ARG318, TYR319, ASN320, LEU321, GLY322, HIS323, ASP324, VAL325, PRO326, PHE327, VAL328, PRO329, TYR330, THR331, ASN332, GLY333, ILE334, VAL335, SER336, TYR337, THR338, GLU339, ILE340, SER341, ASN342, ALA343, SER344, ARG345, GLY346, ALA347, MET348, ARG349, PRO350, THR351, TRP352, GLU353, LEU354, TYR356, SER357, HIS358, TYR359, VAL360.

42	*Candidatus Sulfopaludibacter* sp.	A0A2N9LZH7	94.71	42	89.097	TRP140, TYR141, ALA144, ILE189, TYR192, SER193, TYR194, GLN195, ARG306.
43	*Enterobacter lignolyticus*	E3G3T5	95.47	60	1559.440	LEU34, THR35, TYR36, ASP37, CYS38, ARG39, GLN40, MET41, ILE44, ILE48, TYR55, ALA58, TRP59, ASN61, LEU62, LYS65, TYR92, SER94, PHE95, GLY96, PRO97, TYR98, TYR99, ARG113, ILE118, SER122, LYS123, SER129, LYS123, SER129, LYS130, LEU132, VAL133, SER136, ASP137, ARG140, ALA141, LEU144, TYR148, GLN181, ILE183, ARG190, ILE192, ILE195, ASP196, ARG198, VAL199, ASP202, VAL203, TRP247, ASN249, HIS251, TRP254, ARG300, ARG302, PHE304, HIS305, TYR306, ASN308, PH309, ALA312, ALA315, ARG316, ARG319, GLU322, ILE323, GLU360, LYS362, TYR363, PRO365, GLU367, ALA368, PRO371, LEU372, ALA375, VAL378, TRP379.
44	*Enterobacter cloacae*	V5B691	97.00	48	828.096	SER58, LYS59, TYR60, SER65, ARG67, ILE70, GLU75, PHE78, HIS79, THR82, ALA86, GLU89, LYS90, THR97, ARG101, HIS131, THR132, SER135, MET136, TRP139, THR143, TRP184, SER190, LYS191, ASN193, HIS195, SER196, TRP198, ASN238, ARG241, ARG242, ARG245, ALA248, TYR249, ASN251, TYR252, GLN255, PHE308, LEU309, GLN311, THR313, ASN314, GLN342, ASN343, ARG344, ARG345, GLY348.
45	*Enterobacter kobei*	A0A2J0PFA7	96.99	63	660.488	SER56, LYS57, TYR58, ARG65, LEU68, ASP73, PHE76, HIS77, THR80, THR84, GLU87, LYS88, HIS129, THR130, SER133, MET134, TRP137, ASN191, ASN192, HIS193, TRP196, ARG240, ARG243, SER246, TYR247, ASN249, TYR250, GLN253, PHE306, THR307, GLU309, SER311, SER312, SER341, THR342, ARG343.
46	*Asticcacaulis biprosthecum*	F4QTL1	91.19	62	829.187	SER61, ARG62, TYR63, ARG70, VAL73, SER78, TYR81, GLU82, VAL85, LEU88, ARG89, SER92, GLN93, GLU135, THR136, PHE139, SER140, LEU143, THR144, ASN193, HIS194, TRP197, ARG244, LEU246, SER247, TYR248, ALA250, TYR251, LEU253, ALA254, CYS286, LEU287, LEU290, LEU307, PRO308, ASN309, GLY310, GLU311, PHE312, TYR313, ASP314, GLY316, ASP317, VAL318, ALA319, TRP320, ARG345, SER346, THR347, ASN348, LEU349.
47	*Enterobacter soli*	A0A198GHF4	95.74	46	237.996	SER52, LYS53, TYR54, SER59, ARG61, PHE72, HIS125, THR126, SER129, TRP133, THR180, LYS181, ASN183, ASN184, HIS185, ASN228, LYS231, ARG232, TYR239, TYR242.
48	*Enterobacter huaxiensis*	A0A3R9Q7F1	94.44	56	551.192	SER52, LYS53, TYR54, SER59, ALA60, ARG61, TYR72, ARG73, THR76, THR80, GLU83, HIS125, THR126, SER129, MET130, TRP133, ALA180, LYS181, ASN183, ASN184, HIS185, TRP188, ASN228, LYS231, ARG232, ARG235, ALA238, TYR239, ASN241, TYR242, GLN245, ARG278, ASP283, GLN285, ASP312, ARG313, ARG314.
49	*Kluyvera intermedia*	A0A447MG36	93.07	14	132.676	ASP138, ALA139, ASP140, THR141, MET143, ASN144, THR162, GLY163, LEU164, ARG168, LEU170.
50	*Enterobacter cancerogenus*	A0A0A3YQN6	90.70	73	1598.807	PHE30, LEU31, SER32, GLU35, MET36, THR39, LEU43, PRO50, GLN51, THR52, GLN54, ALA55, TRP56, GLN58, LEU59, GLN61, ALA62, ARG65, TYR89, SER91, LEU92, SER93, ALA94, TYR95, TRP96, ARG110, VAL115, ASN116, SER119, LYS120, GLN123, GLY126, VAL127, LEU129, ALA130, THR133, ALA134, GLN137, ALA138, LEU141, PHE145, GLN178, ARG187, THR189, LEU192, ASP193, ARG195, TYR196, THR199, ARG200, ASP203, ALA204, ASN247, ASN248, HIS249, TRP252, ARG297, ARG299, PHE301, HIS302, TYR303, TYR305, PHE306, GLN309, THR312, SER313, GLN316, ASN357, ASP359, ARG360, VAL361, SER362, LEU363, ARG364, ILE366, PRO367, SER370, LEU371, ARG374, GLN382, ILE385, HIS386, ILE389.

Multiple sequence alignment analysis of alginate lyase sequences

Multiple sequence alignment of the alginate lyase protein sequences revealed the presence of domains AALVPPQGYYEGIEKLKT and AADLVPPPGYYAAVGERK among alginate lyase sequences of *Pseudomonas species* which are the characteristic domains of alginate lyase A1-III. However, the sequences are highly variable among alginate lyases reported in different species.

Phylogenetic analysis of alginate lyase sequences

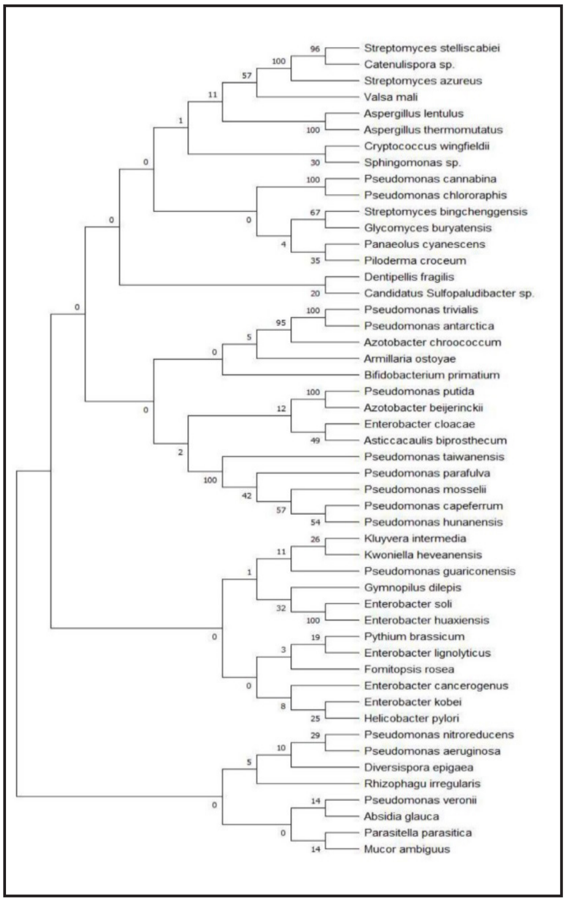


Fig. 1: Phylogenetic Tree of Alginate Lyase Sequences of Different Microorganisms Constructed by the Neighbour-Joining Method

MEGA-X was used to generate the phylogenetic tree using the Neighbor-Joining method. The evolutionary relationship among various alginate lyase sequences reported in different microorganisms (Fig. 1). The boot strap values are mentioned in the nodes of the phylogenetic tree. The alginate lyase of *Pseudomonas cannabina* and *Pseudomonas chlororaphis* form a clade. Similarly, *Pseudomonas taiwanensis, Pseudomonas parafulva, Pseudomonas mosselii, Pseudomonas capeferrum* and *Pseudomonas hunannesis* show evolutionary relationship.

Physicochemical analysis of alginate lyase sequences

Physiochemical parameters like negatively charged residues, positively charged amino acid residues and hydropathicity was also observed among these alginate lyases and were compared in Table 2. From the analysis, it can be viewed that the total number of amino acid residues ranged from 320 to 1059 with varying molecular weight. The pI values ranged from 4.42 to 9.59. The instability index is a measure of a protein's in vivo half-life (10). Proteins with an in vivo half-life of less than 5 hrs have an instability index of more than 40, whereas proteins with a half-life of more than 16 hrs have an instability index of less than 40 (3). Out of 50 alginate lyases, 30 sequences have half-life more than the 16 hrs while rest of the sequences exhibit half-life of less than 5 hrs in vivo. The aliphatic index of a protein is a measurement of the relative volume occupied aliphatic side chains of amino acids like alanine, valine, leucine, and isoleucine. As the aliphatic index rises, so does the thermal stability of globular proteins. The protein sequence-based analysis of aliphatic index among these enzymes revealed that the alginate lyases taken for this study were found to be thermostable due to the high value of aliphatic index.

Table 2: Physicochemical Parameters of Retrieved Alginate Lyase Sequences

S. no	Organisms	Number of amino acids	Molecular weight	Theoretical pI	Total number of negatively charged residues (ASP + GLU)	Total number of positively charged residues (ARG + LYS)	Instability index	Aliphatic index	Grand average of hydropathicity
1	*Pseudomonas nitroreducens*	371	41310.44	8.47	42	45	34.56	63.45	-0.570
2	*Pseudomonas capeferrum*	367	41235.73	9.05	43	50	35.11	71.69	-0.519
3	*Pseudomonas trivialis*	369	41785.60	8.91	44	50	37.85	70.68	-0.566
4	*Pseudomonas parafulva*	367	41041.69	9.53	35	48	25.67	72.78	-0.429
5	*Azotobacter beijerinckii*	372	40953.31	6.14	48	45	47.39	73.90	-0.372
6	*Azotobacter chroococcum*	373	41300.95	6.97	48	48	43.48	75.23	-0.399
7	*Pseudomonas mosselii*	366	41018.59	9.13	41	49	29.23	73.44	-0.484
8	*Armillaria ostoyae*	853	93476.86	5.38	105	84	42.53	66.73	-0.538
9	*Pseudomonas taiwanensis*	367	41184.78	9.35	41	53	34.67	69.29	-0.577
10	*Pseudomonas putida*	363	40683.86	9.08	41	48	32.23	71.87	-0.538
11	*Pseudomonas cannabina*	378	42635.45	8.64	49	53	31.50	68.49	-0.602
12	*Gymnopilus dilepis*	904	97489.85	5.66	94	80	46.20	61.34	-0.183
13	*Cryptococcus wingfieldii*	444	48875.59	4.42	52	24	34.87	79.64	-0.356
14	*Absidia glauca*	953	109226.54	8.32	103	106	45.95	77.61	-0.584
15	*Pseudomonas aeruginosa*	367	40774.11	8.88	42	47	39.02	72.15	-0.447
16	*Pseudomonas guariconensis*	368	41068.54	9.35	39	50	38.02	69.62	-0.579
17	*Parasitella parasitica*	482	50244.32	9.24	58	68	46.1	70.10	-0.475
18	*Streptomyces bingchenggensis*	472	48631.12	6.53	43	40	24.88	75.97	-0.115
19	*Streptomyces stelliscabiei*	509	53545.65	8.53	39	43	34.50	63.63	-0.344
20	*Pseudomonas chlororaphis*	374	41942.47	8.46	47	50	35.56	66.90	-0.571
21	*Catenulispora sp.*	1128	119492.50	5.00	127	96	15.16	71.50	-0.404
22	*Bifidobacterium primatium*	474	52332.63	5.26	44	36	31.55	68.86	-0.317
23	*Panaeolus cyanescens*	1059	117611.30	9.17	80	99	50.71	78.88	-0.277

24	*Pseudomonas hunanensis*	367	41057.63	9.29	40	50	28.39	72.21	-0.504
25	*Dentipellis fragilis*	899	104529.96	6.51	121	112	47.84	78.23	-0.655
26	*Kwoniella heveanensis*	404	42206.93	5.49	27	24	34.17	73.91	-0.049
27	*Mucor ambiguus*	926	107721.88	6.43	121	113	49.86	81.71	-0.556
28	*Rhizophagus irregularis*	902	98308.87	6.64	101	97	43.40	91.42	-0.131
29	*Pseudomonas veronii*	373	42215.10	8.80	45	50	33.36	70.70	-0.555
30	*Pseudomonas antarctica*	369	41549.22	8.66	44	48	36.40	69.11	-0.542
31	*Pythium brassicum*	1010	118528.37	5.94	129	113	40.14	74.32	-0.732
32	*Diversispora epigaea*	641	71003.43	7.98	70	72	32.31	72.96	-0.380
33	*Sphingomonas sp.*	461	50731.59	4.65	34	18	32.37	73.75	-0.154
34	*Piloderma croceum*	501	53492.36	5.86	47	34	43.49	73.95	-0.298
35	*Aspergillus lentulus*	535	56017.21	4.44	50	20	30.93	77.29	-0.079
36	*Valsa mali*	679	72740.45	5.26	74	59	36.34	62.44	-0.457
37	*Fomitopsis rosea*	485	51776.78	6.33	57	53	34.39	73.42	-0.411
38	*Streptomyces azureus*	930	101173.63	4.40	146	70	23.69	69.55	-0.392
39	*Glycomyces buryatensis*	528	57057.71	4.79	56	24	43.66	74	-0.315
40	*Aspergillus thermomutatus*	426	46000.81	9.27	42	52	40.16	65.45	-0.519
41	*Candidatus Sulfopaludibacter sp.*	410	46138.88	6.02	47	43	30.70	78.63	-0.453
42	*Enterobacter lignolyticus*	358	39382.91	9.35	36	32	48.20	82.01	-0.175
43	*Enterobacter cloacae*	352	38862.70	6.67	37	35	45.20	78.61	-0.323
44	*Enterobacter kobei*	374	40316.83	9.68	37	47	42.74	89.47	-0.201
45	*Asticcacaulis biprosthecum*	329	37588.92	8.64	32	36	48.22	81.61	-0.300
46	*Helicobacter pylori*	325	36733.84	8.81	33	38	38.53	79.66	-0.362
47	*Enterobacter soli*	325	36733.84	8	33	38	38.53	79.66	-0.362
48	*Enterobacter huaxiensis*	325	36572.88	9.19	33	41	40.46	85.42	-0.330
49	*Kluyvera intermedia*	379	43846.79	6.23	44	36	46.32	85.96	-0.425
50	*Enterobacter cancerogenus*	422	47544.65	9.49	35	43	43.59	79.24	-0.488

Motif analysis of Alginate lyase sequences

From the analysis, it can be inferred that 16 motifs were distributed among 49 alginate lyase sequences of different microorganisms. Motif location with sequence length ranging from 15 to 50 as shown in Table 3. Motif 1 was observed in *Pseudomonas capeferrum, Pseudomonas trivialis, Pseudomonas parafulva, Azotobacter beijerinckii, Azotobacter chroococcum, Pseudomonas mosselii;* Motif 2 was observed in *Pseudomonas nitroreducens, Pseudomonas capeferrum, Pseudomonas trivialis, Pseudomonas parafulva, Azotobacter beijerinckii, Azotobacter chroococcum, Pseudomonas mosselii, Enterobacter lignolyticus, Enterobacter cloacae, Enterobacter kobei, Asticcacaulis biprosthecum, Helicobacter pylori, Enterobacter soli, Enterobacter huaxiensis, Kluyvera intermedia, Enterobacter cancerogenus;* Motif 3 was observed in *Pseudomonas nitroreducens, Pseudomonas capeferrum, Pseudomonas trivialis, Pseudomonas parafulva, Azotobacter beijerinckii, Azotobacter chroococcum, Pseudomonas mosselii, Enterobacter kobei, Enterobacter soli, Enterobacter huaxiensis;* Motif 4 was observed in *Pseudomonas nitroreducens, Pseudomonas capeferrum, Pseudomonas trivialis, Pseudomonas parafulva, Azotobacter beijerinckii, Azotobacter chroococcum, Pseudomonas mosselii, Enterobacter cloacae, Enterobacter kobei, Enterobacter soli, Enterobacter huaxiensis;* Motif 5 was observed in *Pseudomonas taiwanensis, Pseudomonas capeferrum, Pseudomonas guariconensis, Pseudomonas parafulva, Pseudomonas veronii, Pseudomonas trivialis, Pseudomonas antarctica, Pseudomonas hunanensis, Pseudomonas putida, Pseudomonas mosselii, Pseudomonas chlororaphis, Pseudomonas cannabina, Pseudomonas aeruginosa, Pseudomonas nitroreducens, Azotobacter chroococcum, Azotobacter beijerinckii, Enterobacter huaxiensis, Enterobacter soli, Enterobacter kobei, Enterobacter cloacae ;* Motif 6 was observed in *Streptomyces bingchenggensis, Catenulispora sp., Streptomyces stelliscabiei, Streptomyces azureus, Valsa mali, Aspergillus thermomutatus, Aspergillus lentulus, Bifidobacterium primatium;* Motif 7 was observed in *Fomitopsis rosea, Armillaria ostoyae, Piloderma croceum, Gymnopilus dilepis, Panaeolus cyanescens, Dentipellis fragilis, Cryptococcus wingfieldii, Enterobacter cancerogenus, Kluyvera intermedia, Enterobacter lignolyticus;* Motif 8 was observed in *Pseudomonas taiwanensis, Pseudomonas parafulva, Pseudomonas hunanensis, Pseudomonas mosselii, Pseudomonas capeferrum, Pseudomonas putida, Pseudomonas trivialis, Pseudomonas antarctica, Pseudomonas veronii, Pseudomonas cannabina, Pseudomonas chlororaphis, Pseudomonas guariconensis, Azotobacter chroococcum, Azotobacter beijerinckii, Pseudomonas aeruginosa;* Motif 9 was observed in *Mucor ambiguus, Parasitella parasitica, Absidia glauca, Diversispora epigaea, Rhizophagus irregularis, Pythium brassicum;* Motif 10 was observed in *Aspergillus thermomutatus, Aspergillus lentulus, Streptomyces azureus, Streptomyces bingchenggensis, Catenulispora sp., Valsa mali, Streptomyces stelliscabieii, Bifidobacterium primatium;* Motif 11 *Pseudomonas mosselii, Pseudomonas hunanensis, Pseudomonas guariconensis, Pseudomonas cannabina, Pseudomonas taiwanensis, Pseudomonas parafulva, Pseudomonas chlororaphis, Pseudomonas capeferrum, Pseudomonas putida, Pseudomonas antarctica, Pseudomonas veronii, Pseudomonas trivialis, Azotobacter chroococcum, Pseudomonas aeruginosa, Pseudomonas nitroreducens, Azotobacter beijerinckii, Enterobacter huaxiensis, Enterobacter soli, Enterobacter kobei;* Motif 12 was observed in *Mucor ambiguus, Parasitella parasitica, Absidia glauca, Diversispora epigaea, Rhizophagus irregularis;* Motif 13 was observed in *Mucor ambiguus, Parasitella parasitica, Absidia glauca, Rhizophagus irregularis, Diversispora epigaea;* Motif 14 was observed in *Dentipellis fragilis, Armillaria ostoya, Mucor ambiguuse,* *Parasitella parasitica, Absidia glauca, Diversispora epigaea, Gymnopilus dilepis, Fomitopsis rosea, Enterobacter cancerogenus, Piloderma croceum, Rhizophagus irregularis, Panaeolus cyanescens, Kluyvera intermedia, Enterobacter lignolyticus, Cryptococcus wingfieldii;* Motif 15 was observed in *Dentipellis fragilis, Armillaria ostoyae, Mucor ambiguus, Parasitella parasitica, Absidia glauca, Diversispora epigaea, Gymnopilus dilepis, Fomitopsis rosea, Enterobacter cancerogenus, Piloderma croceum, Rhizophagus irregularis, Kluyvera intermedia, Enterobacter lignolyticus, Cryptococcus wingfieldii;* Motif 16 was observed in *Catenulispora sp., Streptomyces azureus, Enterobacter cancerogenus, Streptomyces stelliscabie, Kluyvera intermediai, Streptomyces bingchenggensis, Cryptococcus wingfieldi, Piloderma croceumi, Valsa mali, Rhizophagus irregularis, Bifidobacterium primatium, Mucor ambiguus, Parasitella parasitica, Aspergillus thermomutatu, Aspergillus lentuluss, Diversispora epigaea, Candidatus Sulfopaludibacter* sp.*, Glycomyces buryatensis, Pythium brassicum, Dentipellis fragilis, Absidia glauca, Helicobacter pylori, Enterobacter lignolyticus, Sphingomonas sp., Fomitopsis rosea, Gymnopilus dilepi, Armillaria ostoyaes, Panaeolus cyanescens*. These 16 motifs were subjected to BLASTp and it was observed that all the 16 motif sequences correspond to mannuronate (Poly M) specific alginate lyases.

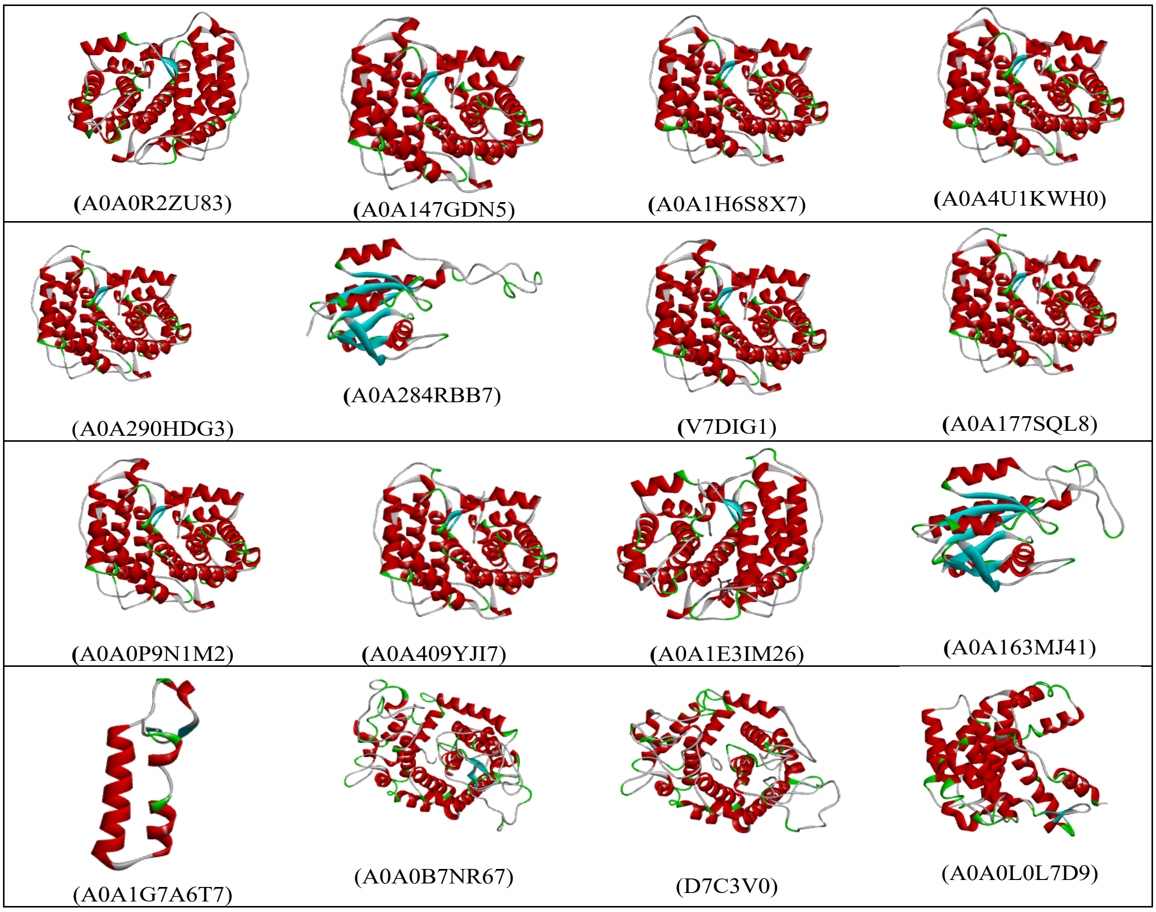
Table 3 Motif Analysis of Alginate Lyases sequences

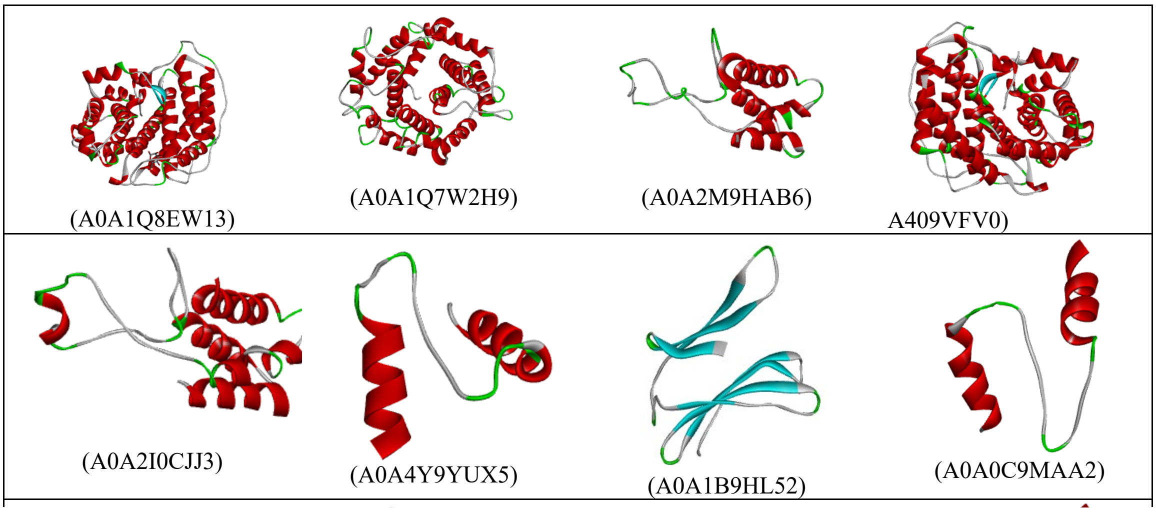
Motif number	Sequence length	Sequences
1.	50	NNHSYWAAWSVMATAVATBRRDLFDWAVKEYKVAANQVDDQGFLPNELKR
2.	50	CALTWLTTWARADALMSTDYNHTGKSMRKWALGSMSGSWLRLKFSNSQPL
3.	50	NFRCEAAPAPYTGSLQFRSKYEGSDKARATLNAQSEKAFRDSTKDITDLE
4.	41	KYAWLEPYCALYTCAPDTLERKHGMQPFKSFRLGGDLTRVY
5.	50	QRALAYHNYALPPLAMIASFAQVNGVDLRQENNGALQRLGERVLAGVKDP
6.	29	AEAZLIEKWFARLADQVVRDWSBLPLEKI
7.	50	LGQWQESGRDQGHSLLGVGLMGTICZMAWNQGEDLYGYDDSRFLKGAEYV
8.	46	FNKQYADQIAADGEQPLEAARTRPFHYRCFNLEAMITNAKJGDYLG
9.	21	AAAALVPPQGYYAGIEKLKTG
10.	50	KYFVVPMTRLLNNSVLLNNLDERPKTPEEPQIIFRHDATEEYNENMRYGR
11.	50	ITHYWANWELCNMASAMAIGVLTDBQAVWDRAVDYFKNGDGNGAIKNAIP
12.	15	RGVSKMVMQYMRDGR
13.	50	YTYHDKNLYAMNNNGGRNTAIEHGRSLPNAKWIMPFDGNCYLSHNGFEEI
14.	50	RDKIILYRIIGNDLPPRHKEGQTLSNLQFILEHEPSFPBTKKIFJLNRII
15.	29	YLEWLVTSPTGIEEASAPNNHGTLYDLQV
16.	21	WYFTGDSRYADKAABIJRAWF

Modeling of alginate lyase structure by homology modeling

The sequences of alginate lyase were submitted in SWISS-MODEL and the structures were generated. The model quality was evaluated by Ramachandran plot analysis and is presented in Table 1. The predicted model structures of alginate lyase of *Absidia glauca, Parasitella parasitica, Panaeolus cyanescens, Piloderma croceum, Aspergillus lentulus, Valsa mali, Glycomyces buryatensis and Aspergillus thermomutatus* have Ramachandran favoured region below 90% during homology modeling. Five structures of alginate lyase retrieved from

SWISS-MODEL repository, while the other structures were modelled using homology modelling as depicted in Fig. 2.





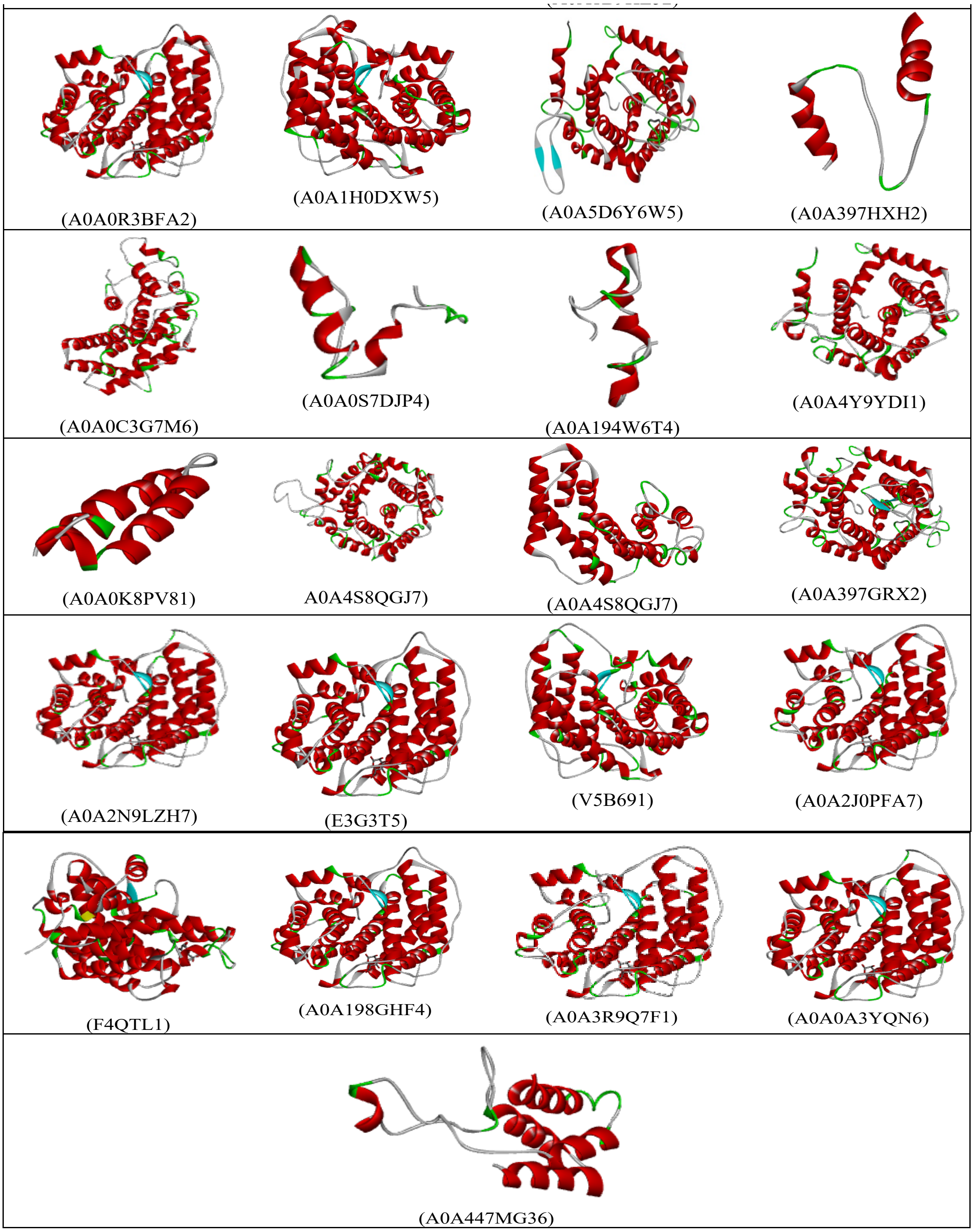


Fig. 2: Structures of Alginate Lyases predicted by Homology Modeling

Prediction of active sites for reported alginate lyases

The number of active sites, surface area of pockets and amino acids present in the pocket of reported alginate lyases was predicted using Computed Atlas of Surface Topography of proteins (CASTp) server. This analysis serves as a basis for protein ligand interaction studies. The number of pockets was calculated for reported alginate lyases ranges from 39-51 as shown in Table 1. The surface area of pockets of the reported alginate lyases were predicted from 500 to 798 using CASTp server. The amino acids present in the pocket of reported alginate lyases were serine, lysine, alanine, arginine, asparagine, aspartic acid, cysteine, leucine, lysine, phenylalanine, proline, serine, threonine and tryptophan and also active site pockets for different alginate lyases was shown as Table 1.

Conclusion

The present work focuses on *in silico* characterization of the different reported alginate lyases. The alginate lyases ranges from UniProt. Multiple sequence alignment revealed the conserved regions among different alginate lyases. The total number of amino acid residues ranged from 320 to 1059 with varying molecular weights. The pI varied between 4.42 and 9.59. Variability was also noted in terms of other physiochemical parameters such as negative charge residues (Asp and Glu), positively charged amino acid residues, and hydropathicity among these alginate lyases (GRAVY). Motifs were analysed using MEME server and the analysis using BLASTp shown as mannuronate (Poly M) specific alginate lyase. 3D structures of the enzyme sequences were modelled using SWISS-MODEL server. Using the Computed Atlas of Surface Topography of Proteins (CASTp) server, the number of active sites, surface area of pockets, and amino acids contained in the pocket of the reported alginate lyase were predicted. The number of pockets was calculated for different reported alginate lyase as ranges from 4-96. This study provides valuable information on different alginate lyase produced by various microbes in terms of structural features and catalysis that can be exploited in designing or engineering of enzymes for various applications.

Acknowledgement

The authors would like to acknowledge the support provided by the management of Kalasalingam Academy of Research and Education to carry out this work.

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