Review

Alginate Lyases from Marine Bacteria: An Enzyme Ocean for Sustainable Future

Noora Barzkar 1,*, Ruilong Sheng 2,3,*, Muhammad Sohail 4,*, Saeid Tamadoni Jahromi 5,*, Olga Babich 6,*, Stanislav Sukhikh 6 and Reza Nahavandi 7

1 Department of Marine Biology, Faculty of Marine Science and Technology, University of Hormozgan, Bandar Abbas 3995, Iran
2 CQM—Centro de Química da Madeira, Campus da Penteada, Universidade da Madeira, 9000-390 Funchal, Portugal; ruilong.sheng@staff.uma.pt
3 Department of Radiology, Shanghai Tenth People’s Hospital, School of Medicine, Tongji University, Shanghai 200072, China
4 Department of Microbiology, University of Karachi, Karachi 75270, Pakistan; msohail@uok.edu.pk
5 Persian Gulf and Oman Sea Ecology Research Center, Iranian Fisheries Sciences Research Institute, Agricultural Research Education and Extension Organization (AREEO), Bandar Abbas 9145, Iran; stamadoni@gmail.com
6 Institute of Living Systems, Immanuel Kant Baltic Federal University, A. Nevskogo Street 14, Kaliningrad 236016, Russia; olich.43@mail.ru (O.B.); stas-asp@mail.ru (S.S.)
7 Animal Science Research Institute of Iran (ASRI), Agricultural Research, Education and Extension Organization (AREEO), Karaj 8361, Iran; mahavandi@gmail.com
* Correspondence: noora.barzkar@gmail.com or barzkar.phd@hormozgan.ac.ir

Abstract: The cell wall of brown algae contains alginate as a major constituent. This anionic polymer is a composite of β-D-mannuronate (M) and α-L-guluronate (G). Alginate can be degraded into oligosaccharides; both the polymer and its products exhibit antioxidative, antimicrobial, and immunomodulatory activities and, hence, find many commercial applications. Alginate is attacked by various enzymes, collectively termed alginate lyases, that degrade glycosidic bonds through β-elimination. Considering the abundance of brown algae in marine ecosystems, alginate is an important source of nutrients for marine organisms, and therefore, alginate lyases play a significant role in marine carbon recycling. Various marine microorganisms, particularly those that thrive in association with brown algae, have been reported as producers of alginate lyases. Conceivably, the marine-derived alginate lyases demonstrate salt tolerance, and many are activated in the presence of salts and, therefore, find applications in the food industry. Therefore, this review summarizes the structural and biochemical features of marine bacterial alginate lyases along with their applications. This comprehensive information can aid in the expansion of future prospects of alginate lyases.

Keywords: alginate; alginate oligosaccharides (AOs); alginate lyase; marine bacteria; brown algae; applications

1. Introduction

The marine ecosystem is considered the largest ecosystem, covering ~70% of this planet [1–10] and giving it a unique feature in the known universe. Reportedly, marine ecosystems make up the habitat of >80% of the living beings found on earth [11–14]. Among marine vegetation, brown algae (Phaeophyceae) holds a distinct position owing to its abundance [15]. Indeed, it plays a major role in CO₂ removal and carbon storage for coastal regions [16].

Primarily, brown algae have a complex sugar composition, mainly including mannitol, laminarin, and alginate [17]. Mannitol is an alcohol derived from mannose, whereas laminarin is a polymer of β-1,3-linked glucose residues branched at 1,6-b [18,19]. Mannitol and laminarin are carbohydrate reserves that are accumulated by the algae during summer, and
the content may reach 25–30% at the onset of autumn [20]. However, the major constituent of the brown algae polysaccharide repertoire is alginate [21], which makes up ~45% of the dry weight. There are various types of alginates according to the arrangement of monomers, β-D-mannuronate (M) and α-L-guluronate (G), either arranged in homopolymeric (polyM, polyG) or heteropolymeric (polyMG) fashion [22,23]. This anionic polymer serves as an important source of carbon for many marine microorganisms [21]. Commercially, alginates are extracted from different species of brown seaweeds, such as Ascophyllum nodosum, Durvillaea potatorum, Ecklonia arborea, Ecklonia radiata, Laminaria digitata, Lessonia nigrescens, Laminaria hyperborea, Lessonia trabeculata, Macrocystis pyrifera, Saccharina japonica, and Sargassum spp. [24,25].

Laminarin and mannitol are chemically less complex and, hence, can be converted by microbes into bioethanol, while alginates do not serve as a readily degradable carbon source. The structural complexity of alginates necessitates the activity of various lyases for its complete degradation; the enzymes are collectively called alginate lyases. These enzymes catalyze the degradation of glycosidic bonds through β-elimination [26] but vary in substrate specificity and, hence, are classified as polymannuronate (M) lyases, polyguluronate (G) lyases, and polyMG-specific lyases. The enzymes can also be distinguished on the basis of catalytic patterns as some act on the terminal residues (exo-enzymes) while others act randomly on the polymer chain (endo-enzymes). Studies on homology in amino acid sequences led to the classification of alginate lyases into polysaccharide lyase (PL) families. Structural elucidation revealed considerable heterogeneity, and the enzymes could be categorized into four groups, including β-jelly roll, (α/α)n toroid, β-helix fold, or (α/α)n toroid + β-jelly roll structures [27,28]. Alginate lyases are also diversified in terms of their molecular masses and grouped into large- (>60 kDa), medium (~40 kDa), and small sizes (25–30 kDa) [29].

Alginate lyases share tremendous applications with other industrial enzymes and are applied in agriculture, food, cosmetics, drug delivery, and biomedicine industries. Various organisms have been reported for the production of alginate lyases with varying substrate specificities. The enzyme producers include marine algae [30,31], marine mollusks [32,33], viruses (Chlorella virus) [34], fungi (Corollospora intermedia) [35], yeast (Meyerozyma guilliermondii) [36], and many terrestrial [29,37–39] and marine [40–42] bacteria. Nonetheless, bacteria are the far most important producers of alginate lyases. Considering the habitat and evolutionary history of the marine organisms, alginate lyases obtained from marine sources often exhibit remarkable salt tolerance and even salt activation [43–46]. Vibrio harveyi-28, a marine isolate, produced alginate lyase with a 24-fold increase in activity in the presence of 1 M NaCl [47]. Interestingly, some marine bacteria, such as Pseudomonas aeruginosa and Azotobacter, have the ability to produce alginate lyases, although they are incapable of utilizing alginate as a carbon source [48]. This review has collected the updated information about enzymatic and biochemical features and the applications of alginate lyases from marine bacteria.

2. Alginate and Alginate Lyases

Alginate is an abundant source of carbon in marine habitats. The cell wall of brown algae (Phaeophyceae) contains alginate, and since there are hundreds of species of brown algae, the material exists in large quantities. Some species, such as Saccharin japonica and Udaria pinnatifida, contain alginate that accounts for up to 45% of their dry weight [21]. Apart from brown algae, some species of bacteria produce alginate as a major component of extracellular polysaccharides or biofilms [49]. The bacterial alginate is constituted by the 1,4-glycoside bond-linked uronic acids, i.e., α-L-guluronic acid (G) and β-D-mannuronic acid (M) [50]. These basic units are arranged in different forms constituting three types of blocks, including poly α-L-guluronic (polyG), poly β-D-mannuronic (polyM), and their heteropolymer (polyGM), in which monomers are linked by a 1-4 glycosidic bond [51,52]. Alginate demonstrates various bioactivities and, hence, is widely employed in food and biomedicine industries. However, the applications are hindered by its high molecular
weight, low water solubility, and unsatisfied bioavailability [53]. Degradation of alginate through chemicals (acid or alkali) or by physical process (such as microwave degradation) or through enzymatic action (by alginate lyases) yields alginate oligosaccharides with varying degrees of polymerization from 2 to 25. Owing to their high solubility and smaller molecular mass, alginate oligomers demonstrate different physiological activities, including antioxidative and immunomodulatory potential, have the capability of regulating blood sugar and blood lipids and can act as plant growth promoters [54,55].

The synthesis of alginate oligosaccharides by physical methods is energy extensive and can result in structural changes in the products. Enzymatic degradation methods are comparatively eco-friendly, energy-saving, and selective, and the products are biologically more active [56]. The enzyme-based methods employ the use of alginate lyases that catalyze β-elimination of glycosidic bonds. The enzymatic degradation of alginate yields various oligosaccharides, such as 4,5-unsaturated uronides, mannanuronate (ΔManUA), and guluronate (ΔGulUA) [57].

Alginate lyases vary in their substrate specificities depending on the amino acid sequence of the enzyme and the arrangement of monosaccharide residues in the substrate. Some lyases recognize mannanurate-containing substrates (PolyMlyases; EC 4.2.2.3), some can act on polymers of guluronoate (PolyGlyases; EC 4.2.2.11), while some are capable of converting heteropolymers, i.e., (PolyMGlyases; EC 4.2.2.-) [56,58,59]. The action of these enzymes is utilized to determine the type of the polymer and to synthesize oligosaccharides of particular types. This is of particular interest as types of linkages between the substrate molecules (M-M, M-G, G-M, and G-G) can also be recognized by these enzymes [51]. These lyases can also be distinguished on the basis of the catalytic pattern as exo- or endo-acting enzymes [60]. Exo-alginate lyases release monomers as the ultimate products, while endo-alginate lyases randomly degrade the polymer and mainly release a mixture of unsaturated oligosaccharides, including di-, tri-, and tetra-saccharides [61]. Based on the amino acid sequence alignment, alginate lyases can be classified into different polysaccharide lyase (PL) families, including PL5, PL6, PL7, PL8, PL14, PL15, PL17, PL18, PL31, PL32, PL34, PL36, PL39, and PL41 families, which are listed in the Carbohydrate-Active enzymes (CAZy) database (http://www.cazy.org/, accessed on 25 September 2021). Alginate lyases also exhibit a great variation in their structure, substrate specificity, and mechanism of action. It is worth noting that the enzymes provide efficient catalysts to produce oligosaccharides of variable length and different types under mild reaction conditions [62]. These functional oligosaccharides are in great demand [63–65], particularly when the raw material does not compete with the food resources [66,67]. The derivatization of the products of these enzymes has the potential to develop new and improved antibiotics with the emphasis on removing biofilms produced by pathogens such as *Pseudomonas* sp. [68].

3. Marine Sources of Alginate Lyase

In the past decades, alginate lyases have been isolated and purified from various marine organisms, including marine bacteria (*Pseudomonas* [69], *Vibrio* [70]), marine fungi [71], marine algae (*Laminaria, Saccharina* [72]), and marine mollusks (*Haliotis discushannai*) [59]. Inoue et al. identified a novel alginate lyase from the brown alga *Saccharina japonica* [31]. Al-
ginate lyase activity has been detected within the extracts from several brown algae species, including *Laminaria digitata* [73], *Pelvetia canaliculata* [74], and *Undaria pinnatifida* [75], and has also been measured in the mid-gut gland of *Turbo cornutus* [76], the hepatopancreas of *Littorina* spp. [32] and *Dolabella auricula* [77], and the crystalline style of marine mussels *Chromotyulus meridionalis* and *Perna perna* [78]. The alginate lyases secreted into the guts of various mollusks may facilitate the digestion process of devoured brown algal tissues. Furthermore, the largest variety of alginate lyases was discovered in marine bacteria, which served as the major sources [79]. For instance, Zhu et al. cloned an alginate lyase FsAlyPL6 from marine bacteria *Flammeovirga* sp. NJ-04 [80]. Zhu et al. reported that *Serratia marcescens* NJ-07 can produce alginate lyase [81]. Furthermore, the alginate lyase-producing marine bacteria are *Pseudomonas* sp. [82], *Photobacterium* sp. [83], *Vibrio* sp. [84], *Defluviitalea phaphypila* [85], *Klebsiella aerogenes* type 25 [86], *Pseudomonas alginovora* XO17 [87], *Bacillus* sp. [42,88], *Corynebacterium* sp. ALY-1 [89], *Zobellia galactanivorans* [90], and *Agarivorans* sp. [91].

4. Alginate Lyase-Producing Marine Bacteria

Large quantities of alginates are produced by various algae in the ocean every year, they serve as nutrient resources for heterotrophic marine bacteria and, thus, play an ecological role in coastal ecosystems, similar to that of cellulose and hemicellulosic biomass in terrestrial environments. Various alginate lyases produced by marine microbes play important roles in marine alginate degradation. A couple of alginate lyases were separated from different kinds of microorganisms in the past several years, especially from the bacteria on brown algae (such as *Bacillus* sp. obtained from rotten seaweed) [88], *Paenibacillus algicola* isolated from rotten brown algae samples collected from China [92], and *Pseudoalteromonas* sp. SM0524 separated from marine kelp residues [93]. Alginate-degrading bacteria were screened and identified from brown algae collected from a French beach and the Arctic region, which belonged to the classes Gamma-proteobacteria and Flavobacteria of the phylum Proteobacteria and Bacteroidetes [94,95]. Wang et al. (2017) reported that 12 different bacterial strains belonging to eight genera were recovered from the three brown algae (*Laminaria japonica*, *Sargassum horneri* and *Sargassum siliquastrum*) samples obtained from the coast of Nanhuangcheng Island, China, capable of excreting alginate lyases [25]. In addition, an alginate lyase-producing bacteria *Vibrio* sp. QD-5 was isolated from rotten kelp [96]. Strain BP-2 producing the alginate lyase was screened and identified from rotten *Sargassum* collected from Weizhou Island, China [97].

5. Enzymatic Properties of Alginate Lyases from Marine Bacteria

Most of the marine-based alginate lyases are endolytic enzymes, which could break down glycosidic bonds of alginate and thus produce unsaturated oligosaccharides (Table 1). Endolytic alginate lyases were employed to prepare AOSs with various DPs. For example, Swift et al. discovered an endo-type alginate lyase AlgMsp from a marine bacterium *Microbulbifer* sp. 6532A, which produces AOSs DP2-5 [46]. Alg7D, an endo-type alginate lyase separated from *Saccharophagus degradans* 2-40T mainly produced oligosaccharides with a DP of 3–5 [98]. It has been disclosed that depolymerized low DP alginate prepared through an enzymatic converter possesses various kinds of biological activities [63,99]. Nguyen et al. prepared a series of AOSs with the potential for efficient production of low DP alginate oligosaccharides by using a new marine actinobacterium-produced alginate lyase AlyD444 *Streptomycetes luridiscabiei* [100]. In addition, Aly-IV from *Vibrio* sp. QD-5 [96] and AlgA from *Pseudomonas* sp. E03 [101] are two novel endolytic alginate lyase enzymes that can release a range of AOSs with low DP. In addition, a few exolytic alginate lyases could directly monomerize alginate to a monosaccharide [102] (Table 1). Interestingly, novel alginate lyases isolated from *Microbulbifer* sp. SH-1 [103] and BP-2 strain [97] demonstrated both exolytic and endolytic cleavage activities.

Substrate-specific alginate lyases are able to be utilized for determining sequences of alginate substrates and producing oligosaccharides with certain structures. The substrate
specificities of these alginate lyases largely rely on their architectures, amino acid residues, and the alignment of the saccharide residues in the substrate. Various alginate lyases could recognize four different types of linkages, including G–G, M–M, G–M, and M–G. The ALG-5 from _Streptomyces_ sp. ALG-5 depolymerizes the polyG substrate [104]. The Alyw203 from _Vibrio_ sp. W2 is also a polyG-specific alginate lyase [105]. High-alkaline alginate lyase, A1m, is a kind of mutant enzyme with cleavage specificity for the G–G linkage [91]. In addition, AlyPB2 from _Photobacterium_ sp. FC615 specifically depolymerizes polyM [83]. However, there are several alginate lyases showing activities in both of them such as the lyases from _Vibrio_ sp. QY108 [106], _Cobetia_ sp. NAP1 [107], _Pseudoalteromonas_ sp. SM0524 [93], _Pseudoalteromonas carrageenovora_ ASY5 [108], _Agarivorans_ sp. L11 [109], and _Streptomyces luridiscabiei_ [100]. Moreover, bifunctional lyases possess different degradation activities toward different substrates. For instance, Aly-SJ02, a bifunctional alginate lyase from _Pseudoalteromonas_ sp. SM0524, was preferable to depolymerizes poly (M) than poly (G) [93]. Aly-SJ02 showed lower $K_m$ to polyG than that of polyM and sodium alginate [93]. Belik et al. reported a bifunctional endolytic alginate lyasesALFA3isolated from _Formosaalgae_ KMM 3553$^T$ [110]. These studies suggested that the bifunctional alginate lyases in alginate-utilizing bacteria could provide an efficient mechanism to utilize rich and reliable alginate sources for producing energy.
Table 1. Alginate lyases separated from various PL families of marine alginolytic bacteria.

| Source                          | Localization | Substrate Specificity | Protein Name | Endo/Exolytic | PL | Main Products (DP) | Cleavage Site | References |
|--------------------------------|--------------|-----------------------|--------------|---------------|----|--------------------|---------------|------------|
| Photobacterium sp. FC615       | Extracellular| polyG                 | AlyPB1       | endolytic     | 6  |                     |               | [83]       |
| Photobacterium sp. FC615       | Intracellular| polyM                 | AlyPB2       | exolytic      | 15 |                     |               | [83]       |
| Vibrio sp. QY108               |              | polyMG                | VsAly7D      | exolytic      | 7  |                     |               | [106]      |
| Streptomyces sp. ALG-5         | Extracellular| polyG                 | ALG-5        | -             | 7  |                     |               | [104]      |
| Cobetia sp. NAP1               |              | polyMG                | AlgC-PL7     | endolytic     | 7  |                     |               | [107]      |
| Sphingomonas sp.               |              | polyMG                | FALy         | endolytic     | 7  | 5–6                |               | [111]      |
| Microbulbifer sp. Q7           | Extracellular| polyG                 | AlyM         | -             | 7  | 2–5                | G–G or G–M   | [112]      |
| Pseudoalteromonas sp. SM0524   |              | polyGM                | Aly-SJ02     | -             | 18 | dimers and trimers  |               | [93]       |
| Microbulbifer sp. 6532A        |              | polyG                 | AlgMsp       | -             | 7  | 2–5                |               | [46]       |
| BP-2 strain                    |              | polyM                 | Alg17B       | endolytic and exolytic | 17 | 2–6                |               | [97]       |
| Vibrio furnissii H1            |              | polyMG                | AlyH1        |                 | 7  | 2–4                |               | [114]      |
| Pseudoalteromonas carrageenovora ASY5 | extracellular | polyGM                | Aly1281      | endolytic     | 7  | 2                  |               | [108]      |
| Pseudoalteromonas carrageenovora ASY5 | extracellular | polyM                | Alg823       | endolytic     | 6  | 2                  |               | [115]      |
| Agarivorans sp. L11            |              | polyGM                | AlyL1        | endolytic     | 7  | 2–4                |               | [109]      |
| Streptomycetes luridiscabiei   |              | polyGM                | AlyDS44      | endolytic     | 7  | 2–4                |               | [100]      |
| Formosaalgae KMM 3553T         |              | polyM                 | ALFA3        | endolytic     | 7  | 1–20               | M–M, M–G, G–M| [110]      |
| Formosaalgae KMM 3553T         |              | polyGM                | ALFA4        | endolytic     | 6  | 1–20               | M–M           | [110]      |
| Vibrio sp. QD-5                |              | polyG                 | Aly-IV       | endolytic     | 7  | 1–3                |               | [96]       |
| Zobellia galactanitorans       | Intracellular| poly-MG               | AlyA1        | endolytic     | 7  | 4–20               | G–M           | [90]       |
| Zobellia galactanitorans       | Intracellular| polyG                | AlyA5        | exolytic      | 7  |                     | M–M, M–G, G–G| [90]       |
| Glaciecolachathamensis S18K6T  |              | polyG                 | AlyGC        | -             | 6  |                     |               | [116]      |
| Vibrio sp. W2                  |              | polyG                 | Alyw203      | endo-type     | 7  | 1–2                |               | [105]      |
According to the amino acid sequence and structural features, alginate lyases could be classified into several polysaccharide lyase (PL) families. As indicated in Table 1, marine bacteria-based alginate lyases are mainly PL6 and PL7 family members, which are endolytic. Moreover, alginate lyases are grouped into families based on the three-dimensional structures, which makes it possible to research the relationship between structure and function. The parallel $\beta$-helix family includes VsAly7D from *Vibrio* sp. QY108 [106], which belongs to the PL-7 family and AlyGC from *Glaciecola chathamensis* S18K6T [116], which belongs to the PL6 family, while the jelly-roll family includes Aly-Sj02 from *Pseudoalteromonas* sp. SM0524 of PL18 [117] and AlyA5 and AlyA1 from *Zobellia galactanivorans* of the PL-7 family [90].

Notably, some alginate-degrading strains could produce several alginate lyases to synergistically degrade exogenous alginate. The *Pseudoalteromonas* sp. strain ASY5 generates two extracellular alginate lyases Alg823 and Aly1281 (Table 1), which have similar action mode and main degradation products but different specificities to substrate. Although Alg823 and Aly1281 are both bifunctional, Alg823 demonstrates the highest activity with polyM [68], while Aly1281 shows higher activity with polyG than that of polyM [108]. The similar action modes and main degradation products may bring them maximum enzyme activity under the same environmental conditions, and the substrate specificity difference leads to a synergistic alginate degradation effect of Alg823 and Aly1281. *Photobacterium* sp. FC615 produces extracellular (AlyPB1) and intracellular (AlyPB2) alginate lyases. Two alginate lyases have different substrate specificities, families, and modes of action. AlyPB1 is an alginate lyase with a preference for polyG, and AlyPB2 is a bifunctional lyase [83]. *Pseudoalteromonas* sp. 0524 secretes two extracellular alginate lyases (AlyPM and Aly-Sj02), which have different substrate specificities and, thus, synergistically facilitate the alginate degradation [93,113]. Additionally, *Formosa algae* KMM 3553 T secretes two endolytic alginate lyases (ALFA3 and ALFA4) with different substrate specificities. ALFA3 is a bifunctional lyase, while ALFA4 degrades only mannuronate blocks [110]. *Zobellia galactanivorans* produce two intracellular alginate lyases (AlyA1PL7 and AlyA5) with different modes of action [90].

6. Biochemical Properties of Marine Bacteria-Produced Alginate Lyases

There are some characteristics of alginate lyases produced from marine bacteria that are shown in Table 2. The optimal working conditions for most of the alginate lyases (especially the PL7 enzyme family) are between pH 7.0 and 8.5. Additionally, several alginate lyases exhibit the optimal activities in alkaline (Alyw203 from *Vibrio* sp. W2 [105]) and acidic (ALFA3 from *Formosa algae* KMM 3553 T [110] and SALy from *Sphingomonas* sp. [107]) environments (Table 2). Lyase Alyw202 has an optimal pH of 9.0, while the optimum pH value for lyases AlyM, AlyA1PL7, and AlyA5 is 7.0. The optimal pH of AlgMsp, AlyPB1, AlyPB2, ALG-5, AlgC-PL7, Aly1281, Alg823, and ALFA4 at pH 8.0 is between those values (Table 2). In addition, VsAly7D from *Vibrio* sp. QY108 showed its maximum activity at a pH of 8.0, and the enzyme stability remained within the pH range of 8.0 to 10.0. Therefore, VsAly7D works as an alkaline-stable alginate lyase that is generally stored under weak alkaline conditions and adapts different environments [106]. AlyPM showed the maximum activity at pH 8.5 and maintained ~70% of the maximum activity from pH 7.0 to 9.5 [113]. AlgC-PL7 retained ~50% of its maximum activity from pH 6 to 9. These results indicated that AlgC-PL7 generally possesses optimal activity under neutral conditions [107]. AlySj-02 from *Pseudoalteromonas* sp. SM0524 demonstrated maximal activity at pH 8.5 and retained >50% activity at pH 7.0–10 after 20 min incubation [93]. Cold-adapted alginate lyase AlyL1 from *Agarivorans* sp. L11 showed the highest activity at a pH of 8.6 and maintained its stability from a pH of 6.0 to 9.6 [109].
| Source                     | Enzyme      | Opt. pH | pH Stability | Opt. Temp (°C) | Thermal Stability | PI   | Activators                                  | Inhibitors                              | Gen Bank Accession No. | References |
|----------------------------|-------------|---------|--------------|----------------|-------------------|------|---------------------------------------------|------------------------------------------|-------------------------------|------------|
| *Photobacterium* sp. FC615 | AlyPB1      | 8.0     | -            | 30             | -                 | 4.88 | -                                           | Hg$^{2+}$, Ni$^{2+}$, Mn$^{2+}$, Zn$^{2+}$, Cu$^{2+}$, SDS, Co$^{2+}$ | MN116685                     | [83]       |
| *Photobacterium* sp. FC615 | AlyPB2      | 8.0     | -            | 20             | -                 | 5.01 | Co$^{2+}$, DTT, β-mercaptopethanol          | Hg$^{2+}$, Ni$^{2+}$, Mn$^{2+}$, Zn$^{2+}$, Cu$^{2+}$, SDS | MN116686                     | [83]       |
| *Vibrio* sp. QY108        | VsAly7D     | 7.6     | stable at pH 7.6–10.6, stable at pH 9.0–10.0 (12 h, with 80% activity) | 35             | 46.5% (20 °C) and 83.1% (30 °C) of the initial enzyme activities | 5.65 | -                                           | Zn$^{2+}$, Fe$^{3+}$, Cu$^{2+}$, SDS and EDTA | QPB15428                    | [106]      |
| *Streptomyces* sp. ALG-5  | ALG-5       | 8.0     | -            | 30             | -                 | -    | -                                           | -                                        | EU137870                     | [104]      |
| *Cobetia* sp. NAP1        | AlgC-PL7    | 8.0     | >50% lyase activity at pH 6–9. | 45             | >90% of the initial enzyme activity (heating at 70–80 °C for 15 min, 80% (heating at 90 °C for 15 min). | -    | -                                           | -                                        | -                            | [107]      |
| *Sphingomonas* sp.        | SALy        | 6.5     | -            | -              | 70% of the initial enzyme activity at 55 °C | -    | -                                           | -                                        | 2CWS                         | [107]      |
| *Flavobacterium* sp.      | FALy        | 7.5     | -            | -              | 30–40% of the initial enzyme activity at 55 °C for 4 h; lost its activity at 60 °C | -    | -                                           | -                                        | JF412659                     | [111]      |
| *Microbulbifer* sp. Q7    | AlyM        | 7       | -            | 55             | 32% of initial enzyme activity at 45 °C for 2 h; 14.7% at 55 °C for 1 h | 4.4  | K$^+$, Ca$^{2+}$, Mg$^{2+}$, glycine        | Zn$^{2+}$, Cu$^{2+}$, Li$^{+}$, Fe$^{3+}$, Fe$^{2+}$, Mn$^{2+}$, EDTA, SDS | WP066959628.1 | [112]      |
| *Pseudoalteromonas* sp. SM0524 | Aly-SJ02  | 8.5     | stable at pH 8.0 ~50% activity at pH 7.0–10 for 20 min | 50             | Remain stable for 41 min at 40 °C and 20 min at 50 °C | -    | Na$^+$, K$^+$, Mg$^{2+}$, Ca$^{2+}$, Co$^{2+}$, Ba$^{2+}$, Ni$^{2+}$, Sr$^{2+}$ | Cu$^{2+}$, Sn$^{2+}$, EDTA | EU548075                     | [93]       |
| *Pseudoalteromonas* sp. SM0524 | AlyPM       | 8.5     | >70% of its highest activity at pH 7.0–9.5 | 30             | 19% of the highest activity at 5 °C, unstable at >30 °C low Tm at 37 °C. | -    | Cu$^{2+}$, Co$^{2+}$                        | Ni$^{2+}$                              | EU548076                     | [113]      |
| Source                | Enzyme | Opt. pH | pH Stability   | Opt. Temp (°C) | Thermal Stability | PI | Activators | Inhibitors          | Gen Bank Accession No. | References |
|----------------------|--------|---------|----------------|----------------|-------------------|----|------------|---------------------|------------------------|------------|
| *Microbulbifer* sp. 6532A | AlgMsp | 8.0     | -              | 50             | activity down by 86 at 60 °C, no activity at 70 °C | -  | -          | Ni<sup>2+</sup>, Ca<sup>2+</sup> | AB603802               | [46]       |
| BP-2 strain          | Alg17B | 7.5–8.0 | stable at pH 7.0–8.0, enzyme activity was reduced to 33% at pH 8.5 | 40–45         | stable at 25–35 °C. 90% of the enzyme activity at 40 °C | -  | Na<sup>+</sup> | Ca<sup>2+</sup>, Zn<sup>2+</sup> | MH820150.1              | [97]       |
| *Bacillus* sp.       | -      | 8.0     | stable at pH 4.0–9.0 | 50             | stable at 45 °C. 50% at 50 °C for 105 min and maintain 100% activity at 45 °C after 180 min | -  | Mg<sup>2+</sup>, Ca<sup>2+</sup>, K<sup>+</sup> | Zn<sup>2+</sup>, Co<sup>2+</sup>, Li<sup>+</sup>, EDTA, PMSF | LC457966               | [88]       |
| *Vibrio furnissii* H1 | AlyH1  | 7.5     | stable at pH 7.0–8.0 for 12 h, >60% activity at pH 6.5–8.5, 80% activity at pH 7.0–8.0 | 40            | stable at <30 °C. >60% of activity at 40 °C for 30 min | Na<sup>+</sup>, Mg<sup>2+</sup>, K<sup>+</sup> | 9.06       | -                      | -                      | MG214325               | [114]      |
| *Pseudoalteromonas* carrageenovora ASY5 | Aly1281 | 8.0     | >65% enzyme activity at pH 6.0–9.5, >70% of the enzyme activities at pH 7.0–9.0 | 50            | >50% of the activity at 45–55 °C | 9.06 | -          | -                    | -                      | [108]      |
| *Pseudoalteromonas* carrageenovora ASY5 | Alg823  | 8.0     | >80% activity at pH 6.0–10.0 (4 °C for 24 h) | 55            | ~75% of the optimal activity at 50 °C for 30 min | Mg<sup>2+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup>, and K<sup>+</sup> | CTAB | -                      | -                      | -                      | [115]      |
| *Agarivorans* sp. L11 | AlyL1  | 8.6     | stable at pH 6.0–9.6 | 40             | 54.5% and 72.1% of optimal activity at 15 °C and 20 °C, respectively | -  | -          | -                    | KM018274               | [109]      |
| *Streptomyces luridiscabiei* AlyDS44 | 8.5     | >70% of the maximum activity at pH 6.5–9.5. | 45             | >80% enzyme activity at 35 °C to 55 °C. | -  | Mn<sup>2+</sup>, Co<sup>2+</sup>, Fe<sup>2+</sup> | Zn<sup>2+</sup>, Cu<sup>2+</sup> | OK169607               | [100]      |
Table 2. Cont.

| Source                     | Enzyme   | Opt. pH | pH Stability                  | Opt. Temp (°C) | Thermal Stability            | PI  | Activators            | Inhibitors            | Gen Bank Accession No. | References |
|----------------------------|----------|---------|-------------------------------|----------------|------------------------------|-----|-----------------------|-----------------------|------------------------|------------|
| *Alteromonas* sp. H-4      | -        | 7.5     | stable at pH 6.6–9.0, <20% activity at pH < 5.0 | 30             | 20% and 40% decrease in the enzyme activity at 30 and 40°C for 5 min, respectively. | -   | MnCl₂ or BaCl₂, EDTA, Na⁺, ZnSO₄, or CdCl₂ | -                     | PRJNA299442            | [118]      |
| *Formosaalgae KMM 3553¹*  | ALFA3    | 6.0     | -                             | 35             | 50% activity at 42°C for 30 min | -   | -                     | -                     | PRJNA299442            | [110]      |
| *Formosaalgae KMM 3553¹*  | ALFA4    | 8.0     | -                             | 30             | stable up to 30°C; 50% activity at 37°C for 1 h 40 min. | -   | -                     | -                     | PRJNA299442            | [110]      |
| *Vibrio* sp. QD-5         | Aly-IV   | 8.9     | >80% activity at pH 7.0–10.0. | 35             | stable at <30°C for 30 min | 5.12 | K⁺, Mg²⁺, Ba²⁺, Al³⁺, Ni²⁺, Zn²⁺, Pb²⁺, EDTA | PRJNA382465            | [96]       |
| *Zobelliagalactanivorans*  | AlyA1    | 7.0     | -                             | 30             | -                             | -   | -                     | -                     | -                      | [90]       |
| *Zobelliagalactanivorans*  | AlyA5    | 7.0     | -                             | -              | -                             | -   | -                     | -                     | -                      | [90]       |
| *Vibrio* sp. W2           | Alyw203  | 10      | >80% of the highest activity at pH 4.0–10.0. | 45             | >90% of its initial activity at 10°C for 20 min | 6.09 | Fe³⁺, Cu²⁺, Zn²⁺, Al³⁺ | SDS, EDTA              | PRJNA382465            | [105]      |
| *Vibrio* sp. W2           | Alyw202  | 9       | >80% activity at pH 5.0–9.0 (4°C) for 12 h, >60% activity at pH 3.0–10.0 (4°C) for 12 h | 45             | -                             | 5.10 | Mn²⁺ and Co²⁺ | Na⁺, Mg²⁺ and Ba²⁺, EDTA and SDS | -                      | [119]      |
As shown in Table 2, the optimal temperature for AlyPB1 from *Photobacterium* sp. FC615 [83], ALG-5 from *Streptomyces* sp. ALG-5 [104], AlyPM from *Pseudoalteromonas* sp. SM0524 [113], ALFA4 from *Formosa alga* KMM 3553 [110], and AlyA1PL7 from *Zobellia galactanivorans* [90] is 30 °C. Alginic lyase produced by *Vibrio furnissii* H1 (AlyH1) [114] and *Agarivorans* sp. L11 (AlyL1) [109] works under a higher optimum temperature at 40 °C. Higher optimal temperatures were found on several alginic lyases produced by *Cobetia* sp. NAP1 (AlgC-PL7) [107], *Streptomyces luridiscabiei* (AlyDS44) [100], *Vibrio* sp. W2 (Alyw202 and Alyw203) [105,119], which had the optimum working temperature of 45 °C. The optimal temperature for Aly1281 from *Pseudoalteromonas carrageenovora* ASY5 [108], AlgMsp from *Microbulbifer* sp. 6532A [46], and Aly-SJ02 from *Pseudoalteromonas* sp. SM0524 [93] are around 50 °C. The highest optimum temperature of 55 °C was observed on alginate lyases produced by *Microbulbifer* sp. Q7. (AlyM) [112] and *Pseudoalteromonas carrageenovora* ASY5 (Alg823) [115]. Although most of the marine bacterial alginic lyases demonstrate an optimum temperature in the range of 30–55 °C, the alginate lyase isolated from *Photobacterium* sp. FC615 depicts optimal activity at 20 °C [83]. In addition, AlyL1 isolated from *Agarivorans* sp. L11 exhibited 54.5% and 72.1% of the maximal activity at 15 °C and 20 °C, respectively, suggesting that AlyL1 was a cold-adapted alginic lyase [109]. Alg17B exhibited different activity at 40–45 °C, and it has 90% of the maximum activity at 40 °C while only 10% of its activity remained at 45 °C; however, Alg17B has good thermal stability at 25–35 °C and maintained 80% of its enzyme activity within this temperature range. It could be seen that, with the temperature increase of 40 to 45 °C, the stability of Alg17B drastically diminished. Alyw203 alginic lyase possessed the maximum activity at 45 °C and the activity remained >80% in the range 40–55 °C [97]. AlyH1 showed high stability below 30 °C, and >60% of its activity could be maintained after incubation at 40 °C for 30 min [114].

It could be noticed that various alginic lyases from different marine biological sources have different molecular weights. Generally, the molecular weight of alginic lyases produced by marine bacteria ranges from 24 to 110 kDa [120]. From an SDS–PAGE analysis, the molecular weight of alginic lyase *Vibrio* sp. QY108 was estimated to be 37 kDa [106]. AlyDS44 has a molecular weight of 28.6 kDa, which belongs to the low molecular weight (25–30 kDa) group of alginic lyases [51]. The alginic lyase produced by *Microbulbifer* sp. ALW1 has a molecular weight of 26.2 kDa [43]. Similar molecular weights were also observed on the alginic lyase extracted from *Isoptericola halotolerans* CGMCC 5336 (28 kDa) [44] and *Streptomyces* sp. ALG-5 (27.5 kDa) [104]. There are several high molecular weights alginic lyases, including AlyM from *Microbulbifer* sp. Q7 (63 kDa) [112], AlyA5 from *Zobellia galactanivorans* (69.5 kDa) [90] and AlyH1 from *Marinimicrobium* sp. H1 (61.3 kDa) [121]. The endolytic alginic lyases, such as ALFA4 from *Formosa alga* KMM3553 and Alg823 from *Pseudoalteromonas carrageenovora* ASY5, possess a high molecular weight as well (Figure 1).

The effects of various cationic/anionic chemical species on alginic lyases enzyme activity are shown in Table 2. Usually, enzyme activity is influenced under the condition of divalent cations, which act as cofactors for increasing/inhibiting enzyme activity by inducing protein conformation change, replacing other enzyme cofactors, and alternating enzyme stability. Ca²⁺ and Mg²⁺ are stimulatory cofactors for regulating the enzyme activity of alginic lyases [60]. As shown in Table 2, in the presence of Mn²⁺ and Co²⁺, the alginic degradation activity of AlyDS44 increased by 242% and 219%, respectively, while Ca²⁺ and Mg²⁺ showed no effect on the AlyDS44 activities; however, Zn²⁺, Cu²⁺, and Fe³⁺ exhibited a slight or moderate enzyme inhibition effect [100]. For Aly-IV, its activity was significantly inhibited by Ba²⁺, Al³⁺, Ni²⁺, Zn²⁺, and Pb²⁺ (1 mM) but was promoted by Ca²⁺ (1 mM), K⁺ (5 mM) and Mg²⁺ (10 mM) [96]. The AlyH1 activity was inhibited by Fe²⁺, Cu²⁺, Zn²⁺, and Mn²⁺ but stimulated by Mg²⁺ (119.25%) and K⁺ (110.31%) [114]. Moreover, enzyme activities of AlyPB1 and AlyPB2 could be largely inhibited by Mn²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Hg²⁺, and SDS. Additionally, AlyPB2 was also inhibited by Ag⁺ and Mg²⁺, but AlyPB1 was not inhibited by them. It could be noticed that, in the presence of
Co\textsuperscript{2+}, DTT, and \(\beta\)-mercaptoethanol, AlyPB2's activity was increased to 158\%, 186\%, and 366\%, respectively. Compared to AlyPB2, these chemicals did not significantly influence the activity of AlyPB1, which was strongly inhibited by Co\textsuperscript{2+} (10 mM) [83]. The metal cations such as Na\textsuperscript{+}, K\textsuperscript{+}, Mg\textsuperscript{2+}, Ca\textsuperscript{2+}, Ba\textsuperscript{2+}, Ni\textsuperscript{2+}, Co\textsuperscript{2+}, and Sr\textsuperscript{2+} could improve Aly-SJ02's enzyme activity and Zn\textsuperscript{2+} showed no effect, while Cu\textsuperscript{2+} and Sn\textsuperscript{2+} could slightly inhibit the activity of Aly-SJ02. In addition, EDTA (1 mM) could decrease the Aly-SJ02 activity to 48.3\% [93]. The metal ion's effect on the activity of AlyPM indicated that Ni\textsuperscript{2+} (2 and 10 mM) could inhibit its activity by ~50\%. Cu\textsuperscript{2+} and Co\textsuperscript{2+} could increase the enzyme activity at a low concentration of 2 mM but inhibit the activity at a higher concentration of 10 mM. However, other metal ions (Mg\textsuperscript{2+}, Ca\textsuperscript{2+}, Ba\textsuperscript{2+}, and Mn\textsuperscript{2+}), had a negligible or low activation effect [113].

**Figure 1.** Molecular weight distributions of various alginate lyases produced by marine bacteria.

7. **Enzyme Kinetics of Alginate Lyases from Marine Bacteria**

Enzyme kinetics is an essential factor in evaluating the catalytic capability of an enzyme toward practical applications. However, since the alginate substrate is biochemically heterogeneous and alginites produced by various seaweeds have different mannuronic/guluronic (M/G) ratios, the enzyme kinetics of alginate lyases was difficult to measure. Additionally, the polyM, polyG, and polyMG subdomains and their frequencies are significantly different in different seaweed sources [60,122]. Alginate lyase-mediated production of alginate usually causes a mixture of polymers with different DP values, and their average length was determined by the preparation methodology and conditions. Therefore, it is hard to compare the enzyme kinetics among different alginate lyases [46].

The kinetic parameters of marine bacteria-based alginate lyases towards different substrates are shown in Table 3. For instance, with the substrate sodium alginate, the \(K_m\) and \(V_{max}\) of AlyH1 were measured as 2.28 mg/mL and 2.81 U/mg, respectively, indicating that AlyH1 (under sodium alginate substrate) possesses high enzyme efficiency [114]. Zhang et al. (2020) investigated the salt effect (NaCl: 300 mM; KCl: 1000 mM) on the enzyme kinetics of Aly1281 (substrate: sodium alginate), and it was found that adding 300 and 1000 mM of NaCl could decrease the \(K_m\) value by 54.9\% and 74.3\%, respectively. Compared to the \(K_m\) values under electrolyte-free conditions, the result indicated that the affinity of
substrate and catalytic activity of alginate lyases could be greatly enhanced by adding salts or electrolytes, which is the salt-activation effect [108]. AlgMsp from *Microbulbifer* sp. 6532A showed a $K_m$ of 3.4 mM for alginate [46]. Additionally, the catalytic efficiency ($k_{cat}/K_m$) of AlyL1 to alginate was calculated as $9952.8 \pm 33.1$ mg mL$^{-1}$ s$^{-1}$. AlyL1 exhibits a $K_m$ value of $0.19 \pm 0.04$ mg/mL with a $V_{max}$ value of $907.8 \pm 72.5$ U/mg protein. The results suggested that AlyL1 possesses a high affinity to alginate and could efficiently degrade alginates into oligosaccharides [109]. Moreover, $K_m$ values of AlyA1 (PL7 family) from *Zobellia galactanivorans* with various seaweed alginate substrates range from 1.7 to 6.2 mM, with increased binding affinity to alginate with higher guluronate composition [90]. In addition, Aly-SJ02, an alginate lyase from *Pseudoalteromonas* sp. SM0524, has a higher $K_m$ of 6.1 mM towards the alginate [93]. For seaweed-intake marine organisms, the low binding affinity of alginate lyases is acceptable due to the high concentration of alginate contents in seaweed (e.g., 17–45% w/w of dried brown seaweed) [21]. There are some notable exceptions of alginate lyases with $K_m$ values in the micromolar range. Alginate lyases from different marine sources could have different polyM, polyG, or polyMG substrate specificities [60]. Typically, some alginate lyases prefer one substrate but still cleave the other substrates at a reduced rate. For example, Aly-SJ02, an alginate lyase from *Pseudoalteromonas* sp SM0524, degrades polyG and polyM with polyG-specific activity and 75% of that against polyM [93]. Additionally, the $K_m$ and $k_{cat}/K_m$ of VsAly7D to alginate were calculated as $0.217$ mM and 227 L mol$^{-1}$ s$^{-1}$, respectively [106]. Bifunctional alginate lyases could degrade different types of alginates, making them potential biocatalysts for industrial application.
Table 3. Kinetic parameters of alginate lyases from marine bacteria toward sodium alginate, polyM, and polyG.

| Enzyme   | Source                     | Substrate Preference | $K_m$         | $V_{max}$ | $k_{cat}$   | References |
|----------|----------------------------|----------------------|---------------|-----------|-------------|------------|
| AlyPM    | *Pseudoalteromonas* sp. SM0524 | polyM                | 3.15 mg/mL (0.5 M NaCl) and 74.39 mg/mL (0 M NaCl) for sodium alginate | -         | -           | [113]      |
| ALFA3    | *Formosa algae* KMM 3553 T | polyGM               | 0.12 ± 0.01 mg/mL | 0.128 × 10^{-3} M/min for G, 0.150 × 10^{-3} M/min for MG, 0.211 × 10^{-3} M/min for M | 3.52 s^{-1} for G, 4.13 s^{-1} for MG and 5.80 s^{-1} for M | [111] |
| ALFA4    | *Formosa algae* KMM 3553 T | polyM                | 3.01 ± 0.05 mg/mL for polyM | 0.314 × 10^{-3} M/min for MG | 2.88 s^{-1} for MG | [111] |
| ALW1     | *Microbulbifer* sp. ALW1   | -                    | 1.03 mg/mL for sodium alginate | 4.63 U/mg for sodium alginate | 69.38 s^{-1} for sodium alginate | [42] |
| Aly1281  | *Pseudoalteromonascarrageenovora ASY5* | -                  | 0.3180 (0.3 M NaCl) and 0.1810 mg/mL (1.0 M NaCl), respectively, 0.2805 (0.3 M KCl) and 0.1631 (1.0 M KCl) for sodium alginate | -         | 2.185 s^{-1} (in 0.3 NaCl), 2.095 s^{-1} (in 1.0 M NaCl), 1.875 s^{-1} (in 0.3 KCl), 1.502 s^{-1} (in 1.0 M KCl) for sodium alginate | [123] |
| AlgNJ–07 | *Serratia marcescens* NJ-07 | -                    | 0.53 mM for sodium alginate, 0.27 mM for polyM | 74, 67 nmol/s for sodium alginate and polyM | 34 for sodium alginate, and 31 s^{-1} for polyM | [81] |
| Aly-IV   | *Vibrio.* sp. QD-5         | -                    | 0.2223 g/mL for sodium alginate, 0.3274 g/mL for polyG | 3.6 OD_{235}/h for sodium alginate, 2.8321 OD_{235}/h for polyG | -           | [97] |
| Aly-SJ02 | *Pseudoalteromonas* sp. SM0524 | bifunctional      | 1.086 for sodium alginate, 0.465 for polyG, 2.751 mg/mL for polyM, | 8.074 OD_{235}/h for sodium alginate, 5.318 OD_{235}/h for polyG, 7.131 for polyM | -           | [93] |
| Alg823   | *Pseudoalteromonascarrageenovora ASY5* | -                  | 0.15 mg/mL for sodium alginate | 1.84 U/g for sodium alginate | 1.19 × 10^6 s^{-1} for sodium alginate | [93] |
| VsAly7D  | *Vibrio* sp. QY108         | -                    | 0.217 mM for alginate | -         | 42.26 s^{-1} for sodium alginate | [107] |
| AlgM4    | *Vibrio weizhoudaoensis* M0101 | bifunctional      | 2.72 mg/mL, for sodium alginate | 2.75 nmol/s for sodium alginate | 30.25 s^{-1} for sodium alginate | [124] |
| AlgH     | *Marinimicrobium* sp. H1   | -                    | 6.6 ± 2.2 mg/mL^{-1} for sodium alginate, 7.6 ± 1.6 mg mL^{-1} for polyG, 9.1 ± 2.4 mg mL^{-1} for polyM | 224.6 ± 33.6, 146.6 ± 15.6, 62.6 ± 8.8 U/mg of protein, respectively, for sodium alginate, polyG and polyM | 260.6 ± 36.2 s^{-1} for sodium alginate, 155.7 ± 17.1 s^{-1} for polyG, 66.8 ± 6.7 s^{-1} for polyM | [121] |
Table 3. Cont.

| Enzyme   | Source           | Substrate Preference          | $K_m$   | $V_{max}$   | $k_{cat}$          | References |
|----------|------------------|-------------------------------|---------|-------------|--------------------|------------|
| AlyH1    | *Vibrio furnissii* H1 | 2.28 mg/mL for sodium alginate | 2.81 U/mg for sodium alginate | -            | [115]             |
| AlgNJU-03| *Vibrio* sp. NJU-03 | bifunctional                  | 8.50 mM for sodium alginate, 10.94 mM for polyM, 4.00 mM for polyG | 1.67 nmol/s for sodium alginate, 0.30 nmol/s for polyM, 2.50 nmol/s for polyG | 30.64, 5.50, 45.87 s$^{-1}$, respectively for sodium alginate, polyM and polyG | [125] |
| AlgNJ-04 | *Vibrio* sp. NJ04  | -                             | 0.49 mM for alginate, 0.86 mM for polyM, 0.24 mM for polyG | 72 pmol/s for alginate, 95 for polyM, 35 pmol/s for polyG | 59 s$^{-1}$ for alginate, 77 s$^{-1}$ for polyM, 29 s$^{-1}$ for polyG | [124] |
| Alys1    | *Tamlana* sp. S12 | polyM                         | 0.20 ± 0.01 mM for sodium alginate | -            | 4.43 ± 0.027 s$^{-1}$ for sodium alginate | [126] |
| AlyC3    | *Psychromonas* sp. C-3 | polyM                         | 0.24 ± 0.05 mg/mL for polyM | 19,704.73 ± 1865.49 U/mg of protein for polyM | -            | [127] |
| AlgMsp   | *Microbulbifer* sp. 6532A | polyG                         | 3.46 ± 0.9 mM for alginate, 1.8 ± 0.4 mM for polyG, 6.8 ± 2.1 mM for polyM | 5765, 3562, 6368 U/mg of protein for alginate, polyG and polyM, respectively | 42 s$^{-1}$ for alginate, 26 s$^{-1}$ for polyG, 46 s$^{-1}$ for polyM | [46] |
| A1m      | *Agarivorans* sp. JAM-A1m | -                             | -       | 38.4, 285.7, 416.7, and 526.3 U/mg of protein (0, 0.1, 0.2, and 0.5 M NaCl, respectively) for sodium alginate | -            | [91] |
8. Application of Alginate Lyases from Marine Bacteria

8.1. Preparation of AOs

Alginate oligosaccharides (AOs) possess various biological properties that provide benefits for improving human health. Their bioactivities, including antitumor [128], antidiabetic [129], antihypertensive [130], anti-inflammatory [131,132], antimicrobial [133], antioxidant [134], anticancer [99], immunomodulatory [135,136] and anti-radiation [43,137] properties, have been comprehensively summarized. Generally, traditional preparation methods for the production of AOs are usually under strong acidic and alkaline conditions [138], thus resulting in severe environmental damage. In contrast, enzyme-based AOs production methods are more “green” and environmentally sustainable. AOs prepared by enzymatic degradation methods showed special bioactivities due to their unsaturated oligosaccharide structures [139,140]. However, there is only one commercially available alginate lyase (CAS number: 9024-15-1, Sigma-Aldrich, St. Louis, MO, USA) with a high pH tolerance, high catalytic activity (>10,000 U/g) and magnificent heat stability, which is expensive and only sold in the form of reagents, and most of the marine bacterial-produced alginate lyases are just investigated at the laboratory level. NitAly obtained from *Nitratiruptor* sp. SB155-2 shows the highest alginate lyase activity at 70 °C [141], while alginate lyase Aly-IV (PL7 family) from *Vibrio* sp. QD-5 [96] and Aly08 from *Vibrio* sp. SY01 [142] are alkaline-stable, with optimal working pH values of 8.9 and 8.35, respectively.

Apart from the above-mentioned pH and thermo-stable alginate lyases, several alginate lyases demonstrated great potential for producing alginate oligomers with various DPs. Since the bioactivities of AOs are largely dependent on their DP values and chemical structures [143,144], endolytic alginate lyase-produced oligosaccharides with various DPs and diverse structures have attracted significant attention. The investigations of new AOs-producing alginate lyases were mostly conducted at the laboratory scale, and it could be seen that the endolytic alginate lyase generally produced alginate oligomers with DPs ranging from 2 to 5 [144]. For instance, the alginate lyase isolated from *Isoptericola halotolerans* CGMCC 5336, purified by gel column chromatography and characterized by TLC and ESI-MS, could perform an elimination reaction on guluronic acid (active sites: G or G-Gresidues) and generate oligomers with DPs of 2–4 [145] (Table 4). Alg2A, an endolytic alginate lyase from *Flavobacterium* sp. S20, can produce oligosaccharides with high yields along with high DP values (e.g., DP5 (penta-), DP6 (hexa-) and DP7 (hepta-)saccharides) [146] (Table 4). Zhu et al. degraded alginate with alginate lyase from *Flameovirga* sp. NJ–04 to prepare oligosaccharides with DP2-4 [58] (Table 4).

Notably, the combination of some endolytic and exolytic lyases could lead to a remarkable synergistic effect on the degradation of alginate. For AOs preparation, the simultaneous application of endolytic lyase AlyPB1 and exolytic lyase AlyPB2 could lead to significantly increased conversion from alginate to unsaturated monosaccharides, which could reach approximately seven-fold that of single AlyPB2 [83] (Table 4). Moreover, substrate-specific alginate lyases could be employed for the preparation of oligosaccharides with a specific molecular structure. Anne et al. constructed a diguluronic acid linkage-cleavable alginate lyase, which could be employed for the preparation of guluronic acid oligosaccharide [147]. Zhu et al. isolated a novel polyM-specific alginate lyase AlgNJ-07 from *Serratia marcescens* NJ-07, which showed good PolyM-degradation efficiency [81] and thus could act as a potential tool for the production of mannanuronic acid oligosaccharide (Table 4).
Table 4. Some applications of alginate lyase from marine bacteria.

| Enzyme          | Source                              | Application                                      | References | Field of Application                                                      |
|-----------------|-------------------------------------|--------------------------------------------------|------------|--------------------------------------------------------------------------|
| ALFA3           | Formosa algae KMM 3553<sup>T</sup>  | Preparation of alginate oligosaccharides          | [110]      | in agriculture, in feed production, to lower cholesterol levels in blood plasma |
| Aly1281         | Pseudoalteromonascarrageeenovora ASY5 | Preparation of alginate oligosaccharides          | [108]      | in agriculture, feed production                                           |
| AlgNJ-07        | Serratia marcescens NJ-07           | Preparation of alginate oligosaccharides          | [81]       | antimicrobials, for the treatment of cystic fibrosis, in medicine for the diagnosis of diseases, to lower cholesterol in blood plasma |
| AlgNJ-07        | Serratia marcescens NJ-07           | Preparation of alginate oligosaccharides          | [81]       | antimicrobials for the treatment of cystic fibrosis, in medicine for the diagnosis of diseases, to lower cholesterol in blood plasma |
| FsAlgB          | Flammeovirga sp. NJ-04              | Preparation of alginate oligosaccharides          | [58]       | in medicine for the diagnosis                                             |
| Aly             | Pseudomonas sp. HZJ 216             | Preparation of alginate oligosaccharides          | [148]      | antimicrobials, in medicine for the diagnosis of diseases                 |
| Alg2A           | Flavobacterium sp. S20              | Preparation of alginate oligosaccharides          | [146]      | to lower plasma cholesterol levels                                       |
| Aly5            | Flammeovirga sp. Strain MY04        | Preparation of alginate oligosaccharides          | [149]      | in medicine for the diagnosis                                             |
| AlyPB1 and AlyPB2 | Photobacterium sp. FC615            | Preparation of unsaturated monosaccharide         | [83]       | antimicrobials for the treatment of cystic fibrosis                       |
| Alg7A           | Vibrio sp. W13                      | Preparation of alginate oligosaccharides          | [144]      | inhibition of lipid oxidation in food emulsions                          |
| Alginate lyase  | Isoptericolahalotolerans CGMCC 5336 | Preparation of alginate oligosaccharides          | [145]      | in feed production                                                        |
| AlyP1400        | Pseudoalteromonas sp. 1400          | The degradation of biofilms                       | [150]      | in biofuel production                                                     |
| AlyL1           | Agarivorans sp. L11                 | Produce TPC for bioenergy production              | [151]      | inhibition of lipid oxidation in industrial emulsions                    |
| Alg7D           | Saccharophagusdegradans 2–40<sup>T</sup> | Produce DEH for bioenergy production              | [123]      | inhibition of lipid oxidation in industrial emulsions                    |
| AlyPB2          | Photobacterium sp. FC615            | Alginate Sequencing                               | [83]       | in the production of alginates                                            |
| Aly SM0524      | Pseudoalteromonas sp. SM0524        | Preparation of bioethanol                         | [152]      | antimicrobials for the treatment of cystic fibrosis, for lowering plasma cholesterol levels |
| Alg17C          | Cobetia sp. NAP1                    | Biofuels and chemicals production                | [107]      | in agriculture                                                            |
| Alginate lyase  | Shewanella sp. Kz7                  | Biofuel production                                | [153]      | in agriculture                                                            |
| Alginate lyase  | Gracilibacillus sp. A7              | Disposal of seaweed waste                         | [154]      | in agriculture                                                            |
8.2. Anti-Biofilm Activity

It is difficult for normal antibiotics to kill some pathogenic bacteria with complex biofilms on their surfaces. It was disclosed that alginate components in the biofilm of Pseudomonas aeruginosa could protect them from being recognized and cleared by the immune system and resisting antibiotic treatment [124,155]. Therefore, using a purified alginate lyase-antibiotic complex to synergistically treat Pseudomonas aeruginosa infections is a possible therapeutic method [125,156]. Recently, a purified alginate lyase (AlyP1400) from a marine Pseudoalteromonas sp. 1400 bacterium demonstrated the capability of disrupting the formation of biofilms of Pseudomonas aeruginosa by decomposing alginate within the extracellular polysaccharide matrix and thus enhancing the bactericidal activity of tobramycin, which may act as a promising strategy for combinational therapy [150] (Table 4).

8.3. Bioethanol Production

The alginate lyases are also employed as a potential tool for producing bioethanol. The exo-type alginate lyase depolymerizes the alginate oligomers into unsaturated monosaccharides and subsequently non-enzymatically converted to 4-deoxy-L-erythro-hexoseulose uronic acid (DEH), which was then reduced into 2-keto-3-deoxy-gluconate (KDG) by DEH reductase and was further connected to the Entner–Doudoroff (ED) pathway [157]. Normally, industrial microorganisms cannot directly utilize alginate as a starting resource to produce ethanol due to the lack of an alginate-mediated metabolic pathway. For a long time, it has been difficult to achieve efficient production of ethanol from brown algae. In 2012, Wargacki et al. [152] designed and prepared a bio-ethanol synthesis microbial platform using E. coli as a producer to secrete alginate lyase SM0524Aly from Pseudoalteromonas sp. SM0524 by an auto transporter (Table 4). Additionally, in Vibrio splendidus 12B01, an alginate lyase-encoding large gene cluster was introduced along with alginate catabolism-auxiliary gene clusters for achieving appropriate metabolism pathways. Finally, a pyruvate decarboxylase (Pdc) and an alcohol dehydrogenase B (AdhB)-encoding gene cluster was integrated into the E. coli chromosome to produce bioethanol. Moreover, endogenous E. coli genes, which encode fermentative byproducts, were removed. Accordingly, the fermentative yield of alginate, mannitol, and glucon could reach 0.28 g ethanol/per g dry brown algae (>80% of the maximum theoretical yield) [152]. Yagi et al. (2016) utilized Alg17C, an exo-oligoalginate lyase (PL7 family) isolated from halophilic Gram-negative bacterium Cobetia sp. NAP1 (brown algae Padina arborescens Holmes, as the bacterium resource) to depolymerize alginate into a monomeric sugar acid. Furthermore, Yagidis concluded that Alg17C could serve as the key enzyme to produce alginate monomers in the process of utilizing alginate for the production of biofuels and chemicals [107] (Table 4). It has been reported that the alginate lyase from Shevanella sp. Kz7 could degrade polyG blocks of alginate and accordingly produce monosaccharides such as 6-tetrahydroxy tetrahydro-2H-pyran-2-carboxylic acid (TPC), a useful intermediate for biofuel production [153] (Table 4).

8.4. Disposal of Seaweed Waste

In recent years, the amount of seaweed waste has drastically increased worldwide. One of the main organic components in seaweed is alginate, the content of which is as high as 50% in seaweed species such as wakame (Undaria pinnatifida) [158]. The disposal and re-utilization of seaweed waste are essential issues for the protection of marine environments and recycling of sustainable biomass. However, the degradation of alginate by general microorganisms is not easy to realize, mainly due to the complicated structures and molecular alignments of alginate. Thus, the isolation of specific microorganisms for alginate degradation is highly demanded, which is essential for the effective disposal of seaweed wastes. Tang et al. (2009) utilized alginate lyase-producing bacteria strain A7 (Gra-cilibacillus sp.) to degrade alginate in the wakame composting process. In a laboratory-scale test, after 72 h of composting, the alginate content in the wakame remarkably diminished from an initial value of 36.0% to 14.3%, suggesting the effectiveness of A7 for alginate decomposition [154] (Table 4).
8.5. Elucidate the Structure of Alginate

To profoundly understand the influence of the polymer architecture on the physico-chemical properties of alginate, alginate lyases have been utilized to analyze the fine polymer architecture, especially the alignment of $\alpha$-L-guluronate (G) and $\beta$-D-mannuronate (M) units of alginate. It is also very necessary to investigate the fine architecture of alginate for the preparation of tailor-made alginate. Lu et al. combined $^1$H NMR spectroscopy with exolytic alginate lyase AlyPB2 to establish a method for sequencing alginate oligosaccharides [83] (Table 4). Compared with the traditional sequencing method, this method provides a simple strategy for characterizing the structure of alginate oligosaccharides.

The O-antigenic polysaccharide of the *P. algicola* alga is composed of branched pentasaccharide repeating units containing monosaccharides quite common in nature (Figure 2a). *L. japonica* synthesizes a sulfated oligopolysaccharide composed of branched trisaccharide repeating units with the following structure: (Figure 2c). *S. horneri* also produces sulfated oligopolysaccharide, a galactan composed of linear trisaccharide repeat units and containing a pyruvic acid (Pyr) residue. *U. pinnatifida* produces a sulfated OPS composed of branched trisaccharide repeat units and has the following structure (Figure 2c). We isolated and analyzed another sulfated oligopolysaccharide from the *P. arborescens Holmes* alga. The repeating unit of the oligopolysaccharide of this algae is a branched pentasaccharide composed of the residues of 2,4-diacetamido-2,4,6-trideoxy-\(\text{D}\)-glucose (\(\text{D}\)-QuiNAc4NAc), L-rhamnose (1-Rha), 3-(4-hydroxybutyramido)-3,6-dideoxy-\(\text{D}\)-glucose, sulfated at the second position (b-\(\text{D}\)-Qui2SO3-3N(4Hb)), and two residues of 2-acetamido-2-deoxy-\(\text{D}\)-glucuronic acids (\(\text{D}\)-GlcpNAcA) (Figure 2d). *I. halotolerans* algae is an O-antigenic polysaccharide consisting of linear pentasaccharide repeating units containing residues of 2,4-diacetamido-2,4,6-trideoxy-\(\text{D}\)-glucose (\(\text{D}\)-QuiNAc4NAc), 2-acetamido-2-deoxy-\(\text{D}\)-galactose (\(\text{D}\)-GalNAc), 4-amino-4,6-dideoxy-\(\text{D}\)-glucose (\(\text{D}\)-Qui4N), N-acetyl-\(\text{D}\)-alanine (\(\text{D}\)-AlaAc), and two residues of 2-acetamido-2-deoxy-galacturonic acids (GalpNAcA) (Figure 2e) [159].

When studying the mechanism of action of alginate lyases, it was found that most of the studied alginate lyases function endolytically, i.e., they split the alginate molecules from the inside and do not produce significant amounts of oligomers at the beginning of the reaction [48]. If the reaction proceeds, the end products are typically dimers, trimers, tetramers, or pentamers [85]. However, several exoliases were described that remove single residues from the polymer end [48,160].

Gacesa [161] was the first to propose a reaction mechanism for alginate lyases. First, the negative charge on the carboxylate anion is shielded by the enzyme. This allows the proton to be abstracted from C-5. It is proposed to stabilize the intermediate enolate ion by resonance. Finally, electron transfer from the carboxyl group results in the formation of a double bond between C-4 and C-5 and cleavage of the O-glycosidic bond. It was found that cleavage is promoted by an amino acid residue acting as an acid [162]. The new non-reducing end will contain 4-deoxy-\(\text{L}\)-erythro-hex-4-enepyranosyluronate (\(\Delta\)). This double bond is absorbed at 235 nm and is used to quantify alginate lyase activity [48]. The negative charge of most alginate lyases is stabilized by glutamine, arginine, or asparagine. It is important for the catalytic mechanism that, for M-residues, the C-5-proton and the departing oxygen on C-4 lie syn relative to each other, while for G residues they lie anti relative to each other. For the studied alginate lyases, it was found that for M-specific lyases, the C-5 proton is abstracted by tyrosine, which also acts as an acid facilitating the cleavage of the O-glycosidic bond. For lyases acting on G-residues, the C-5 proton is abstracted by histidine, while tyrosine again acts as an acid [162]. Alginate lyases belonging to the PL6 family do not follow this pattern. They use Ca\(^{2+}\) as a neutralizer, lysine as a proton abstracting residue, and arginine as an acid [162].
Figure 2. Alginate lyase structures of algae [159]. (a) Alginate lyase from *P. Algicola*; (b) alginate lyase from *L. Japonica*; (c) alginate lyase from *U. Pinnatifida*; (d) alginate lyase from *P. arborescens Holmes*; (e) alginate lyase from *I. halotolerans*.

9. Conclusions Remarks

Thus, each year, various algae in the ocean produce large amounts of alginates, which serve as nutrient resources for heterotrophic marine bacteria and thus play an ecological role in coastal ecosystems similar to that of cellulose and hemicellulose biomass in terres-
trial environments. Various alginate lyases produced by marine microbes have played an important role in the degradation of marine alginate, and several alginate lyases have been isolated from various types of microorganisms over the past few years, especially from brown algae bacteria. Alginate lyases derived from marine bacteria serve as a stable pool of enzymes in the process of alginate degradation and marine carbon utilization. Alginate lyases derived from marine bacteria have great potential for application in the pharmaceutical industry, biofuel production, and environmental protection. It is vital to discover more new alginate lyases and explore their structure, functions, and structure-function relationship in order to advance marine enzymology and biotechnology. Almost no alginate lyase product has been developed for therapeutic applications (such as antibacterial, anticancer, and other diseases). Based on the foregoing review, extensive research in the field of alginate lyases derived from marine bacteria in the direction of advanced biotechnologies is expected.

Author Contributions: Conceptualization, writing and original draft preparation, N.B.; editing, S.T.J., M.S., R.S., R.N.; reviewing, O.B.; funding acquisition, S.S. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by the Russian Foundation for Basic Research (Project No. 19-316-60002/19 dated 22 August 2019).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare that there are no conflict of interest regarding the publication of this paper.

References
1. Barzkar, N.; Jahromi, S.T.; Poorsaheli, H.B.; Vianello, F. Metabolites from marine microorganisms, micro, and macroalgae: Immense scope for pharmacology. Mar. Drugs 2019, 17, 464. [CrossRef] [PubMed]
2. Jahromi, S.T.; Barzkar, N. Marine bacterial chitinase as sources of energy, eco-friendly agent, and industrial biocatalyst. Int. J. Biol. Macromol. 2018, 120, 2147–2154. [CrossRef] [PubMed]
3. Barzkar, N.; Homaei, A.; Hemmati, R.; Patel, S. Thermostable marine microbial proteases for industrial applications: Scopes and risks. Extremophiles 2018, 22, 335–346. [CrossRef] [PubMed]
4. Jahromi, S.T.; Barzkar, N. Future direction in marine bacterial agarases for industrial applications. Appl. Microbiol. Biotechnol. 2018, 102, 6847–6863. [CrossRef] [PubMed]
5. Barzkar, N. Marine microbial alkaline protease: An efficient and essential tool for various industrial applications. Int. J. Biol. Macromol. 2020, 161, 1216–1229. [CrossRef]
6. Barzkar, N.; Khan, Z.; Jahromi, S.T.; Pourmozaffar, S.; Gozari, M.; Nahavandi, R. A critical review on marine serine protease and its inhibitors: A new wave of drugs? Int. J. Biol. Macromol. 2021, 170, 674–687. [CrossRef]
7. Barzkar, N.; Sohail, M. An overview on marine cellulolytic enzymes and their potential applications. Appl. Microbiol. Biotechnol. 2020, 104, 6873–6892. [CrossRef]
8. Barzkar, N.; Sohail, M.; Jahromi, S.T.; Gozari, M.; Pourmozaffar, S.; Nahavandi, R.; Hafezieh, M. Marine bacterial esterases: Emerging biocatalysts for industrial applications. Appl. Biochem. Biotechnol. 2021, 193, 1187–1214. [CrossRef]
9. Barzkar, N.; Jahromi, S.T.; Vianello, F. Marine Microbial Fibrinolytic Enzymes: An Overview of Source, Production, Biochemical Properties and Thrombolytic Activity. Mar. Drugs 2022, 20, 46. [CrossRef]
10. Barzkar, N.; Sohail, M.; Jahromi, S.T.; Nahavandi, R.; Khodadadi, M. Marine microbial 1-glutaminase: From pharmaceutical to food industry. Appl. Microbiol. Biotechnol. 2021, 105, 4453–4466. [CrossRef]
11. Barzkar, N.; Fariman, G.A.; Taheri, A. Proximate composition and mineral contents in the body wall of two species of sea cucumber from Oman Sea. Environ. Sci. Pollut. Res. 2017, 24, 18907–18911. [CrossRef] [PubMed]
12. Jahromi, S.T.; Pourmozaffar, S.; Jahanbakhshi, A.; Rameshi, H.; Gozari, M.; Khodadadi, M.; Sohrabipour, J.; Behzadi, S.; Barzkar, N.; Nahavandi, R. Corrigendum to “Effect of different levels of dietary Sargassum cristaefolium on growth performance, hematological parameters, histological structure of hepatopancreas and intestinal microbiota of Litopenaeus vannamei”. Aquaculture 2021, 535, 736376. [CrossRef]
13. Choi, A.H.; Ben-Nissan, B. Marine-Derived Biomaterials for Tissue Engineering Applications; Springer: Berlin/Heidelberg, Germany, 2019; Volume 14.
14. Ozturk, M.; Egamberdieva, D.; Pešić, M. Biodiversity and Biomedicine: Our Future; Academic Press: Cambridge, MA, USA, 2020.
15. Chakravarty, R.; Hong, H.; Cai, W. Positron Emission Tomography Image-Guided Drug Delivery: Current Status and Future Perspectives. *Mol. Pharm.* **2014**, *11*, 3777–3797. [CrossRef] [PubMed]

16. Chung, I.K.; Beadall, J.; Mehta, S.; Sahoo, D.; Stojkovic, S. Using marine macroalgae for carbon sequestration: A critical appraisal. *J. Appl. Phycol.* **2011**, *23*, 877–886. [CrossRef]

17. Roessjadj, G.; Jones, S.B.; Snowden-Swan, L.J.; Zhu, Y. *Macroalgae as a Biomass Feedstock: A Preliminary Analysis*; Pacific Northwest National Lab. (PNNL): Richland, WA, USA, 2010.

18. Fleming, M.; Manners, D.; Masson, A. The enzymic degradation of laminarin. *Biochem. J.* **1967**, *104*, 32.

19. Horn, S.; Aasen, I.; Østgaard, K. Production of ethanol from mannitol by *Zymobacter palmei*. *J. Ind. Microbiol. Biotechnol.* **2000**, *24*, 51–57. [CrossRef]

20. Jensen, A.; Haug, A. Geographical and seasonal variation in the chemical composition of *Laminaria hyperborea* and *Laminaria digitata* from the Norwegian coast. *Akademik Trykkeriensentral* **1956**, *14*, 20.

21. Mabeau, S.; Kloareg, B. Isolation and analysis of the cell walls of brown algae: *Fucus spiralis*, *F. ceranoides*, *F. vesiculosus*, *F. serratus*, *Bifurcaria bifurcata* and *Laminaria digitata*. *J. Exp. Bot.* **1987**, *38*, 1573–1580. [CrossRef]

22. Gacesa, P. Enzymic degradation of alginites. *Int. J. Biochem.* **1992**, *24*, 545–552. [CrossRef]

23. Haug, A.; Larsen, B.; Smidsrod, O. Studies on the sequence of uronic acid residues in alginic acid. *Acta Chem. Scand.* **1967**, *21*, 691–704. [CrossRef]

24. Peteiro, C. Alginate production from marine macroalgae, with emphasis on kelp farming. In *Alginites and Their Biomedical Applications*; Springer: Berlin/Heidelberg, Germany, 2018; pp. 27–66.

25. Wang, M.; Chen, L.; Zhang, Z.; Wang, X.; Qin, S.; Yan, P. Screening of alginate lyase-excreting microorganisms from the surface of brown algae. *AMB Express* **2017**, *7*, 1–9. [CrossRef] [PubMed]

26. Gacesa, P. Alginites. *Carbohydr. Polym.* **1988**, *8*, 161–182. [CrossRef]

27. Itoh, T.; Nakagawa, E.; Yoda, M.; Nakaichi, A.; Hibi, T.; Kimoto, H. Structural and biochemical characterisation of a novel alginate lyase from *Paenibacillus* sp. str. FPU-7. *Sci. Rep.* **2019**, *9*, 1–14. [CrossRef]

28. Xu, F.; Wang, P.; Zhang, Y.-Z.; Chen, X.-L. Diversity of three-dimensional structures and catalytic mechanisms of alginate lyases. *Appl. Environ. Microbiol.* **2018**, *84*, e02040-17. [CrossRef] [PubMed]

29. Osawa, T.; Matsubara, Y.; Muramatsu, T.; Kimura, M.; Kakuta, Y. Crystal structure of the alginate (poly α-L-guluronate) lyase from *Corynebacterium* sp. at 1.2 Å resolution. *J. Mol. Biol.* **2005**, *345*, 1111–1118. [CrossRef] [PubMed]

30. Inoue, A.; Mashino, C.; Uji, T.; Saga, N.; Mikami, K.; Ojima, T. Characterization of an eukaryotic PL-7 alginate lyase in the marine red alga *Pyropia yezoensis*. *Curr. Biotechnol.* **2015**, *4*, 240–258. [CrossRef] [PubMed]

31. Inoue, A.; Ojima, T. Functional identification of alginate lyase from the brown alga Saccharina japonica. *Sci. Rep.* **2019**, *9*, 1–11. [CrossRef] [PubMed]

32. Elyakova, L.A.; Favorov, V.V. Isolation and certain properties of alginate lyase VI from the mollusk *Littorina sp.* *Biochim. Biophys. Acta (BBA)-Enzymol.* **1974**, *358*, 341–354. [CrossRef]

33. Hata, M.; Kumagai, Y.; Rahman, M.M.; Chiba, S.; Tanaka, H.; Inoue, A.; Ojima, T. Comparative study on general properties of alginate lyases from some marine gastropod mollusks. *Fish. Sci.* **2009**, *75*, 755–763. [CrossRef] [PubMed]

34. Suda, K.; Tanji, Y.; Hori, K.; Unno, H. Evidence for a novel Chlorella virus-encoded alginate lyase. *EMS Microbiol. Lett.* **1999**, *180*, 45–53.

35. Schaumann, K.; Weide, G. Enzymatic degradation of alginate by marine fungi. In *Thirteenth International Seaweed Symposium*; Springer: Berlin/Heidelberg, Germany, 1990.

36. Zhang, W.; Xia, X.; Zhang, Z. Alginate lyase of a novel algal fermentation strain. *Chem. Biochem. Eng. Q.* **2019**, *33*, 125–131. [CrossRef]

37. Yamasaki, M.; Moriwaki, S.; Miyake, O.; Hashimoto, W.; Moriwaki, S.; Miyake, O. Overproduction of the alginate lyase in *Actinoplanes sp.* at 1.2 Å resolution. *J. Mol. Biol.* **2005**, *345*, 31863–31872. [CrossRef] [PubMed]

38. Caswell, R.; Gacesa, P.; Luttrell, K.; Weightman, A. Molecular cloning and heterologous expression of a Klebsiella pneumoniae gene encoding alginate lyase. *Gene* **1989**, *75*, 127–134. [CrossRef]

39. Haraguchi, K.; Kodama, T. Purification and properties of poly (β-D-mannurionate) lyase from Azotobacter chroococcum. *Appl. Microbiol. Biotechnol.* **1996**, *46*, 576–581. [CrossRef]

40. Yu, Z.; Zhu, B.; Wang, W.; Tan, H.; Yin, H. Characterization of a new oligoalginate lyase from marine bacterium *Vibrio* sp. *Int. J. Mol. Macromol.* **2018**, *112*, 937–942. [CrossRef] [PubMed]

41. Chu, Y.J.; Kim, H.S.; Kim, M.S.; Lee, E.Y.; Kim, H.S. Functional Characterization of a Novel Oligoalginate Lyase of *Stenotrophomonas maltophilia* KJ-2 Using Site-Specific Mutation Reveals Bifunctional Mode of Action, Possessing Both Endolytic and Exolytic Degradation Activity Toward Alginate in Seaweed Biomass. *Front. Mar. Sci.* **2020**, *7*, 420. [CrossRef]

42. Chen, P.; Zhu, Y.; Men, Y.; Zeng, Y.; Sun, Y. Purification and characterization of a novel alginate lyase from the marine bacterium *Bacillus* sp. Alg07. *Mar. Drugs* **2018**, *16*, 86. [CrossRef]

43. Chu, Y.; Wu, L.; Chen, Y.; Ni, H.; Xiao, A.; Cai, H. Characterization of an extracellular biofunctional alginate lyase from marine *Microbulbifer* sp. ALW1 and antioxidant activity of enzymatic hydrolysates. *Microbiol. Res.* **2016**, *182*, 49–58. [CrossRef]

44. Dou, W.; Wei, D.; Li, H.; Li, H.; Rahman, M.M.; Shi, J.; Xu, Z.; Ma, Y. Purification and characterization of a bifunctional alginate lyase from novel *Isopericola halotolerans* CGMCC 5336. *Carbohydr. Polym.* **2013**, *98*, 1476–1482. [CrossRef]
74. Madgwick, J.; Haug, A.; Larsen, B. Ionic requirements of alginate-modifying enzymes in the marine alga *Pelvetia canaliculata* (L.) Dene. et Thur. *Bot. Mar.* 1978, 21, 1–4. [CrossRef]

75. Watanabe, T.; Nisizawa, K. Enzymatic studies on alginate lyase from *Undaria pinnatifida* in relation to texture-softening prevention by ash-treatment (Haiboshi) [Algae]. *Bull. Jpn. Soc. Sci. Fish.* 1982, 2, 243–249. [CrossRef]

76. Muramatsu, T.; Hirose, S.; Katayose, M. Isolation and properties of alginate lyase from the mid-gut gland of wreath shell *Turbo cornutus*. *Agric. Biol. Chem.* 1977, 41, 1939–1946.

77. Nisizawa, K.; Fujibayashi, S.; Kashiwabara, Y. Alginate lyases in the hepatopancreas of a marine mollusc, Dolabella auricularia Solander. *J. Biochem.* 1968, 64, 25–37. [CrossRef] [PubMed]

78. Seiderer, L.; IJ, S.; RC, N.; PA, C. Quantitative significance of style enzymes from two marine mussels (*Choromytilus meridionalis* Krauss and *Perna perna* Linnaeus) in relation to diet. *Mar. Biol. Lett.* 1982, 3, 257–272.

79. Xue, X.; Zhou, Y.; Gao, X.; Yan, P. Advances in application of alginate lyase and its enzymatic hydrolysate. In *IOP Conference Series: Materials Science and Engineering*; IOP Publishing: Bristol, UK, 2019.

80. Zhu, B.; Ni, F.; Sun, Y.; Yao, Z. Biochemical characterization and degradation pattern of a unique pH-stable polyM-specific alginate lyase from newly isolated *Serratia marcescens* NJ-07. *Mar. Drugs* 2018, 16, 129. [CrossRef] [PubMed]

81. Min, K.H.; Sasaki, S.F.; Kashiwabara, Y.; Suzuki, H.; Nisizawa, K. Multiple components of endo-polyguluronide lyase of *Pseudomonas* sp. *Biochem. Biophys. Res. Commun.* 1977, 81, 539–546. [CrossRef] [PubMed]

82. Lu, D.; Zhang, Q.; Wang, S.; Guan, J.; Jiao, R.; Han, N.; Han, W.; Li, F. Biochemical characteristics and synergistic effect of two novel alginate lyases from *Photo bacterium* sp. FC615. *Biotecnol. Biofuels* 2019, 12, 1–17. [CrossRef] [PubMed]

83. Sugimura, I.; Sawabe, T.; Ezura, Y. Cloning and sequence analysis of *Vibrio halioticoli* genes encoding three types of alguronic acid lyase. *Mar. Biotechnol.* 2000, 2, 65–73. [CrossRef] [PubMed]

84. Iwamoto, M.; Kurachi, M.; Nakashima, T.; Kim, D.; Yamaguchi, K.; Oda, T.; Ishimoto, Y.; Muramatsu, T. Structure–activity relationship of alginate oligosaccharides in the induction of cytokine production from RAW264.7 cells. *FEBS Lett.* 2005, 579, 4423–4429. [CrossRef] [PubMed]

85. Xu, X.; Zhou, Y.; Gao, X.; Yan, P. Advances in application of alginate lyase and its enzymatic hydrolysate. In *IOP Conference Series: Materials Science and Engineering*; IOP Publishing: Bristol, UK, 2019.

86. Boyen, C.; Bertheau, Y.; Barbeyron, T.; Kloareg, B. Preparation of guluronate lyase from Pseudomonas alginovora for protoplast isolation in Laminaria. *Enzym. Microb. Technol.* 2009, 579, 123–129. [CrossRef] [PubMed]

87. Zhang, R.; Zhou, J.; Jia, Z.; Zhang, Y.; Gu, G. Hypoglycemic effect of *Rehmannia glutinosa* oligosaccharide in hyperglycemic and alloxan-induced diabetic rats and its mechanism. *J. Ethnopharmacol.* 2004, 90, 39–43. [CrossRef] [PubMed]
102. Hirayama, M.; Hashimoto, W.; Murata, K.; Kawai, S. Comparative characterization of three bacterial exo-type alginate lyases. *Int. J. Biol. Macromol.* **2016**, *86*, 519–524. [CrossRef]

103. Yang, J.; Cui, D.; Ma, S.; Chen, W.; Chen, D.; Shen, H. Characterization of a novel PL 17 family alginate lyase with exolytic and endolytic cleavage activity from marine bacterium *Microbulbifer* sp. SH-1. *Int. J. Biol. Macromol.* **2021**, *169*, 551–563. [CrossRef]

104. Kim, D.E.; Lee, E.Y.; Kim, H.S. Cloning and characterization of alginate lyase from a marine bacterium *Streptomyces* sp. ALG-5. *Mar. Biotechnol.* **2009**, *11*, 10–16. [CrossRef]

105. Belik, A.; Silchenko, A.; Malyarenko, O.; Rasin, A.; Kiseleva, M.; Kusaykin, M.; Ermakova, S. Two new alginate lyases of PL7 and PL6 families from polysaccharide-degrading bacterium *Formosa* sp. KM553T: Structure, properties, and products analysis. *Mar. Drugs* **2020**, *18*, 130. [CrossRef] [PubMed]

106. Zhang, F.; Fu, Z.; Tang, L.; Zhang, Z.; Han, F.; Yu, W. Biochemical Characterization of a Novel Exo-Type PL7 Alginate Lyase VsaAly7D from Marine *Vibrio* sp. QY108. *Int. J. Mol. Sci.* **2021**, *22*, 8402. [CrossRef]

107. Yagi, H.; Fujise, A.; Ibashii, N.; Ohshiro, T. Purification and characterization of a novel alginate lyase from the marine bacterium *Colletia* sp. NAP1 isolated from brown algae. *Biosci. Biotechnol. Biochem.* **2016**, *80*, 2338–2346. [CrossRef]

108. Zhang, Y.-H.; Shao, Y.; Jiao, C.; Yang, Q.-M.; Weng, H.-F.; Xiao, A.-F. Characterization and application of an alginate lyase, Aly1281 from marine bacterium *Pseudoalteromonas carrageenovora* ASYS. *Mar. Drugs* **2020**, *18*, 95. [CrossRef] [PubMed]

109. Li, S.; Yang, X.; Zhang, L.; Yu, W.; Han, F. Cloning, expression, and characterization of a cold-adapted and surfactant-stable alginate lyase from marine bacterium *Agarivorans* sp. L11. *J. Microbiol. Biotechnol.* **2015**, *25*, 681–686. [CrossRef] [PubMed]

110. Manns, D.; Nyttegenegger, C.; Saake, B.; Meyer, A.S. Impact of different alginate lyases on combined cellulase–lyase saccharification of brown seaweed. *RSC Adv.* **2016**, *6*, 45392–45401. [CrossRef]

111. Yang, M.; Yu, Y.; Yang, S.; Shi, X.; Mou, H.; Li, L. Expression and characterization of a new polyG-specific alginate lyase from marine bacterium *Microbulbifer* sp. Q7. *Front. Microbiol.* **2018**, *9*, 2894. [CrossRef]

112. Dong, X.-L.; Dong, S.; Xu, F.; Dong, F.; Li, P.-Y.; Zhang, X.-Y.; Zhou, B.-C.; Zhang, Y.-Z.; Xie, B.-B.; Qin, Q.-L.; Zhang, X.-Y.; Pang, X.-H.; Zhou, B.-C. Molecular insight into the role of the N-terminal extension in the maturation, substrate recognition, and catalysis of a bacterial alginate lyase from *Pseudoalteromonas* sp. SM0524. *Front. Microbiol.* **2016**, *7*, 1120. [CrossRef]

113. Chen, X.-L.; Dong, S.; Xu, F.; Dong, F.; Li, P.-Y.; Zhang, X.-Y.; Zhou, B.-C.; Zhang, Y.-Z.; Xie, B.-B. Characterization of a new cold-adapted and salt-activated polysaccharide lyase family 7 alginate lyase from *Pseudoalteromonas* sp. SM0524. *Front. Microbiol.* **2016**, *7*, 1120. [CrossRef]

114. Li, S.; Yang, X.; Zhang, L.; Yu, W.; Han, F. Cloning, expression, and characterization of a cold-adapted and surfactant-stable alginate lyase from marine bacterium *Agarivorans* sp. L11. *J. Microbiol. Biotechnol.* **2015**, *25*, 681–686. [CrossRef] [PubMed]

115. Belik, A.; Silchenko, A.; Malyarenko, O.; Rasin, A.; Kiseleva, M.; Kusaykin, M.; Ermakova, S. Two new alginate lyases of PL7 and PL6 families from polysaccharide-degrading bacterium *Formosa* sp. KM553T: Structure, properties, and products analysis. *Mar. Drugs* **2020**, *18*, 130. [CrossRef] [PubMed]

116. Manns, D.; Nyttegenegger, C.; Saake, B.; Meyer, A.S. Impact of different alginate lyases on combined cellulase–lyase saccharification of brown seaweed. *RSC Adv.* **2016**, *6*, 45392–45401. [CrossRef]

117. Yang, M.; Yu, Y.; Yang, S.; Shi, X.; Mou, H.; Li, L. Expression and characterization of a new polyG-specific alginate lyase from marine bacterium *Microbulbifer* sp. Q7. *Front. Microbiol.* **2018**, *9*, 2894. [CrossRef]

118. Dong, S.; Wei, T.-D.; Chen, X.-L.; Dong, F.; Li, C.-Y.; Wang, P.; Xie, B.-B.; Qin, Q.-L.; Zhang, X.-Y.; Pang, X.-H.; Zhang, Y.-Z.; Chen, X.-L. Novel molecular insights into the catalytic mechanism of marine bacterial alginate lyase AlyGC from polysaccharide lyase family 6. *J. Biol. Chem.* **2017**, *292*, 4457–4468. [CrossRef]

119. Zhang, Y.-H.; Shao, Y.; Jiao, C.; Yang, Q.-M.; Weng, H.-F.; Xiao, A.-F. Characterization and application of an alginate lyase, Aly1281 from marine bacterium *Pseudoalteromonas carrageenovora* ASYS. *Mar. Drugs* **2020**, *18*, 95. [CrossRef] [PubMed]

120. Hirayama, M.; Hashimoto, W.; Murata, K.; Kawai, S. Comparative characterization of three bacterial exo-type alginate lyases. *Int. J. Biol. Macromol.* **2016**, *86*, 519–524. [CrossRef]

121. Yan, J.; Chen, P.; Zeng, Y.; Men, Y.; Mu, S.; Zhu, Y.; Chen, Y.; Sun, Y. The characterization and modification of a novel bifunctional and robust alginate lyase derived from *Marinimicrobium* sp. H1. *Mar. Drugs* **2019**, *17*, 545. [CrossRef]

122. McHugh, D.J. Production, properties and uses of alginates. Production and Utilization of Products from Commercial Seaweeds. *FAO. Fish. Tech. Pap.* **1987**, *58*, 521–527.

123. Tomoo, S.; Yoshio, E.; Takahisa, K. Purification and Characterization of an Alginate Lyase from Marine Alteromonass. *Nippon Suisan Gakkaishi* **1992**, *58*, 521–527.

124. Wiens, J.R.; Vasil, A.I.; Schurr, M.J.; Vasil, M.L. Iron-regulated expression of alginate production, mucoid phenotype, and biofilm formation by *Pseudomonas* aeruginosa. *MBio* **2014**, *5*, e01010-13. [CrossRef]

125. Daboor, S.M.; Rohde, J.R.; Cheng, Z. Disruption of the extracellular polymeric network of *Pseudomonas* aeruginosa biofilms by alginate lyase enhances pathogen eradication by antibiotics. *J. Cyst. Fibros.* **2021**, *20*, 264–270. [CrossRef]

126. Yagi, H.; Fujise, A.; Ibashii, N.; Ohshiro, T. Purification and characterization of a novel alginate lyase from the marine bacterium *Colletia* sp. NAP1 isolated from brown algae. *Biosci. Biotechnol. Biochem.* **2016**, *80*, 2338–2346. [CrossRef]

127. Xu, F.; Chen, X.-L.; Sun, X.-H.; Dong, F.; Li, C.-Y.; Li, P.-Y.; Ding, H.; Chen, Y.; Zhang, Y.-Z.; Wang, P. Structural and molecular basis for the substrate positioning mechanism of a new PL7 subfamily alginate lyase from the arctic. *J. Biol. Chem.* **2020**, *295*, 16380–16392. [CrossRef]
128. Chen, J.; Hu, Y.; Zhang, L.; Wang, Y.; Wang, S.; Zhang, Y.; Guo, H.; Ji, D.; Wang, Y. Alginase oligosaccharide DP5 exhibits antitumor effects in osteosarcoma patients following surgery. Front. Pharmacol. 2017, 8, 623. [CrossRef] [PubMed]

129. Hao, J.; Hao, C.; Zhang, L.; Liu, X.; Zhou, X.; Dun, Y.; Li, H.; Li, G.; Zhao, X.; An, Y. OM2, a novel algomannurionate-chromium (III) complex, promotes mitochondrial biogenesis and lipid metabolism in 3T3-L1 adipocytes via the AMPK-PPC1α pathway. PLoS ONE 2015, 10, e0131930. [CrossRef] [PubMed]

130. Ueno, M.; Tamura, Y.; Toda, N.; Yoshinaga, M.; Terakado, S.; Otsuka, K.; Numabe, A.; Kawahata, Y.; Murota, I.; Sato, N. Sodium alginate oligosaccharides attenuate hypertension in spontaneously hypertensive rats fed a low-salt diet. Clin. Exp. Hypertens. 2012, 34, 305–310. [CrossRef] [PubMed]

131. Khan, S.; Tøndervik, A.; Sletta, H.; Klinkenberg, G.; Emanuel, C.; Onsøyen, E.; Myrvold, R.; Howe, R.A.; Walsh, T.R.; Hill, K.E. Overcoming drug resistance with alginate oligosaccharides able to potentiate the action of selected antibiotics. Antimicrob. Agents Chemother. 2012, 56, 5134–5141. [CrossRef]

132. Saigusa, M.; Nishizawa, M.; Shimizu, Y.; Saeki, H. In vitro in vivo anti-inflammatory activity of digested peptides derived from salmon myofibrillar protein conjugated with a small quantity of alginate oligosaccharide. Biosci. Biotechnol. Biochem. 2015, 79, 1518–1527. [CrossRef]

133. Powell, L.C.; Pritchard, M.F.; Emanuel, C.; Onsøyen, E.; Rye, P.D.; Wright, C.J.; Hill, K.E.; Thomas, D.W. A nanoscale characterization of the interaction of a novel alginate oligomer with the cell surface and motility of Pseudomonas aeruginosa. Am. J. Respir. Cell Mol. Biol. 2014, 50, 485–492. [CrossRef]

134. Inoue, A.; Anraku, M.; Nakagawa, S.; Ojima, T. Discovery of a novel alginate lyase from Flavobacterium sp. strain MY04, effects of module truncation on biochemical characteristics, alginate degradation patterns, and alginate oligosaccharide-yielding properties. FEBS Lett. 2013, 577, 79–84. [CrossRef] [PubMed]

135. Tusi, S.K.; Khalaj, L.; Ashabi, G.; Kiaei, M.; Khodagholi, F. Alginate oligosaccharide protects against endoplasmic reticulum-and mitochondrial-mediated apoptotic cell death and oxidative stress. Biomaterials 2011, 32, 5438–5448. [CrossRef]

136. Liu, J.; Yang, S.; Li, X.; Yan, Q.; Reaney, M.J.; Jiang, Z. Alginate oligosaccharides: Production, biological activities, and potential applications. Compr. Rev. Food Sci. Food Saf. 2019, 18, 1859–1881. [CrossRef]

137. Jiang, Z.; Zhang, X.; Wu, L.; Li, H.; Chen, Y.; Li, L.; Ni, H.; Li, Q.; Zhu, Y. Exolytic products of alginate by the immobilized alginate lyase confer antioxidant and antiapoptotic bioactivities in human umbilical vein endothelial cells. Carbohydr. Polym. 2021, 251, 116976. [CrossRef]

138. Gao, J.; Lin, L.; Sun, B.; Zhao, M. A comparison study on polysaccharides extracted from Laminaria japonica using different methods: Structural characterization and bile acid-binding capacity. Food Funct. 2017, 8, 3043–3052. [CrossRef] [PubMed]

139. Kawada, A.; Hiura, N.; Shiraiwa, M.; Tajima, S.; Hiruma, M.; Harai, K.; Ishibashi, A.; Takahara, H. Stimulation of human keratinocyte growth by alginate oligosaccharides, a possible co-factor for epidermal growth factor in cell culture. FEBS Lett. 1997, 408, 43–46. [CrossRef]

140. Wilcox, M.D.; Brownlee, I.A.; Richardson, J.C.; Dettmar, P.W.; Pearson, J.P. The modulation of pancreatic lipase activity by alginites. Food Chem. 2014, 146, 479–484. [CrossRef] [PubMed]

141. Liao, H.; Anraku, M.; Nakagawa, S.; Ojima, T. Discovery of a novel alginate lyase from Nitratiruptor sp. SB155-2 thriving at deep-sea hydrothermal vents and identification of the residues responsible for its heat stability. J. Biol. Chem. 2016, 291, 15551–15563. [CrossRef]

142. Wang, Y.; Chen, X.; Bi, X.; Ren, Y.; Han, Q.; Zhou, Y.; Han, Y.; Yao, R.; Li, S. Characterization of an alkaline alginate lyase with pH-stable and thermo-tolerance property. Int. J. Biol. Macromol. 2021, 201, 118158. [CrossRef]

143. Vasudevan, U.M.; Lee, O.K.; Lee, E.Y. Alginate derived functional oligosaccharides: Recent developments, barriers, and future outlooks. Carbohydr. Polym. 2021, 267, 118138. [CrossRef]

144. Zhu, B.; Li, K.; Wang, W.; Ning, L.; Tan, H.; Zhao, X.; Yin, H. Preparation of trisaccharides from alginate by a novel alginate lyase Alg7A from marine bacterium Vibrio sp. W13. Int. J. Biol. Macromol. 2019, 139, 879–885. [CrossRef]

145. Chen, Y.; Dou, W.; Li, H.; Shi, J.; Xu, Z. The alginate lyase from Isoptericola halotolerans CGMCC 5336 as a new tool for the production of alginate oligosaccharides with guluronic acid as reducing end. Carbohydr. Res. 2018, 470, 36–41. [CrossRef]

146. Huang, L.; Zhou, J.; Li, X.; Peng, Q.; Lu, H.; Du, Y. Characterization of a new alginate lyase from newly isolated Flavobacterium sp. S20. J. Ind. Microbiol. Biotechnol. 2013, 40, 113–122. [CrossRef]

147. Tøndervik, A.; Klinkenberg, G.; Aarstad, O.A.; Drablos, F.; Ertesvåg, H.; Ellingsen, T.E.; Skjåk-Bræk, G.; Valla, S.; Sletta, H. Isolation of mutant alginate lyases with cleavage specificity for di-guluronic acid linkages. J. Biol. Chem. 2010, 285, 35284–35292. [CrossRef]

148. Li, L.; Jiang, X.; Guan, H.; Wang, P. Preparation, purification and characterization of alginate oligosaccharides degraded by alginate lyase from Pseudomonas sp. HZJ216. Carbohydr. Res. 2021, 470, 1094–1098. [CrossRef]

149. Han, W.; Gu, J.; Cheng, Y.; Liu, H.; Li, Y.; Li, F. Novel alginate lyase ( Aly5) from a polysaccharide-degrading marine bacterium, Flavmeovirga sp. strain MY04, effects of module truncation on biochemical characteristics, alginate degradation patterns, and oligosaccharide-yielding properties. Appl. Environ. Microbiol. 2016, 82, 364–374. [CrossRef] [PubMed]

150. Daboor, S.M.; Raudonis, R.; Cohen, A.; Rohde, J.R.; Cheng, Z. Marine bacteria, a source for alginolytic enzyme to disrupt Pseudomonas aeruginosa biofilms. Mar. Drugs 2019, 17, 307. [CrossRef] [PubMed]

151. Dharani, S.R.; Srinivasan, S.; Sarath, R.; Ramya, M. Recent progress on engineering microbial alginate lyases towards their versatile role in biotechnological applications. Folia Microbiol. 2020, 65, 937–954. [CrossRef]
152. Wargacki, A.J.; Leonard, E.; Win, M.N.; Regitsky, D.D.; Santos, C.N.S.; Kim, P.B.; Cooper, S.R.; Raisner, R.M.; Herman, A.; Sivitz, A.B. An engineered microbial platform for direct biofuel production from brown macroalgae. *Science* **2012**, *335*, 308–313. [CrossRef]

153. Wang, L.; Li, S.; Yu, W.; Gong, Q. Cloning, overexpression and characterization of a new oligoalginate lyase from a marine bacterium, *Shewanella* sp. *Biotechnol. Lett.* **2015**, *37*, 665–671. [CrossRef]

154. Tang, J.C.; Taniguchi, H.; Chu, H.; Zhou, Q.; Nagata, S. Isolation and characterization of alginate-degrading bacteria for disposal of seaweed wastes. *Lett. Appl. Microbiol.* **2009**, *48*, 38–43. [CrossRef]

155. Maurice, N.M.; Bedi, B.; Sadikot, R.T. *Pseudomonas aeruginosa* biofilms: Host response and clinical implications in lung infections. *Am. J. Respir. Cell Mol. Biol.* **2018**, *58*, 428–439. [CrossRef]

156. Tavafi, H.; Ali, A.A.; Ghadam, P.; Charavi, S. Screening, cloning and expression of a novel alginate lyase gene from *P. aeruginosa* TAG 48 and its antibiofilm effects on *P. aeruginosa* biofilm. *Microb. Pathog.* **2018**, *124*, 356–364. [CrossRef]

157. Preiss, J.; Ashwell, G. Alginic acid metabolism in bacteria: I. Enzymatic formation of unsaturated oligosaccharides and 4-deoxy-L-erythro-5-hexoseulose uronic acid. *J. Biol. Chem.* **1962**, *237*, 309–316. [CrossRef]

158. Skriptsova, A.; Khomenko, V.; Isakov, V. Seasonal changes in growth rate, morphology and alginate content in *Undaria pinnatifida* at the northern limit in the Sea of Japan (Russia). *J. Appl. Phycol.* **2004**, *16*, 17–21. [CrossRef]

159. Kokoulin, M.S.; Tomshich, S.V.; Kalinovsky, A.I.; Komandrova, N.A. O-antigens of marine gram-negative bacteria. *Bull. Far East. Branch Russ. Acad. Sci.* **2015**, *6*, 132–139. (In Russian)

160. Park, D.; Jagtap, S.; Nair, S.K. Structure of a PL17 family alginate lyase demonstrates functional similarities among exotype depolymerases. *J. Biol. Chem.* **2014**, *289*, 8645–8655. [CrossRef] [PubMed]

161. Gacesa, P. Alginate-modifying enzymes. A proposed unified mechanism of action for the lyases and epimerases. *Fed. Eur. Biochem. Soc. Lett.* **1987**, *212*, 199–202. [CrossRef]

162. Garron, M.L.; Cygler, M. Uronic polysaccharide degrading enzymes. *Curr. Opin. Struct. Biol.* **2014**, *28*, 87–95. [CrossRef]