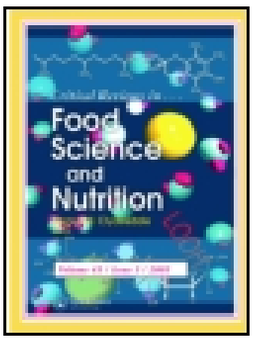

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REVIEW

Process and applications of alginate oligosaccharides with emphasis on health beneficial perspectives

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ABSTRACT

Alginates are linear polymers comprising 40% of the dry weight of algae possess various applications in food and biomedical industries. Alginate oligosaccharides (AOS), a degradation product of alginate, is now gaining much attention for their beneficial role in food, pharmaceutical and agricultural industries. Hence this review was aimed to compile the information on alginate and AOS (prepared from seaweeds) during 1994–2020. As per our knowledge, this is the first review on the potential use of alginate oligosaccharides in different fields. The alginate derivatives are grouped according to their applications. They are involved in the isolation process and show antimicrobial, antioxidant, anti-inflammatory, antihypertension, anticancer, and immunostimulatory properties. AOS also have significant applications in prebiotics, nutritional supplements, plant growth development and others products.

KEYWORDS

Alginate oligosaccharides; health beneficial effects; marine foods; nutrient enhancementstructure-activity relationship; synthesis

Introduction

Ocean occupies 70% of the earth surface with more than one billion unicellular and one million multicellular organisms (Burgess 2012) and provides enormous resources to marine researchers to explore new natural products with diverse applications. Recent advances in algal biotechnology have yielded various biologically active natural products such as biopolymers and its derivatives, proteins and amino-acids, carotenoid pigments, terpenoids, phlorotannins, and alkaloids, polyunsaturated fatty acids, polyamines, growth hormones and other essential minerals (Rengasamy et al. 2014). Seaweeds are frequently reviewed for their potential as antimicrobial, antioxidant, anti-inflammatory, anticancer, antidiabetes, anti-obesity, anti-Alzheimer's and potent enzyme inhibiting agents (Rengasamy et al., 2014). Alginate is a kind of acidic linear polysaccharide which consists of alpha-L-guluronate and its C-5 epimer, beta-D-mannuronate, linking with 1,4-O-glycosidic bonds. It usually exists in three different ways: poly-alpha-L-guluronate (pG), poly-beta-D-mannuronate (pM), and heteropolymeric regions (pMG) (Zhu, Chen, et al. 2016). Alginate has been extensively used in various industries as a thickening, gelling and stabilizing agent and pharmaceutical, cosmetic, and textile industries (Yang et al. 2012; Lee and Mooney 2012).

Seaweeds or marine macroalgae are among the major alginate resources predominantly from genus *Laminaria*, *Macrocystis* and *Ascophyllum,* comprising up to 40% of dry weight. The global production of alginates and its derivatives has elevated from 48,731 MT (2010) to 58,270 MT (2015) implying more than 3.65% of an average growth rate. In year 2020, global market value of alginate and its derivatives reached US$442.6 million and is projected to reach US$547.2 million till year 2027 (Industry Research 2021). Recently, the U.S. Food and Drug Administration has declared and considered alginate salts, including alginate in the form of sodium and potassium alginate, as safe products (FDA 2012). This will boost the functional food industry to develop new products from various alginate source, including alginate oligosaccharides. Alginate oligosaccharides (AOS) are the degradation product of alginate polymers with low molecular weight. AOS is formed through cleavage of glycosidic linkages through endo active ß-elimination and double bonds between the C4 and C5 carbons in the non-reducing terminal residues during hydrolysis. Several methods are being used for depolymerizing alginate into low molecular alginates, alginate oligomers and its derivatives such as reducing (Smidsrød et al., 1963), acidic (Haug et al., 1963), radiation (Lee et al. 2003; Şen 2011), thermal treatments (Aida et al. 2010; Holme et al. 2008; Kelishomi et al. 2016) and various enzymatic methods (Zhao et al. 2011).

Alginate oligosaccharides (AOS) are oligomers comprising 2–25 monomers prepared from higher molecular weight alginate polymer (Liu et al. 2019). AOS has lots of similarity in its physicochemical properties with its parent polymer

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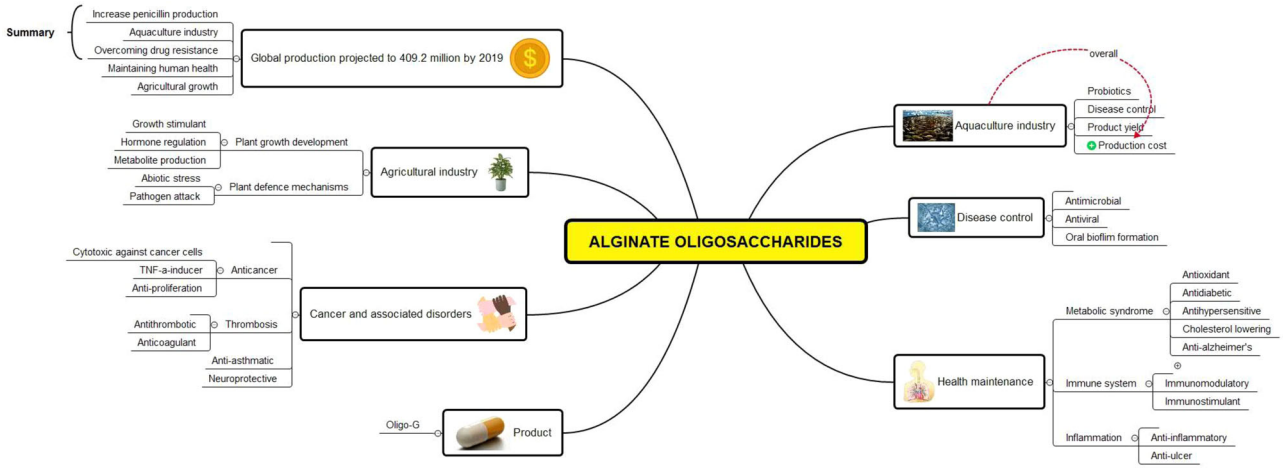

Figure 1. Applications of alginate oligosaccharides.

(Boucelkha et al. 2017; Xing et al. 2020). Similar to alginate, alginate oligomers show affinity with monovalent and divalent ions but lacks gel formation in the presence of divalent cations. A high concentration of alginate oligosaccharide solutions is formed with a non-significant elevation in viscosity due to the low molecular weight of alginate oligosaccharides (Rye et al. 2018). AOS (Alginate oligosaccharides) derived from marine alginate have shown significant bioactivities and therapeutic potential (Ushasree, Lee, and Lee 2021). The alginate oligosaccharides have been reported to have various biological activities, including immunostimulant (Wang et al. 2014), plant growth-promoting (Zhang et al. 2014), antioxidant (Falkeborg et al. 2014), antifungal (Tøndervik et al. 2014) and neurogenerative (Zhou, Shi, Bi et al. 2015) and anti-allergic activities (Uno et al. 2006). Nevertheless, there are limitations in the multidimensional application of this polymer due to its higher viscosity and low water solubility when used in high concentrations to attain certain health benefits (Liu et al. 2019). However, alginate oligosaccharides have been extensively studied for biological properties.

Literature has revealed various bioactivities of alginate oligosaccharides, such as antioxidant, antimicrobial, antiinflammatory, and immunomodulatory properties (Ming et al. 2021). Summary of applications of alginate oligosaccharides (AOS) in various industries are shown in Figure 1. Recently, the research focus has shifted from alginate to alginate oligosaccharides due to their bioactivities (Liu et al. 2019; Xing et al. 2020). The application of AOS in improving gut health has opened new opportunities for its usage as a functional metabolite in curtailing various diet-related metabolic syndromes (Li, He, and Wang 2019; Li, Wang, Liu, et al. 2020; Wang et al. 2020). AOS can be employed as a functional ingredient in treating tumors and minimizing the side effects of chemotherapy (Chen et al. 2017). Furthermore, alginate oligosaccharides have also shown potential in the agriculture and food sectors due to their plant growth promotion and shelf-life extension properties, respectively (Salachna et al. 2018; Zhang, Yao, et al. 2020; Liu et al. 2020; Zhang et al. 2019; Zhang, Wang, et al. 2020). Keeping in view the potential of AOS, this review provides a comprehensive overview of the production of AOS and their application in food, agriculture, and pharmacology.

Production of alginate oligosaccharides from seaweeds

In order to depolymerize alginate to get low molecular weight oligosaccharides and various oligo derivatives, various protocols were used, including acid hydrolysis, enzymatic degradation, photolysis, oxidative-reductive depolymerization, thermal degradation, thermolysis under acid or alkaline conditions (Burana-Osot et al. 2009). Tables 1 and 2 shows the production of alginate oligosaccharides from various physical, chemical, and biotechnological approaches. The schematic representation of various processes involved in the production of AOS are described in Figure 2.

A newly purified endo-type alginate lyase obtained from *Cellulophaga sps* was used to hydrolyze sodium alginate (Zhu, Chen, et al. 2016). The resulting AOS possessed excellent thermal stability and bioactivity. Another study by Zhang et al. (2006) explained the use of negative-ion electrospray tandem mass spectroscopy (ESI-MS) to characterize the AOS prepared using both mild acid hydrolysis and *Vibrio* sp. derived alginate lyase. Both acid and enzyme hydrolyzed oligosaccharides were further purified with Bio-Gel P6 and obtained 15 homo and hetero oligosaccharides including ΔGG, ΔMG, ΔMM, ΔMGG, ΔGMG, MM, MMM, MMMM, MMMMM, GG, GGG, GGGG and GGGGG and were dominated by intense B-, C- Y- and Z type ions together with A residues. Yang, Li, and Guan (2004) reported degradation of polymannuronates through application of hydrogen peroxide (30%) (Yang, Li, and Guan 2004). The results suggest that 0.5 h reaction time at 90 °C was found to produce more saccharides (1–15 saccharides) with a maximum yield of 58%. The structure of resulted oligomannuronates that were identified using ESI-MS and NMR analysis. Similar results were reported by Mao et al. (2012), where low molecular weight alginate oligosaccharides were obtained through oxidative degradation using H_2O_2 (1%) with 2 h of reaction time. They found that depolymerization occurred in the first hour, and the process was slow down and constant after 2 h. These findings were supported by a previous study where oxidation of alginate occurred during

Table 1. Depolymerization of alginate through physical and chemical methods.

Methods	Synthesis method	Synthesized product	References
Physical	Cobalt-60 gamma rays (20–100 kGy)	Low molecular weight alginate oligomers	Abd El-Mohdy (2017)
	Periodate oxidation	Low molecular weight saccharides	Balakrishnan et al. (2005)
	Photochemical UV/titanium dioxide process	ManA and GluA rich fractions and GM alternating sequence	Burana-Osot et al. (2009)
	Gamma-ray irradiation	Oligomers	Hien et al. (2000)
	Thermal degradation at 22.5, 36, 66 and 80°	Mixture of oligosaccharides	Holme et al. (2008)
	Thermal degradation of alginic acid sodium salt at 140 °C for 1.5, 4.5 and 7.5h	Low molecular weight alginate	Kelishomi et al. (2016)
Chemical	Hydrogen peroxide	Molecular weight ($\leq$ 5.5 kDa DP 2-24	Soukaina et al. 2020
	Ultra-sonication (59 kHz)	Low molecular weight alginate polymer (100 kg/mol)	Dodero, Vicini, and Castellano (2020)
	Soaking in NaOCl (5%) followed by addition of KCl (0.13M) and ethanol (96%)	Low molecular weight alginate	Yudiati et al. (2018)
	Polymannuronate blocks were hydrolyzed using hydrogen peroxide (30%) at 5% (v/v).	low-molecular-weight mannuronates	Yang, Li, and Guan (2004)
	Sodium alginate was degraded using hydrogen peroxide (1%)	Low molecular weight oligomers	Mao et al. (2012)
	100ml of 1% poly-G (adjusted to pH4.0 with 0.3N HCl) hydrolyzed at 120 °C for 30min	Oligo-guluronic acids (DP = 1-9)	Shimokawa et al. (1996)
	Hydrolysis of M-block and G-block with 1M and 2M Trifluoroacetic acid at 100 °C and 120 °C for 24h	ManA and GluA	Lu et al. (2015)
	Sodium alginate (1.5%) was hydrolyzed using hydrogen peroxide (30%) at various reaction temperature and time	Low molecular weight alginate	Li, Li, and Guo (2010)
	Sodium alginate (10 g) was dissolved 1000 mL distilled water with 0.3 M HCl and heated at 100 °C for 6 h	Oligogluronate blocks	Ariyo, Bucke, and Keshaverz (1997)
	Partial acid hydrolysis: Alginate was dissolved in 0.3-0.6M HCl reflux at 100 °C for 4-6h	Low molecular weight OM and OG fractions	Asilonu et al. (2000)
	Acid hydrolysis of 1% alginate in 200 ml of an acidic solution (0.3 M HCl, pH 4.0) at 121° for 80 min	Oligomers mainly consist of monomer (176Da), dimer (352Da) and trimer (528Da)	Yamasaki et al. (2012)

the first hour and decreased after 1 hour (Balakrishnan et al. 2005).

Among various approaches practised, depolymerization by acid hydrolysis is one of the most rapid methods for depolymerization of alginate; however, this procedure is costly and not eco-friendly (Salachna et al. 2018). On the other hand, the use of H_2O_2 (hydrogen peroxide) for oxidative depolymerization of alginate is preferred as just water is the by-product of this procedure. Even though oxidative depolymerization is thought to be a random procedure, recently, alginate oligosaccharides from *B. bifurcate* (brown algae) demonstrated the degree of polymerization in between 2 & 24 and controlled molecular weight ($\leq$ 5.5 kDa) (Soukaina et al. 2020). Some other methods, including ultrasonic, UV (ultraviolet), and gamma irradiation, are also extensively used to degrade alginate. Among these, gamma irradiation is known to be the most energy-efficient and effective alginate degradation procedure (Abd El-Mohdy 2017). There are no special requirements to control additives, environment, and temperature for alginate de-polymerization (Abd El-Mohdy 2017). Besides these, sub-critical water hydrolysis, hydrothermal treatment, and plasma treatment are few other physical methods used to depolymerize alginate. In a solution plasma process, an electrical discharge applied to the reactor produces reactive species, resulting in the breakdown of alginate polysaccharide chains (Liu et al. 2019).

As mentioned earlier, alginate is a linear polysaccharide abundantly produced by marine brown algae. Therefore, enzymes like alginate lyases have also been used for alginate degradation through β-elimination reaction. Alginate lyases play a vital role in the recycling of marine carbon and hence have significant industrial applications. Alginate lyases adopt two different catalytic mechanisms and three conformational changes during degradation (Xu et al. 2018). Compared to physical and chemical processes, degradation of alginate via enzymes (alginate lyases) is eco-friendly, energy-efficient, selective, and AOS formed is more bioactive (Cheng et al. 2020). These edolytic alginate lyases help cleave the glycosidic linkages present within the alginate, liberating unsaturated oligosaccharides with a different degree of polymerization (DP). Mostly, the main degradation products released by alginate lyases are small alginate oligosaccharides, i.e., DP2 (disaccharide), DP3 (trisaccharide), DP4 (tetrasaccharide), and DP5 (pentasaccharide) (Li, Shang, et al. 2017; Gao et al. 2018). The CAZy (Carbohydrate-Active enZYmes) database categorizes alginate lyases into twelve PL (polysaccharide lyase) families, i.e., PL5-7, 14, 15, 17, 18, 31, 32, 34, 36, & 39 accordingly their primary structure (http://www.cazy.org/) (Cheng et al. 2020).

In alginate lyases, the depolymerization pattern and substrate specificity depend upon specified functional components, residues of the active site, and conservative motifs, even though it is unraveled in polysaccharide lyase families (Cheng et al. 2020; Xu et al. 2018). Moreover, in alginate lyases, the CBM (non-catalytic carbohydrate-binding module) is critically responsible for selecting substrate and

Table 2. Biotechnological production of alginate oligomers and its derivatives.

Alginate source	Enzyme source	Enzyme	Alginate/enzyme ratio	Conditions	Analytical condition	References
Rotten brown algae	*Microbulbifer sp. ALW1*	AlgL17	10 μl of Enzyme solution (0.6 mg mL^{-1}) was added to 390 μl of sodium phosphate buffer (50 mM, pH: 8) having Na-alginate (2.5 mg mL^{-1})	pH: 8, temperature: 35°C	ESI-MS	Jiang et al. (2019)
Macrocystis pyrifera	*Vibrio sp. W13*	Alg7A	Enzyme (0.1 mL) and alginate (1%, 0.9 mL) in Tris-HCl (20 mM, pH: 7.5) were incubated for 0.2 h at 30°C	pH: 7, temperature: 30°C	TLC, ESI-MS	Zhu, Li, et al. (2019)
Macrocystis pyrifera	*Flammeovirga sp. NJ-04*	FsAlgB	Enzyme (0.042 mg) and alginate (1%, 0.9 mL) in Tris-HCl (50 mM, pH: 8.0) were incubated for 10 min at 40 °C	pH: 8, temperature: 40°C	TLC, ESI-MS	Zhu, Ni, et al. (2019)
Sodium alginate derived from brown seaweed	*Bacillus sp. Alg07*	AlgA	The diluted enzyme (100 μL) was mixed with substrate solution (pH: 7.5, 1900 μL) having sodium alginate (10 g L^{-1}), sodium chloride (200 mM), and Tris-HCl (20 mM)	pH: 7.5, temperature: 40°C	ESI-MS	Chen et al. (2018)
Sodium alginate derived from brown seaweed	*Flammeovirga sp. strain MY04*	Aly2	The enzyme (2 µg) was mixed with sodium alginate (1 mg/ml) in NaAc-HAc buffer (50 mM, pH 6.0) and was incubated at 40 °C	pH: 6.0, temperature: 40°C	Gel-filtration HPLC, NMR	Peng et al. (2018)
Brown algae	*Thalassotalea crassostreae*	TcAlg1	0.5 mM of the enzyme was added to 200 µL of sodium phosphate buffer (20 mM, pH: 6) having sodium alginate (2%)	pH: 7, temperature: 40°C	TLC, MALDI–TOF MS, NMR	Wang et al. (2018)
Sodium alginate	*Marinimicrobium sp. H1*	AlgH	The diluted enzyme (50 μL) was mixed with substrate solution having sodium alginate (10 g L^{-1}) and was incubated for 30 min at 45°C	pH: 10, temperature: 45°C	HPLC, NMR, ESI-MS	Yan et al. (2019)
Alginate (M/G ratio 0.85)	*Microbulbifer sp. Q7*	AlyM	100 μl of Enzyme solution (0.6 mg mL^{-1}) was added to substrate solution (900 μL, 0.5%) in phosphate buffer (50 mM, pH: 7) and was incubated for 10 min at 45°C	pH: 7, temperature: 55°C	HPLC, NMR, ESI-MS, IR	Yang et al. (2018)
Saccharina cichorioides	*Formosa algae KMM 3553^T*	ALFA3 & ALFA4	100 µL of the enzyme solution was mixed with substrate solution (200 µL, 4 mg mL^{-1}) in buffer (300 µL)	pH: 6.0, temperature: 35°C pH: 8.0, temperature: 30°C	NMR, SDS-PAGE	Belik et al. (2020)
Rotten Sargassum	*Vibrio weizhoudaoensis M0101*	AlgM4	900 µL of sodium alginate solution (1%) was added to an enzyme (100 µL) and incubated for 10 min at 30°C	pH: 8.5, temperature: 30°C	UPLC–QTOF–MS/MS, TLC and ESI-MS	Huang et al. (2018)
Rotten Sargassum	*Marine BP-2 strain*	Alg17B	Enzyme solution (0.1 mg mL^{-1}) was mixed with sodium alginate (0.2 g) in Tris-HCL buffer (100 mL, 50 mM, pH: 7.5) and was incubated for 10 min at 37°C	pH: 8, temperature: 45°C	UHPLC-Q-Extractive analysis, TLC, & ESI-MS	Huang et al. (2019)
Alginate (M/G ratio 0.6)	*Bacteroides cellulosilyticus CRE21*	BcelPL6		pH: 7.5, temperature: 30°C	MALDI-TOF/TOF MS, LC-ESI-MS,	Stender et al. (2019)
Sodium alginate	*Pseudoalteromonas carrageenovora ASY5*	Aly1281	Enzyme (200 μL) was added to 0.5% sodium alginate (800 μL, pH 8.0) and incubated for 40 min at 50 °C	pH: 8.0, temperature: 50°C	TLC and ESI-MS	Zhang, Yao, et al. (2020)
Sodium alginate from brown algae	*Photobacterium sp. FC615*	AlyPB1 & AlyPB2	100 μl of 150 mM NaH2PO4–Na2HPO4 buffer (pH 8.0); 100 μl of 3 mg/ml sodium alginate,	pH: 8.0, temperature: 30°C; pH: 8.0, temperature: 20°C	NMR, LCMS-IT-TOF, TLC and ESI-MS	Lu et al. (2019)
Laminaria japonica	*Vibrio sp. SY01*	OalV17				

(*continued*)

Table 2. Continued.

Alginate source	Enzyme source	Enzyme	Alginate/enzyme ratio	Conditions	Analytical condition	References
			Enzyme solution (100 μL) was added to pre-incubated sodium alginate solution (900 μL, 0.3%) in phosphate buffer (50 mM) and incubated for 10 min at 40 °C	pH: 7.2, temperature: 40°C	SE-HPLC & TLC-ESI-MS	Li, Wang, Jung, et al. (2020)
Sodium alginate from *Macrocystis pyrifera* (M/G ratio 77/23)	*Cellulophaga sp NJ-1*	Alginate lyase	1ml enzyme added to 100 ml 1% sodium alginate	30° C for 2-72 h	TLC and ESI-MS	Zhu, Chen, et al. (2016)
Rotten brown seaweed	*Microbulbifer sp. ALW1*	Alginate lyase	0.5 U of enzyme added to 100 ml of 50 mM Tris–HCl buffer (pH 7.0) containing 1% (w/v) sodium alginate	37 °C for 9 h	ESI-MS	Zhu, Wu, et al. (2016)
Poly-α-guluronic acid	*NA*	Alginate lyase	50 U of enzyme added to 1000 ml PolyG (10 g) dissolved in50 mM Tris–HCl buffer at pH 7.5	30 °C for 10 h	HPAEC, ESI-MS and NMR	Liu et al. (2002)
Sodium alginate	*Flavobacterium sp. LXA*	Alginase	4 ml enzyme added to 200 volume of 1% sodium alginate 2.5% phosphate-citrate buffer pH 7.0	40 °C for 15 to 360 min	HPLC	An et al. (2009)
Laminaria japonica, (M/G: 2.28)	*Vibrio sp. 510*	Alginate lyase	50U enzyme added to 5 g alginate dissolved in 1000 ml 50 mM Tris-HCl buffer pH 7.5	28 °C for 24 h	HPTLC, NMR and ESMS	Zhang et al. (2016)
Sodium alginate from Laminaria (M/G: 2.28)	*Pseudomonas sp. HZJ 216*	Alginate lyase	200 U enzyme added to alginate (5 g) dissolved in 500 mL of 50 mmol L1 Tris–HCl buffer (pH 7.0)	30 °C for 6 h	ESI-MS and NMR	Li et al. (2011)
Sodium alginate (M/G: 0.2)	*Flavobacterium multivolume*	Alginate lyase	14 U enzyme added to 2 g Poly-G dissolved in 100 ml 10 mM phosphate buffer pH7.0	37 °C for 30 min	–	Shimokawa et al. (1996)
Oligomannuronate	*Haliotis tuberculata*	Alginate lyase	0.2U/ml enzyme	20 °C	HPLC and ^{1}H NMR	Heyraud et al. (1996)
Sodium alginate	*Sphingobacterium*	Alginate lyase S	5% w/w enzyme added to 150 ml alginate dissolved in ammonium acetate buffer	30 °C for 72 h	TLC, ESI-TOF-MS, and FTIR	Falkeborg et al. (2014)
Alginate (M/G,2.28;MW: 300 kDa)	*Vibrio sp. 510*	Alginate lyase	50 U enzyme added to 5 g alginate dissolved in 1000 ml 50 mM Tris-HCl buffer pH 7.5	28 °C fr 24 h	–	He et al. (2013)
Sodium alginate	*Vibrio sp. 510*	Alginate lyase	80U enzyme added to 1 g of 2% alginate solution	28 °C	IR	Hu et al. (2004a)
Alginate	*Alginate lyases (EC 4.2.2.3)*	Alginate lyase	0.08U /ml	28 °C	–	Hu et al. (2004b)
PG and PM from Sodium alginate (1000-cps grade)	*Pseudoalteromonas sp. strain No. 272*	Alginate lyase	0.4 ml of 50 μg/ml enzyme added to 10 g PG or PM dissolved in water	30 °C for 2 h	–	Iwamoto et al. (2005)
Sodium alginate (M/G,0.05;MW: 500 kDa)	*Corynebacterium sp.*	Poly (α-L-guluronate)lyase	0.1% enzyme added to 10 g/l alginate solution	35 °C for 2 h	–	Iwasaki and Matsubara (2000)
Sodium alginate	*Alteromonas sp. No. 1786*	Alginate lyase	Enzyme solution mixed with 0.5% (w/v) sodium alginate in 0.05 M sodium phosphate buffer, pH 7.5	40 °C for 6 h	HPLC, ^{1}-NMR and 13 C-NMR	Kawada et al. (1997)
Sodium alginate	*Pseudoalteromonas sp. strain No. 272*	Alginate lyase	1μg/ml enzyme added 1% alginate solution	40 °C for 3-7 days	–	Kurachi et al. (2005)
alginate (viscosity, 1010 mPa s)	*S. violaceoruber*	Alginate lyase	Enzyme hydrolysis followed by ethanol precipitation and supernatant was used to determine AOS	50 °C for 10 min	–	Liu et al. (2013)
Sodium alginate (MW:9600, M/G: 47/53)	*Alteromonas naacleodii (FEARP-9218)*	Alginate lyase	The enzyme (133 ml, 45.3 U) was added to 367 ml aqueous solution of 50 g alginate, pH 7.0	35 °C for 20 h	HPLC, ^{1}-NMR and 13 C-NMR	Natsume et al. (1994)
Sodium alginate	*Alginate lyase from Sigma, A1603*	Alginate lyase	0.001 mg/ml enzyme added to 50 ml of 2% alginate solution	6,12 and 24 h	–	Park et al. (2016)
Alginate	*Gracilibacillus A7*			30 °C for 300 min	–	Tang et al. (2011)

(*continued*)

Table 2. Continued.

Alginate source	Enzyme source	Enzyme	Alginate/enzyme ratio	Conditions	Analytical condition	References
		Partially purified alginase	Ten ml enzyme added to 200 mL of 0.5% (w/v) alginate dissolved in 0.02 mol/L Phosphate-citrate buffer solution			
Sodium alginate	*Flavobacterium sp. LXA*	Alginase	Enzyme 30 μg/ml added to a different amount of alginate solution (each 100 ml)	–	–	Tusi et al. (2011)
Sodium alginate	*alginate lyase (EC 4.2.2.3)*	Alginate lyase	Batch reactor (conditions not specified)	–	–	Wang et al. (2007)
Sodium alginate (1000 cps grade)	*Pseudoalteromonas sp. Strain No. 272*	Alginate lyase	Enzyme solution 0.08 ml, 0.05 mg/ml added to 5 g of PG or PM dissolved in 50 ml distilled water	30 °C	–	Xu et al. (2003)
Alginate	*Vibrio sp. 510*	Alginate lyase	80 U of enzyme added to the substrate solution containing 1 g of alginate	The batch reactor, 28 °C	Gel permeation chromatography	Xu et al. (2014)
Sodium alginate (1000 cps grade)	*Pseudoalteromonas sp. strain No. 272*	Alginate lyase	5% of alginate in aqueous solution was digested with 1 μg/ml enzyme	40 °C for 3 days	–	Yamamoto et al. (2007b)
Sodium alginate (MW 25700) from Eisenia bicyclis	*Genetically engineered Escherichia coli*	Alginate lyase	1.5 U enzyme added to 1.5 g sodium alginate dissolved in 30 ml water	37 °C		Yonemoto et al. (1993)
Alginate (viscosity, 1010 mPas;MW 32,000e 2,00,2000)	*Sphingomonas sp*	Alginate lyase	–	45 °C for 10 min	–	Zhang, Liu, et al. (2013)
Alginate	*Streptomyces violaceoruber*	Alginate lyase	–	–	–	Zhang et al. (2013c)
Alginate	*Flavobacterium sp. LXA*	Alginate lyase	Enzyme added to the 2.5% sodium alginate at a ratio of 1:50 in 20 mmol/L phosphate-citrate buffer (pH 7.0)	40 °C for 360 min	–	Zhang et al. (2015)

product pattern (Hu et al. 2019). Therefore, substrate specificity to form targeted alginate oligosaccharide products may be achieved due to site-specific engineering in catalytic module and active site. Bi-functional endolytic alginate lyases effectively prepare distinctive alginate oligosaccharides due to their high activity and broad-spectrum substrate specificity (Cheng et al., 2017). Nevertheless, alginate lyases for production of alginate oligosaccharides are hampered due to limited knowledge regarding varied mechanisms of actions (Cheng et al. 2020). Table 2 depicts the biotechnological production of alginate oligomers and their derivatives.

Identification of oligosaccharides derived from pM, pG or pMG and structural determinations are reported to be essential for the molecular understanding of the structure-activity relationship (Li et al. 2011). In order to achieve this goal, sodium alginate from brown seaweed *Laminaria* was degraded through enzymatic hydrolysis with alginate lyase from the marine bacterium *Pseudomonas* sp. HZJ216 (Li et al. 2011). Six oligosaccharides such as DM, DMM, DGM, DG, DGG and DMG were isolated and purified using anion exchange chromatography. All the six oligosaccharides were identified using ESI-MS and NMR. Shimokawa et al. (1996) reported two series of oligo-guluronic acids from sodium alginate. Two different methods were used, acid hydrolysis of poly-G with HCL obtaining oligo-guluronic acid with a degree of polymerization 1–9 and enzymatic degradation of poly-G using alginate degrading enzyme isolated from *Flavobacterium multivolume* obtaining oligo-guluronic acids with 4-deoxy-L-erythro-hex-4-enopyranosyluronic acid residues with the non-reducing end with DP 2–7.

The mannuronic to guluronic acid (M/G) ratio is considered as one of the important factors for the selection of appropriate application of alginate (Şen 2011) due to its significance in the physiochemical properties of alginate (Murata et al. 2000; Stevens et al. 2004; Pawar and Edgar 2012; Lu et al. 2015). In order to detect the M/G ratio in alginate and alginate derivatives, various hydrolysis conditions such as sample concentration, temperature, acid concentration and reaction time were employed (Lu et al. 2015). They also developed an effective method to determine the M/G ratio using High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD). The M/G value 6.6–6.8 was recorded at the sample concentration 2.5 or 5 mg/ml and trifluoroacetic acid (TFA) concentration of 1 M or 2 M at 100 °C with M block, while M/G value 0.3 was recorded at the sample concentration 2.5 or 5 mg/ml and TFA concentration of 1 M at 120 °C with G block. They also suggest that the use of HPAEC-

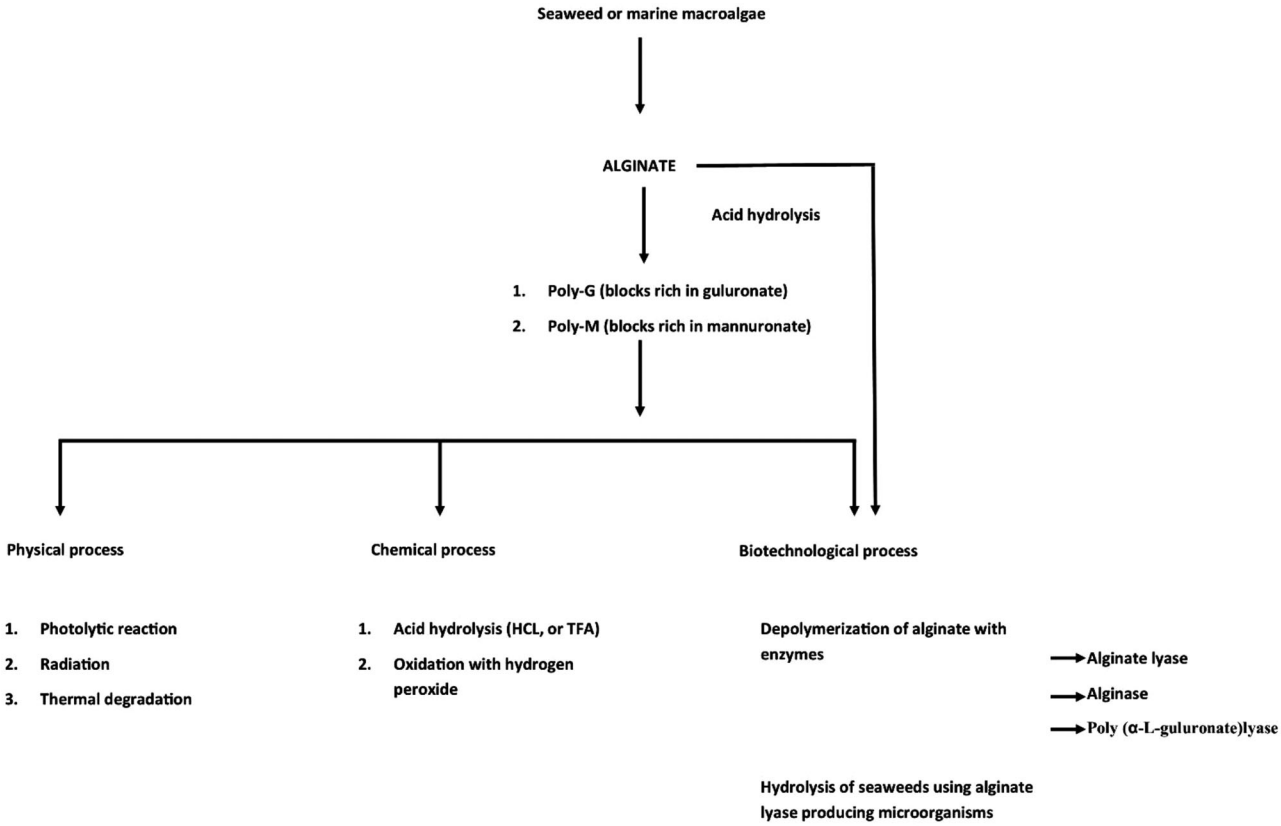

Figure 2. Schematic representation of production of alginate oligosaccharides.

PAD was a robust and accurate method to analyze the M/G ratio of alginate. The homopolymeric blocks of guluronic acids were hydrolyzed using alginate lyase and purified (Liu et al. 2002). The structure of resultant hydrolysates consisting of two oligosaccharides such as diguluronate (ΔG) and tryguluronate (ΔGG), with a molecular weight of 396 and 594, respectively.

The mutant *Pseudomonas mendocina* NK-01 was effectively studied for the production of alginate oligosaccharides, and mass spectrum revealed that the obtained AOS consists of*β*-D-mannuronic acid and/or α-L-guluronic acid, and *β*-D-mannuronic acid and/or α-L-guluronic acid residues (Guo et al. 2011). Similarly, two mutant strains, *Pseudomonas mendocina,* were used to produce AOS (Guo, Feng, et al. 2012) and characterized using mass spectroscopy and gel permeation chromatography. The results demonstrated that AOS produced using *P. mendocina C7* and *P. mendocina NK-01* were identical in monomer composition and molecular weight. The results suggest that *P. mendocina C7* can be used for large scale fermentation of AOS. They also speculated that the accumulation of acetyl-CoA and efficient carbon flux resources accelerated the synthesis of AOS.

The hydrolysis of alginate using elevated temperature, mechanisms of depolymerization and kinetics were studied (Holme et al. 2008). Alginate with guluronate content was also studied. They found that alginate degradation was mainly influenced by oxidative, reductive depolymerization at pH 5–8. This result suggests that the stability of alginate solution was affected by impurities as transition metal ions and the solution's oxygen and pH. A similar result was observed by Smidsrød et al. (1963), where the presence of oxygen affects the stability of alginate due to the presence of phenolic reducing substances, which increases ORD reaction. Extensive chromatographic analysis was carried out with sodium alginate isolated from *Laminaria* and *Macrocystis*, polyguluronate (G blocks) and polymannuronate (M blocks) obtained from *Laminaria digitata*, saturated and unsaturated oligogluronate and oligomannuronate (Heyraud et al. 1996). In addition, the catalytic properties of *Haliotis tuberculatoa* alginate lyase were also studied. The results of the kinetic analysis suggested that the catalytic site of alginate lyase suitable to accommodate oligomannuronate pentamer and capable of cleaving the G-M linkages. Burana-Osot et al. (2009) developed a photochemical reaction method for the degradation of alginate where ultraviolet lights with titanium dioxide catalyst were used, which reduce 40% of the average molecular weight of alginate. A high yield of GluA-rich, ManA-rich alginate and GM alternating sequence blocks were reported.

Xiao et al. (2011) explained the production of AOS by using 1805 enzyme and found mixtures of AOS with a degree of polymerization ranging from 2 to 10. The monomers consisted of 2–5 glucose units subjected to silica gel column chromatography and analyzed with TLC and ESI-MS. Ying et al. (2012) established various detection methods for AOS and suggested that Fluorophore assisted Carbohydrate Electrophoresis (FACE), HPLC and TLC were ideal methods to detect AOS. Shan et al. (2011) explained various methods to determine the molecular weight of AOS.

Table 3. Biomedical properties of alginate derivatives reported during 1989–2020.Han, Zhang, et al. (2019); Chen et al. (2017); Xing et al. (2020); Hu et al. (2004a); Iwamoto et al. (2005); Bang et al. (2015); Liu, Liu, and Yi (2015)

Pharmacological properties	Mode of action	References
Antimicrobial	Inhibition of quorum sensing controlled virulence factors and biofilm formation brings structural changes in microbial cells Impairing microbial adherence. Modifies hyphal infiltration Inhibits the expression of SAP4, SAP6 and phospholipase B activity Alters EPS Disrupts DNA-Ca^{2+}-DNA bridges	Powell et al. (2018); Jack et al. (2018); Pritchard, Jack et al. (2017); Pritchard, Powell, Jack et al. (2017); Pritchard, Powell, Khan et al. (2017); Zhu, Chen, et al. (2016); Hengzhuang et al. (2016); Hu et al. ; Khan et al. (2012); Powell, Pritchard et al. (2013); Powell, Sowedan et al. (2013); Roberts et al. (2013); TØndervik et al. (2014)
Antioxidant	Scavenges free radicals Enhances the activity of anti-oxidative enzymes Minimizes oxidative damage of cell Increases the activity of superoxide dismutase	Wang et al. (2020); Xing et al. (2020); Liu et al. (2019); Wang et al. (2007); Huafang et al. (2010); Tusi et al. (2011); Falkeborg et al. (2014); Zhu, Wu, et al. (2016); Wu (2014)
Anticoagulant	It prevents the platelet thromboembolism	Xin et al. 2016; Wu, Zhang, et al. (2016); Li, Shang, et al. (2017)
Immunostimulant	Improves keratinocyte growth Enhances [^{3}H] thymidine uptake Reduced NO and TNF-α production Activates the phosphorylation of Akt Stimulates NF-κB & mTOR Activates the MAPK signaling pathway Inhibits the production of IgE	Xing et al. (2020; Fang et al. (2017); Kawada et al. (1997); Xu et al. (2014); Xu et al. (2015); Uno, Hattori, and Yoshida (2006); Kurachi et al. (2005); Yamamoto et al. (2007a); Yamamoto et al. (2007b); Hattori et al. (2004)
Antitumor	Induces TNF-a secretion Attenuates α2,6-sialylation modification Suppresses tumorgenicity Activates Hippo/YAP-pathway Reduced MDA, IL-1 & IL-6 contents Modulates host-mediated immune response	
Hypolipidemic and anti-obesity	Decreases LDL-c Regulates the expression of LDLR Reduces the serum content of triglycerides and total cholesterol Decreases BMI, body & liver weight Increases AMPKα and ACC phosphorylation in adipocytes Modulates Gut microbiome Inhibits lipogenesis gene expression Decreases obesity-related inflammation Suppresses over-expression of STOML2 Enhances immune functionality	Li, Wang, Liu, et al. (2020); Wang et al. (2020); Tran et al. (2019); Li, He, and Wang (2019); Yang et al. (2015)
Neuroprotective & Anti-inflammatory	Blocks H_2O_2 induced oxidative stress and caspase-dependent apoptotic cascades Reduces neurotoxicity Decreases the secretion of pro-inflammatory secretions Diminishes the NO, PGE2 & ROS production Decreases iNOS and COX-2 levels Down-regulated the expression of IL1β and CD11c Decreased the expression of BACE1, APP, cytochrome c, & Bax/Bcl-2	Bi et al. (2020); Wang et al. (2020); Bi et al. (2018); Tusi et al. (2011); Eftekharzadeh et al. (2010); Zhou, Shi, Gao et al. (2015); Zhou, Shi, Bi et al. (2015)
Anti-hypertensive and cardioprotective	Inhibited ER and oxidative stress-mediated apoptosis Protects against myocardial I/R injury Reduces infarct size and cardiac injury Decreases cardiac troponin-I content Suppressed C/EBP homologous protein Upregulated B-cell lymphoma-2 Inhibited the TGF-β1/p-Smad2-signaling pathway Suppressed the upregulation of 4-HNE & NOX2	Feng et al. (2020); Guo et al. (2017); Ueno et al. (2012); Terakado et al. (2012); Hiura, Chaki, and Ogawa (2001); Kimura et al. (2005); Moriya et al. (2013)
Anti-diabetic	Inhibited the activity of α-glucosidase activity Upregulate AMPK-PGC1α signaling pathway Increases insulin sensitivity Upregulated GLUT-4 and insulin receptor expression Stimulated PI3K/Akt signaling pathway Decreased the levels of fasting glucose Regulated IRS1/PI3K & JNK pathways	Wang et al. (2020); Yang et al. (2019); Lan et al. (2017); Hao et al. (2015); Hao et al. (2011); Ying et al. (2014)
Miscellaneous	inhibitory effects on HBsAg and HBeAg Activates NF-kB and Raf/MEK/ERK signaling pathways Modulates collagen expression Regulated serum miR-155	Qu et al. (2017); Zhao et al. (2007); Blaine (1947); Barnett and Varley (1987); Attwood (1989); Tajima et al. (1999); Schmidt et al. (1993); Rosdy and Clauss (1990); He et al. (2013)

Table 4. Applications of alginate oligomers and its derivatives in agriculture.

Role	Plant	Physiological process	References
Growth and development	*Eucomis autumnalis*	Bio-stimulator, enhance plant tolerance	Salachna et al. (2018)
	Faba bean seeds	Promotes seed germination rate & plant growth	Abd El-Mohdy (2017)
	Brassica campestris	Seed germination, seedling growth and stress responses	Zhang, Yin, et al. (2013)
	Soybean cotyledon	Elicitor activity and antimicrobial activity Promotes synthesis of phenolic compounds	An et al. (2009)
	Soybean	Elicitor activity Induces the glyceollins production	Hu et al. (2012); Xian-Zhen et al. (2009); Park et al. (2004)
	Microalgae *Nannochloropsis oculata*	Increased growth rate Alleviated algicidal effect of Cu^{+2}	Yokose et al. (2009)
	Brassica, rice and tobacco	Germination and shoot elongation	Tomoda et al. (1992); Yonemoto et al. (1993)
	Tobacco cells	Growth promotion mechanisms	Guo, Yin, et al. (2012)
	Oryza sativa L	Elicitor activity and anti-pathogenic effect against *Magnaporthe grisea*	Zhang et al. (2015)
	Oryza sativa L	Increases IAA biosynthesis and diminished IAA oxidase activity Increased level of OsIA11 and OsPIN1 genes	Zhang et al. (2014)
	Brassica campestris	Nitrogen metabolism Increased net photosynthetic rate Elevated water use efficiency Enhanced the NR, GS, GDH, and EP activity	Zhang et al. (2011); Zhang, Liu, et al. (2013)
	Green alga, *Clamydomonas reinhardtii*	Growth and fatty acid composition	Yamasaki et al. (2012)
	Maize	Seed germination and stimulates the enzyme responsible for germination Increased seed viability	Hu et al. (2004b)
	Carrot and rice	Root elongation	Hu et al. (2012)
	Parley	Root elongation	Tomoda et al. (1994); Natsume et al. (1994)
	Lettuce	Root elongation	Iwasaki and Matsubara (2000)
	Opium poppy (*Papaver somniferum L*)	Growth, yield and alkaloid production	Khan et al. (2011)
Defense mechanisms	Cucumber (*Cucumis sativus*)	Reduced drought-stressed cucumbers and significantly increased the fresh weight, stomatal conductance, transpiration rate, and photosynthesis rate. Enhanced antioxidative properties and upregulated genes responsible for ABA (abscisic) signaling	Li et al. (2018)
	Eucomis autumnalis	Curtailed the adverse effects of salinity, less weight reduction in different parts	Salachna et al. (2018)
	Brassica campestris	Growth promotion under salt stress	Tang et al. (2011)
	Triticum aestivum	Polyethylene glycol induced drought stress Improved seed growth, fresh weight and root length	Liu et al. (2013)

Tai Hong-bo, Dai-di, and Irbis (2015) detailed the various processes involved in AOS production, including physical, chemical, and enzymatic degradation.

On the other hand, using specific microbes is considered an effective alternative method for depolymerization and production of alginate oligosaccharide (AOS). *Bacterodies ovatus* and *Bacteroides uniformis* present in human gut microbiota and are found to depolymerize alginate yielding alginate oligosaccharides. These bacteroidetes secretes enzymes that hydrolyze alginate (Li, Shang, et al. 2017). Depolymerization of alginate has also been successfully achieved by using different microbes, for instance, *Flavobacterium* sp. strain LXA, *Gracilibacillus* A7, *Bacillus litoralis* strain M3, *Pseudoalteromonas agarovorans* CHO-12, and *B. subtilis* KCTC 11782BP (Liu et al. 2019). Purity and cost of processing are the main concerns in the production of alginate oligosaccharides through various methods. Therefore, different bioengineering techniques have been studied to ensure the safe production of AOS. Purposely, Li, Wang, Jung, et al. (2020) used engineered *Yarrowia lipolytica* (yeast strain) having alginate lyase activity for depolymerization of alginate (Li, Wang, Jung, et al. 2020). This procedure concluded that AOS had high purity (92.60%) and yield (91.70&). Hence, applying biotechnological approaches to produce highly pure AOS from alginate polymer may open new horizons in the pharmaceutical sciences field.

Food and industrial applications

Prebiotic, gut health and feed supplementation

Biopolymers and derived oligosaccharides have been successfully used in the aquaculture industry as feed supplementation to increase the growth of aquaculture species by improving their immunity (Huang, Zhou, and Zhang 2006; Jiang et al. 1999; Li et al. 2003; Zhou et al. 2006; Wang et al. 2014). The possible mechanism of immunostimulant effect includes that carbohydrate polymers activate the intracellular signal transduction system through interacting with corresponding receptors on the cell membrane, leading to the release of multiple cytokines (Janeway and Medzhitov 2002;

Dauphinee and Karsan 2006; Wang et al. 2014). The non-specific immunostimulatory effect of three different molecular weight AOS prepared from *Laminaria japonica* on sea cucumber *Apostichopus japonicus* was investigated (Wang et al. 2014). The low molecular weight (<1 kD and DP of 2–5) AOS) displayed better effects on phagocytic capacity, lysozyme activity, peroxidase activity, total nitric acid synthase coelomocytes than the other two group of AOS possess DP ranged between 5 and 20. This result would add value to the aquaculture industry as a potential immunostimulant to control the disease burden. The effect of AOS on the quality characteristics of shrimp *Litopenaeus vannamei* was reported by Yun-fei et al. (2013) in terms of positive effect on weight, firmness, springiness and chewiness of shrimp meat with DP3 and DP6 compared to control, sodium pyrophosphate.

Moreover, they suggest that AOS might improve the bound state of water molecules in the muscles. The supplementation of AOS leads to improved goblet cell size and lipase activity of Turbot (*Scophthalmus maximus* L) (Pan Jin-lu et al. 2016). They also reported no improvement with protease and amylase activities, apparent digestibility, intestinal villi length, and mucosal fold height of turbot.

Alginate oligosaccharides have played a significant role in nutritional supplementation and increased the production of both aquaculture and poultry. A recent study by Yan et al. (2011) revealed that diet supplemented with AOS inhibited bacterial growth and stimulated the immune system of broiler chickens. Interestingly, AOS at 0.2% significantly reduces the *Salmonella* colonization and increases lactic acid bacteria. They also reported that 0.2% AOS dosage at five days significantly increased cecal Salmonella enteritidis IgA production. The supplementation at 0.04% displayed an anti-inflammatory effect by upregulating interleukin (IL)-10 expression in the cecal tonsils. The impact of alginate oligosaccharide supplementation on the growth and physiological changes of Manila calm *Ruditapes philippinarum* was investigated (Yamasaki et al. 2016). The AOS supplementation at 4 mg/ml promotes the growth of calm. The exact mechanism for this growth promotion was not explored, but they suggest that much of the AOS taken through the incurrent siphon was absorbed by the alimentary canal and acts as a biologically active substance.

In recent times, alginate oligosaccharides are promoted as potential prebiotic candidates (Pritchard, Powell, Jack et al. 2017). The addition of AOS as a prebiotic in the diet helps strengthen the intestinal barrier integrity and stimulate the production of intestinal health-modulating bacteria (Wan et al. 2018). Enzymatically (alginate lyase) produced AOS have been used as prebiotics due to the breakdown of long-chain alginates. Bacillus megaterium has been employed for the production of alginate lyase via a discontinuous fermenter. The growth of probiotics (lactobacillus & bifidobacteria) was increased due to addition of 1% AOS to a media compared to media lacking AOS. This study showed that alginate oligosaccharides could be used as good prebiotics (Afni et al. 2017). According to an in-vitro study conducted by Han, Zhang, et al. (2019), alginate oligosaccharide was supplemented to a culture medium and fermented for 24 hours using pig fecal microbiota. AOS increased the content of SCFAs (short-chain fatty acids), specifically butyric acid and altered the gut macrobiotic composition. Furthermore, AOS reduced the growth of *Shigella, Escherichia,* and *Peptoniphilus* in supplemented media. They concluded that AOS have prebiotic potential and can be used as a functional ingredient in improving gastrointestinal health and regulating the composition of gut-microbiota (Han, Yang, et al. 2019).

Algal-derived oligosaccharides are considered valuable sources of prebiotics as they improve gut health and increase the growth of beneficial microorganisms present in the gut. Prebiotic effect of AOS derived from *Laminaria* sp. have been evaluated on the intestinal microbiota of *Salmo salar* (Atlantic salmon). Fish ingesting feed supplemented with 0.5% of AOS imparted prebiotic effect on distal microbiota in intestines of *Salmo salar.* This study revealed AOS application in the feed and food industries (Gupta et al. 2019). Studies have shown the health-promoting benefits associated with the consumption of alginate oligosaccharides. A study conducted on C57BL/6J mice administrated with chow, high fat, high fat + AOS diet for ten weeks revealed a reduction in the concentration of triglyceride, low-density lipoprotein cholesterol, and inhibited lipogenesis gene expression. Moreover, AOS imparted modulating effect on gut microbiota and significantly enhanced *Lactobacillus gasseri, Lactobacillus reuteri,* and *Akkermansia muciniphila*. In addition, AOS supplementation elevated the levels of SCFAs, i.e., butyric acid, propionic acid, and acetic acid (Wang et al. 2020).

Industrial application

Elicitors stimulate biological systems through various metabolic pathways, resulting in increased production of specific biologically active molecules or substances. Oligosaccharides are reported to have an elicitation mechanism in secondary metabolite production in plants and some microorganisms (Albersheim et al. 1977; Tamerler et al. 2001; Nair et al. 2005; Murphy et al. 2007; Nair et al. 2008).

The oligomannuronate blocks (OM) of AOS prepared from *Laminaria hyperborean* was used as elicitor to produce penicillin. Various concentrations of AOS were tested to determine the elicitation mechanism in *Penicillium chrysogenum*, a wild type low penicillin producing strain (Ariyo, Bucke, and Keshaverz 1997). The exciting results were observed that OM blocks at 100 µg/ml remarkably increased the penicillin G production, and the yield was 150% higher than the control. The higher production of δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine (ACV), a precursor for penicillin G, was also observed in *P. chrysogenum*. Similar results were found when AOS added to the stirred tank reactor cultures of P. chrysogenum (Ariyo et al. 1998). The addition of oligomannuronate and oligogluronate blocks increased the production of penicillin G by 47% and 49%, respectively. They also reported difference in the accumulation pattern of ACV. Nair et al. (2009) studied the AOS mode of AOS on penicillin G production in P. chrysogenum and found increased penicillin G production rate and transcript copy

numbers of three major penicillin G biosynthesis genes pcbAB, pcbC and pcbDE compared to control.

Preservation and storage of food material are major problems in food industries, especially with seafood and aquaculture industries. Currently, numerous methods are employed to maintain the self-life of these food products, including chemical preservatives. Recently, an attempt was made to use alginate and its oligosaccharides as additives and studied the effects of loss of texture, color and chemical characteristics, especially that changes with functional proteins of peeled shrimp *Litopenaeus vannamei* on thawing (Ma et al. 2015). Commercially available food additives trehalose and sodium pyrophosphate was used to compare the effect during six weeks of storage. Compared to other preservatives and high molecular weight alginate, low molecular weight alginate oligosaccharides displayed an excellent cytoprotective effect by preventing decreased thawing loss, degradation of textural and color properties and denaturation of myofibrillar proteins. The mechanism behind this cytoprotective effect is that alginate oligosaccharides replace the water molecules by forming hydrogen bonds with polar residues of lipid or proteins in muscles, and hence it stabilizes their structure in the absence of water during the storage process (Sola-Penna and Meyer-Fernandes 1998; Tadanori et al. 2002; Ma et al. 2015).

Recently, *Laminaria japonica* derived oligosaccharides (LJOs) was reported to have potent antibacterial and antioxidant activities (Wang et al. 2008; Li, Li, and Guo 2010; Xu et al. 2010; Chen et al. 2012; Peng et al. 2012; Wu 2014). The effect of cherry preservation with different concentrations of LJOs-incorporated pullulan coatings was evaluated, and the LJOs used was prepared by the H_2O_2 hydrolysis method with 15% extraction yield (Wu, Lu, and Wang 2016). The effects of LJOs was dose-dependent and found to be effective in reducing respiratory intensity, vitamin C loss, weight loss, softening and 0.2% LJOs significantly increased titratable acid content. Various scientific reports have shown alginate oligosaccharides (AOS) as a suitable ingredient during postharvest storage (Zhang, Wang, et al. 2020). Alginates are natural macromolecule that has shown a strong affinity toward the water and therefore can be used as a coating material to enhance the shelf life of different food commodities (Bose et al. 2019; Chiabrando and Giacalone 2017). Recently, alginate oligosaccharides have gained importance owing to their utilization in food, agriculture, and pharmaceutical fields. Purposely, the results of a study concluded that AOS treated frozen shrimps have demonstrated reduced cooking losses & thawing, better texture & myofibrillar proteins (Zhang et al. 2017).

Health beneficial effects

Antimicrobial properties

Alginate oligosaccharide was prepared by alginate degradation using alginate lyase from *Flavobacterium sp.*LXA and tested for antibacterial activity (An et al. 2009). The results displayed that AOS S7 with DP 6.8 possessed effective inhibition against *Pseudomonas aeruginosa*. The synergistic study of alginate and chitin oligosaccharides in conjunction with antibiotic azithromycin against wild-type antibiotic-resistant *Pseudomonas aeruginosa* was investigated recently (He et al. 2014). The results demonstrated that azithromycin supplemented with 2 mg mL AOS suppressed the growth of *P. aeruginosa* in parallel with the inhibition of quorum sensing controlled virulence factors and biofilm formation. This is the first report on the synergistic effect of AOS against bacterial growth. Many researchers reported similar findings (Tin et al. 2009; Khan et al. 2012; Powell, Sowedan et al. 2013). Recently Hengzhuang et al. (2016) investigated the effect of low OligoG CF-5/20 on the disruption of mucoid *P. aeruginosa* biofilm in a murine lung infection model system. OligoG CF-5/20 is a low molecular weight alginate oligomer produced from the enrichment of sodium alginate. This oligomer comprised of α-L-guluronic acid (<85%) and β-D-mannuronic acid (<15%). They reported that the biofilm disruption was dose-dependency, and 5% OligoG CF-5/20 significantly reduced the minimum biofilm eradication concentration from 512 to 4 μg/ml after 8 h of treatment. These findings suggest that OligoG CF-5/20 have clinical value by reducing the microbial burden in chronic biofilm infections. In total 10 AOS derivatives, including mannuronic and guluronic fractions, were prepared from alginate using alginate lyase hydrolysis. The isolated fractions were tested for antibacterial activity against 19 different bacterial pathogens. The results revealed that mannuronic fractions were more effective than guluronic fractions in which M3 fraction with a molecular weight of 4.235 kDa exhibited prominent inhibitory activity against ten pathogens. It was found to be more effective against *Escherichia coli* (MIC, 0.312 μg/ml), *Salmonella paratyphi* (MIC, 0.225 μg/ml), *Staphylococcus aureus* (MIC, 0.016 μg/ml) and *Bacillus subtilis* (MIC, 0.325 μg/ml). The molecular mechanism explained this higher activity with M fractions because the G-block fraction usually forms stiff chains, leading to gel formation while M-block fractions form flexible chains with high water solubility (Ertesvåg and Valla 1998). This high water solubility of M-block fractions makes it easier to pass through bacterial cells to exert antibacterial activity.

Multidrug resistance is now increasing worldwide, and it has become a massive challenge for developed and developing countries since it affects the global economy. Hence, it is necessary to discover a new class of multidrug-resistant antibiotics that need to overcome this problem. Researchers have now diverted their focus on marine-based antibacterial compounds, including biopolymers and polymer derived saccharides. The extensive study by Khan et al. (2012) investigated the effect of OligoG, a low molecular alginate oligosaccharide nanomedicine prepared by hydrolysis of *Laminaria hyperborean* alginate. The prepared OligoG was screened against 21 different multidrug-resistant clinical isolates, including *Pseudomonas aeruginosa*, *Burkholderia spp.*, *Acinetobacter spp.*, *Enterobacteriaceae spp.*, *Staphylococcus aureus* and *Streptococcus oralis*. The results revealed that OligoG increased the efficacy of conventional antibiotics against these significant clinical isolates. OligoG at 2, 6 and 10% were reported to directly affect the biofilm quality and health of the cells within the biofilm. These results also suggest that OligoG could be a novel nanomedicine target for

multidrug-resistant clinical pathogens shortly. These results were further confirmed by Powell, Pritchard et al. (2013) where they detailed the mechanism behind the antibacterial effect with OligoG. Powell, Sowedan et al. (2013) also reported the effect of alginate oligosaccharides on the mechanical properties of gram-negative biofilms (Powell, Sowedan et al. 2013) and found that OligoG potentially inhibits the biofilm formation and structural changes in the bacterial cells. In another study, the *in-vitro* effect of OligoG against oral pathogen-related biofilm alone or in combination with triclosan, a conventional antibacterial agent, was evaluated (Roberts et al. 2013). OligoG at 0.3% potentiated the antibacterial effect of triclosan against *Streptococcus mutants* grown on artificial saliva, but it was not active against *Porphyromocas gingivalis*. These results highlighted the potential of OligoG on the management of peri-implantitis due to its capability of inhibiting growth and impair bacterial adherence.

The potential of OligoG as antibacterial agents has acquired researchers attention to look at its efficacy against fungal infections. Tøndervik et al. (2014) studied the effect of OligoG against 11 clinical strains of *Candida* and 3 strains of *Aspergillus*. The in vitro testes indicated that OligoG modulates the fungal growth and fungal biofilm formation. In addition, phase I and phase IIa clinical studies demonstrate that OligoG is safe for human use, in vivo testing has no intolerance to inhaled doses of up to 540 mg/day (http://ClinicalTrails.gov, NCT00970346 and NCT01465529; EudraCT, 2009-009330-33).

In order to improve the oral drug delivery against microbial pathogens, Park et al. (2016) incorporated alginate and its oligosaccharides in artificial gastric juice. The immobilization efficiency and lysozyme stability in gastric juice were also evaluated by *E. coli* and found that the antimicrobial activity of incorporated biopolymer controlled enzyme was comparable to the pure lysozyme (50–90% cell mortality). OligoG CF-5/20 (novel alginate oligosaccharide) has shown potential anti-fungal properties against diverse fungal strains. In an in-vitro model of epithelial invasion of *Candida albicans*, treatment of alginate oligomer (OligoG CF-5/20) modified the hyphal infiltration and inhibited the phospholipase activity of *Candida albicans* in a dose-dependent manner. Accordingly, the alginate oligosaccharide treatment decreased the expression of SAP4, SAP6, PLB2 (phospholipase B), and virulence factors by *Candida albicans* (Pritchard, Jack et al. 2017). Likewise, an in-vitro model was formed using AS (artificial-sputum) medium to assess the impact of OligoG CF-5/20 on the growth of *Pseudomonas aeruginosa*. Treatment of low molecular weight alginate oligosaccharide on pseudomonal microcolony formation in AS medium revealed reduced quorum sensing signaling and biofilm disruption in a dose-dependent manner. Conclusively, in AS, decreased antimicrobial activity of colistin was restored due to OligoG CF-5/20 (Pritchard, Powell, Jack et al. 2017). Alginate oligomer (OligoG CF-5/20) significantly impaired the motility and assembly of *Pseudomonas aeruginosa* biofilms by altering the quorum-sensing systems (*lasI-lasR* & *rhlI-rhlR*) in *P. aeruginosa* (Jack et al. 2018). On the other hand, in gram-negative bacteria, this novel alginate oligomer did not alter the cell membrane permeability and lipopolysaccharide structure, although weak Ca-mediated binding of alginate oligosaccharide was noticed with the cell surface (Pritchard, Powell, Khan et al. 2017).

The optimum conditions for the degradation of alginate into AOS were aimed to get AOS with the degree of polymerization within 10 and reported that 3% H_2O_2, the temperature at 80 °C and reaction time 1.5–2 h was found suitable (Yong and Pei-hong 2009). The prepared AOS was tested for their antimicrobial activity and found that AOS was inactive against fungal pathogens. The minimum inhibitory concentration of *Staphylococcus aureus*, *Escherichia coli* and *Micrococcus luteus* were 0.625% and *Bacillus subtilis* at 2.5%. In contrast, Guo-feng et al. (2015) reported that the optimum conditions for the degradation of alginate (1.5) were 5.0% H_2O_2, 50 °C for 50 minutes and that AOS prepared under these conditions has the highest antimicrobial activity. The AOS at 0.25% (w/v) inhibited beer bacteria *Lactobacillus brevis* 49, which leads to the prevention of beer turbidity. OligoG (OligoG CF-5/20) obtained from brown seaweed inhibits the activity of gram-negative bacteria whereas increases the colistin activity against *Pseudomonas aeruginosa*, therefore, aids patients having cystic fibrosis. In established biofilms, this alginate oligosaccharide alters EPS (extracellular polymeric substances), disrupts DNA-Ca^{2+}-DNA bridges, and synergistically increases antibiotic potential (Powell et al. 2018). Additional research is required to precisely understand the mechanistic approach for inhibiting microbial infections due to the administration of alginate oligosaccharides and their derivatives.

Antioxidant properties

The antioxidant activities of AOS were reported frequently, and most of the published work stated that AOS has remarkable activity against free radicals compared to other oligosaccharides. For example, Wang et al. (2007) reported that compared to chitosan and fucoidan oligosaccharides, AOS displayed a high radical scavenging effect but did not show any activity in the Fe^{2+} chelating assay. Oligoalginate prepared using alginate lyase obtained from *Acenitobactor junii* was tested for its antioxidant activity through the oxygen radical absorbance capacity (ORAC) method. It was found that antioxidant capacity was related to DP (Huafang et al., 2010). Similarly, Wu Hai-ge, Qian, and Zi-ang (2015) found the potential of AOS on in vitro antioxidant activity through scavenging ROS. They continued normal cell morphology, improving enzymatic activities for SOD and GSH-Px and finally relieving the cellular oxidative damage caused by H_2O_2.

Alginate oligosaccharides prepared using alginate were evaluated for antioxidant effect (Tusi et al. 2011). They also studied the effect of prepared AOS on the H_2O_2 induced cell death and molecular mechanism in neuron-like PC12 cells. The in vitro results showed that the AOS treated PC12 cells blocked H_2O_2-induced oxidative stress and caspase-dependent apoptotic cascades originating from the endoplasmic reticulum

and mitochondria. These results were further confirmed in vivo, where the AOS possessed a neuroprotective effect against Aβ-induced neural damage. Alginate oligosaccharides have been identified to scavenge free radicals effectively and can be utilized as antioxidants (Liu et al. 2019).

Alginate lyase degrades alginate polymer isolated from different brown algae into AOS (alginate oligosaccharides) with various bioactivities. Purposely, Wang et al. (2020) cloned Aly1281 (novel alginate lyase) from *P. carrageenovora* (ASY5) and reported it as a member of the PL7 family. Compared to other alginate lyases, this novel alginate lyase (Aly1281) possessed more degradation specificity and yielded di-alginate oligosaccharides with potent antioxidant properties. Resultant AOS scavenged highest (88.8%) hydroxyl, DPPH (69.6%), and $ABTS^{+}$ (81.5%) radicals at 20 mg mL^{-1} concentration. Further, the reported EC0 values of AOS was found to be 7.84, 5.65, & 8.70 mg mL^{-1} for DPPH, ABTS and hydroxyl radicals, respectively (Wang et al. 2020). The enzymatically depolymerized AOS using alginate lyase S displayed potent radical scavenging activity against ABTS (95.5%), hydroxyl (90%), and superoxide radicals (87%). The formation of conjugated alkene acid structure during enzymatic polymerization claimed the observed activity (Falkeborg et al. 2014). Alginate-derived oligosaccharides prepared using alginate lyase from marine bacterium *Microbulbifer sp*. ALW1 consists of disaccharides and trisaccharides with low molecular weight and low DP by Zhu, Wu, et al. (2016). The prepared AOS was a potent radical scavenger against DPPH, ABTS + and hydroxyl and possessed high reducing power.

Wang et al. (2007) compared the antioxidant activities of fucoidan (FOS) and chitosan oligosaccharides (COS) with alginate oligosaccharide (4388 Da) prepared by enzymatic hydrolysis. The results revealed that AOS possessed higher hydroxyl radical scavenging activity than FOS and COS, but AOS failed to scavenge Fe^{2+} and anti-lipid peroxidation. Wu et al. (2014) optimized the reaction conditions for the production of alginate oligosaccharides from *Laminaria japonica* by hydrolysis using H_2O_2 and determined its antioxidant activity. They proposed the optimum hydrolysis condition for maximum yield (17.65%) was 24 h reaction time at 75 °C with 4% H_2O_2 concentrations. The hydrolyzed AOS displayed intense hydroxyl radical scavenging activity (91.31%) at 100 µg/ml. Presently, even though the antioxidant properties of alginate oligosaccharides are well-documented, quite a few investigations are highlighting its mechanism of action. Knowledge regarding the molecular basis of radical scavenging activity of AOS can help modify its structure, keeping in view their targeted applications. These modifications may aid in the food and pharmaceutical industries use of alginate oligosaccharides (Xing et al. 2020).

Anticoagulant activity

Marine biopolymers have been extensively reported for their anticoagulant activities, including seaweed polysaccharides such as fucoidan, laminarin, alginate, and other polymers. Propylene glycol alginate sodium sulfate (PSS), a sulfated polysaccharide derivative of alginate, is a potent heparinoid drug currently being used to prevent hyperlipidemia and cardio-cerebrovascular disease in China (Xin et al. 2016). However, PSS has been reported to have serious side effects by bleeding, and researchers speculated that the molecular weight of PSS might be responsible for bleeding (Li 1999; Xin et al. 2016). Xin et al. (2016) prepared low molecular weight PSS derivatives and tested them for anticoagulant and antithrombotic activities. The results revealed that there was a correlation between molecular weight and observed activity. Among the four low molecular derivatives, FP-6k possessed remarkable coagulation activity and prevented platelet thromboembolism.

In contrast to these results, Wu, Wang, et al. (2016) proposed that high molecular weight and a high degree of sulfation is needed for the anticoagulant activity. They tested thirteen PSS oligosaccharide derivatives on anticoagulant and cell proliferation activity. They found that low molecular PSS oligosaccharides did not show any anticoagulant activity, but PSS oligosaccharides with DP above eight uronic acid units showed significant cell proliferation stimulation by activating FGF/FGFRic signaling in the BaF3 cells.

Recently, series of eight low-molecular-weight heparinoid oligosaccharide derivatives prepared by chemical modification of polymannuronate and polyguluronates, including LPM/LPG phosphate (LPMP/LPGP), LPM/LPG-H-phosphate (LPMHP/LGPHP), LPM/LPG sulfate (LPMS/LPGS) together with low molecular weight mannuronate (LPM) and low molecular weight polyguluronate (LPG) (Li, Zeng, et al. 2017). All the oligosaccharide derivatives were subjected to anticoagulant activity and FGF/FGFR1c signaling activation abilities to prove their heparin-like effect. The heparin-like abilities were improved with oligosaccharide having sulfate group compared with other two anionic groups, and it is suggested that sulfate group was the best substitution to improve the anticoagulant activities. Also, higher activity was recorded with sulfated derivatives based on polyguluronate structure than polymannuronate structure.

Immunostimulatory/immunomodulatory activities

Alginate and alginate-derived oligosaccharides are extensively reported to be potent immunostimulants/immunomodulators (Xing et al. 2020). The enzymatically depolymerized sodium alginate oligosaccharides using alginate lyase from *Alteromonas* sp. was investigated for immunostimulant activity (Kawada et al. 1997). The prepared AOS was tested for the growth of human keratinocyte. Interestingly, they found remarkable growth improvement of keratinocyte and [^{3}H] thymidine uptake in the presence of epidermal growth factor, and the activity was much more significant than bovine pituitary extract, which is used in human keratinocyte culture. This finding also suggests that AOS can be developed as a possible co-factor for epidermal growth factor-dependent stimulator in keratinocyte culture medium.

In order to evaluate Immunostimulatory properties, polyguluronate and polymannuronate fractions with DP 20–24 were prepared from sodium alginate using alginate lyase hydrolysis (Xu et al. 2014). The mechanism of immunostimulatory effect was assayed on murine macrophage

RAW264.7 cells, and structure-activity relationships also explained. The results showed that AOS derivatives prepared through enzymatic hydrolysis induced nitric oxide production, stimulated ROS and TNF-αproduction but AOS prepared through other method showed less activity. Comparative proteomic analysis and western blot were employed to confirm molecular mechanism associated with AOS (Xu et al. 2015). GOS significantly increases the cell proliferation of RAW264.7 cells and induces the TNF-αsecretion and NO production. These findings revealed that different mechanisms might be associated with the macrophages activating of GOS and GOS controls over inflammatory reaction cell homeostasis. It was already reported that AOS significantly enhances human endothelial cells and keratinocytes (Kawada et al. 1997, 1999). Literature reveals that alginate-derived GOS (guluronate oligosaccharide) significantly stimulates macrophages. GOS is recognized, upregulated, and taken up by TLR4 (Toll-like receptor-4) on RAW264.7 macrophages. The endocytosis of GOS dependent on TLR4 activates the phosphorylation of Akt followed by stimulation of NF-κB (nuclear factor-κB) & mTOR (mechanistic target of rapamycin). Furthermore, this alginate-derived oligosaccharide activates the MAPK (mitogen-activated protein kinase)-signaling pathways and therefore implies significant immunostimulatory activities (Fang et al. 2017).

ALGO, alginate oligosaccharides produced from lyase degradation with a molecular weight of 713, was further fractionated to get four different ALGO derivatives. These derivatives were tested for anti-allergic properties through intraperitoneal injection on mice. They found ALGO derivatives effectively suppressed Th2 development and IgE production through the induction of IL-12 production and suggested ALGO can be used to prevent IgE-mediated allergic disorders. Uno, Hattori, and Yoshida (2006) further confirmed this anti-allergic reaction where mice were fed with different concentrations of AOS and studied the production of IgE and induction of oral tolerance. The IgE production was inhibited in mice fed with AOS and suggested that AOS could be potentially anti-allergic food material or dietary supplement.

The high molecular weight alginates (food and pharmacological grade) with varied viscosities were hydrolyzed using *Pseudo alteromonas sp*. alginate lyase to obtain alginate oligomers with different DP. Both alginate and its oligomers were tested for cytokine-inducing activity (Kurachi et al. 2005). The results demonstrated that alginate induces the TNF-αsecretion from mouse macrophage cell RAW264.7, and the activity was significantly influenced by molecular weight and M/G ratio. It was also reported that a mixture of enzyme hydrolyzed AOS induced TNF-αsecretion from mouse macrophage cell RAW264.7 was 10-fold higher than alginate polymer. This study was further extended to see the cytokine levels in the mouse serum after intraperitoneal injection of AOS mixture (Yamamoto et al. 2007a). They noticed the dose-dependent effect of AOS, and the high serum level was detected at 70 mg/kg of AOS mixture. These findings were further confirmed by Yamamoto et al. (2007b), where guluronate and mannuronate oligomers with a wide range of DP on cytokine inducing activity. They noticed that mannuronate oligomers were potent cytokine inducer than guluronate oligomers, and structure-activity relationship suggest that the activity mainly influenced by molecular conformation and molecular weight of the oligomers.

Conjugation of alginate oligosaccharides with other biomolecules is of great interest in food industries to develop a new food source with reduced pathogenicity with improved bioactivity. Likewise, β-lactoglobin (β-LG) – a major whey protein consist of essential amino acids valued for its great potential as emulsifying, foaming and gelling properties (McKenzie 1971; Shimizu and Yamauchi 1985; Waniska and Kinsella 1988; Foegeding, Kuhn, and Hardin 1992) but it was reported to be potent allergen of milk allergy and also losses it emulsifying efficacy in acidic pH. In order to improve emulsifying quality and reduce allergenicity, β-LG was conjugated with AOS and investigated for immunogenicity (Hattori et al. 2004). The results revealed that β-Lg-AOS conjugate enhanced the thermal stability, significantly reduced the anti-β-LG antibody response in mice. These results suggested that AOS conjugates are edible and could be helpful in functional food industries.

Antitumor properties

Antitumor activities were observed with AOS prepared using alginate lyase from *Vibrio* sp.510 sulfated with the formamide-chlorosulphonic acid. Both AOS and sulfated derivatives were assayed for antitumor properties in vitro and in vivo (Hu et al. 2004a). The results suggested that oligosaccharide A containing a molecular weight of 3798 Da displayed 70.4 and 66.0% of tumor inhibition against solid Sarcoma 180 at doses of 100 and 50 mg/kg, respectively. It also suggested the indirect antitumor effect via modulation of the host-mediated immune response. The structure-activity relationship of two alginate oligomers, namely guluronate and mannuronate, produced by enzymatic degradation of *Pseudoalteromonas sp* alginate lyase polyguluronate (PG) and polymannuronate (PM) was investigated (Iwamoto et al. 2005). The enzymatically produced unsaturated AOS induced tumor necrosis factor (TNF)-a secretion from RAW264.7 cells compared to saturated AOS produced by acid hydrolysis. The structure relationship activity concluded that the unsaturated end-structure of alginate oligomers was necessary for the TNF-a-inducing activity. Recently, Bang et al. (2015) used alginate from *Laminaria hyperborean* to produce AOS with the aid of *Bacillus subtilis* and tested for asthma effect. They found remarkable results where AOS showed an anti-asthma effect by modulating Th1/Th2 cytokines, which plays a significant role in asthma pathogenesis.

It is well known that vanadium ions and complexes having various pharmacological functions, including antioxidant, antidiabetic, anticancer and insulin-like effects (Bran, 2004; Ashiq et al. 2008; Etcheverry et al. 2008; Naso et al. 2011; Evangelou 2002) and at the same time these vanadium salts have poor gastrointestinal absorption and severe side effects (Li et al. 2008). Vanadyl alginate polysaccharides (VAPS) and vanadyl alginate oligosaccharides (VOPS) were

prepared, characterized and tested for in vitro antioxidant, PTP1B enzyme inhibitory activity and anticancer properties (Liu, Liu, and Yi 2015). Both the VAPS and VOPS exerted a potent hydroxyl and DPPH radical scavenging effect than the alginate and alginate oligosaccharides. Also, both VAPS and VAOS were shown to have potent inhibitors against PTB1B with IC50 values of 6.9 μg/ml and 16.5 μg/ml, respectively. They proposed two mechanism for the observed PTB1P inhibition as follows; (i) vanadium acid radicals can inhibit PTP1B activity by competition, decreasing its dephosphorylation of phosphorylation-tyrosine, (ii) vanadium generated the reactive oxygen species (ROS), and this ROS oxidizes the cysteine in the PTP1B domain forming disulfide bond, and finally, the PTP1B loses its activity due to changes in the active site. They also reported that both VAPS and VOPS possessed a strong anti-proliferation effect.

AOS has shown antitumor properties in osteosarcoma (musculoskeletal tumor) patients. According to a study, alginate lyase was used to prepare different fractions (DP2 to DP5) of alginate oligosaccharides. Patients in this study were divided into control (placebo) and experimental (AOS: 10 mg daily) groups, respectively. In osteosarcoma cells, only DP5 revealed antitumor properties among all the prepared fractions of AOS. Pre-surgery's clinical data showed no significant difference among both the experimental and control group, while two years post-surgery, the average tumor volume noticed in the AOS-treated group was less (214.6 ± 145.7 c.c.) compared to the control group (467.2 ± 225.3 c.c.). Similarly, the local recurrence rate in AOS-treated and control group was noticed to be 44.90 and 68.70&, respectively. Alginate oligosaccharides elevated the content of HDL-c, GSH, SOD, and decreased MDA (malondialdehyde), IL-1 (interleukin-1) β, IL-6, LDL-c, & TC (total cholesterol) levels. Conclusively, alginate oligosaccharides decreased osteosarcoma progression due to enhanced antioxidative and antiinflammatory properties of patients, hence possessing the potential for drug development to treat osteosarcoma (Chen et al. 2017). Recently, Han, Zhang, et al. (2019) reported the anticancer effect of marine-derived AOS in PC-3 and DU145 prostate cancer cells. AOS attenuated α2,6-sialylation modification and therefore inhibited the cellular growth of prostate cancer cells. Additionally, alginate polysaccharide affected the transcriptional process by inhibiting the activity of the ST6Gal-1 promoter. In prostate cancer cells, alginate oligosaccharide inhibited tumorigenicity via activation of Hippo/YAP-pathway (Han, Zhang, et al. 2019). Currently, antitumor and anticancer properties of AOS focuses on the elimination of tumorous cells due to immunomodulatory and anti-inflammatory abilities. Despite extensive research on the antitumor potential of AOS, there is a dire need to study an in-depth mechanism of action, limiting advances and application of alginate oligosaccharide in cancer therapy (Xing et al. 2020).

Hypolipidemic and anti-obesity properties

The low–density lipoprotein and its receptor play an essential role in reducing blood cholesterol which diminishes the cardiovascular disease risk. For the first time, the impact of alginate oligosaccharides on cholesterol metabolisms was studied both in vitro and in vivo (Yang et al. 2015). The alginate obtained from *Laminaria hyperborean* was degraded to produce low molecular weight AOS (< 10 kDa) and was tested for cholesterol regulation. The results showed improved low-density lipoprotein (LDL) uptake by regulating LDLR and proprotein convertase subtilisin/kexin type 9 (PCSK9) expression. The proposed mechanism for this activity was AOS lowered plasma LDL-cholesterol level through regulation of LDLR expression. They also suggested that AOS could be used to develop new potent cholesterol-lowering drugs shortly.

Obesity, hyperlipidemia and their associated complications are one of the leading causes of various metabolic syndromes. On the other hand, current anti-obesity drugs possess side effects and are costly. Therefore, it is a need for time to evaluate alternative food natural components to minimize these problems. Accordingly, anti-obesity effects of UAOS (unsaturated alginate oligosaccharides) produced by enzymatic hydrolyzes of brown algae (*Laminaria japonica*) have been reported in a study comprising of fat-rich diet mouse model by Li, He, and Wang (2019). As compared to saturated alginate oligosaccharides produced by acid hydrolysis, UAOS helped significantly in reducing serum TG (triglyceride), TC (total cholesterol), AST (aspartate aminotransferase), and ALT (alanine aminotransferase) contents. Moreover, body weight, adipose mass, liver weight, and formation of ROS in the high-fat diet-induced mice model were also decreased.

Elevation in AMPKα (AMP-activated protein kinase-α) and ACC (acetyl-CoA carboxylase) phosphorylation in adipocytes refers to the fact that UAOS predominantly possesses anti-obesity potential via AMPK-signaling. They concluded that unsaturated alginate oligosaccharides could treat hyperlipidemia, fatty liver and related diseases (Li, He, and Wang 2019).

Furthermore, in another study, this research group suggested that UAOS attenuated the high-fat diet-induced obesity and related complications via modulation of the gut microbiome, signifying the use of UAOS as a potential component in the treatment of obesity and related physiological aliments (Li, Wang, Liu, et al. 2020). In high-fat diet-induced mice (C57BL/6J), supplementation of alginate oligosaccharides amended lipid metabolism by decreasing the content of LDL-c & TC and due to inhibitory expression of lipogenesis genes. AOS also elevated the concentration of serum insulin and reduced the content of blood glucose (fasting). The growth of Akkermansia muciniphila (mucin degrading gut-microbe) linked with curtailment of obesity was also increased due to intervention of AOS. Further, AOS supplementation also elevated the SCFAs levels in experimental groups. Obesity-associated inflammation was also reduced owing to the reduction in endotoxin level (Wang et al. 2020). AOS also helps in improving metabolic systems. Alginate oligosaccharide (AOS) improves immunometabolic systems by suppressing the over-expression of STOML2. A study conducted on HFD (high-fat diet)-

induced obese zebrafish evaluated the potent effect of AOS in modulating immune-metabolic pathways. Outcomes of this study revealed the anti-obesity effect of AOS due to a reduction in BMI, body weight, and serum glucose levels. Mechanistically, AOS inhibited the expression of stomatin-like protein-2 (STOML2), decreased inflammation, down-regulated apoptotic genes, and improved immune functionality in HFD-induced obese zebrafish (Tran et al. 2019).

Anti-inflammatory and neuroprotective effects

Six alginate oligosaccharides were prepared using alginate lyase from *Pseudomonas sp*. HZJ 216 and were characterized (Li et al. 2011). The effects of AOS on H_2O_2-induced cell death and molecular mechanisms underlying neuron-like PC12 cells was determined (Tusi et al. 2011). AOS treated PC12 cells blocked H_2O_2 induced oxidative stress and caspase-dependent apoptotic cascades originating from ER and mitochondria. It was also noticed that AOS exhibited a neuroprotective effect against Ab-induced neural damage. Eftekharzadeh et al. (2010) have already reported the neuroprotective effect of alginate on NT2 neurons against H_2O_2 -induced neurotoxicity. Saigusa et al. (2015) also noticed that Salmon myofibrillar protein (MP) conjugated with alginate oligosaccharide significantly reduced the secretion of proinflammatory mediators at 19 μg/mg protein.

The guluronate oligosaccharides (GOS-OD) obtained from sodium alginate through oxidative hydrolysis was investigated for anti-inflammatory activity in lipopolysaccharide-activated murine macrophage RAW 264.7 cells (Zhou, Shi, Gao et al. 2015). GOS-OD effectively diminished the production of nitric oxide (NO), prostaglandin E2 (PGE2), and free radical species, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), and also secretion of pro-inflammatory cytokines in LPS-activated murine macrophage RAW 264.7 cells. Zhou, Shi, Gao et al. (2015) further confirmed the anti-inflammatory effect with AOS prepared by oxidative degradation and had carboxyl group at the 1-position of the reducing end. Similar anti-inflammatory effect of AOS on the lipopolysaccharide (LPS)/β-amyloid (Aβ)-induced neuroinflammation and microglial phagocytosis of A$\beta$was reported (Zhou, Shi, Bi et al. 2015).

Alginate oligosaccharide treatment was found to lower the expression of markers of inflammation, including IL1β and CD11c (Wang et al. 2020). Alginate-derived polymannuronate is sulfated and then followed by selenylation replacement reaction to form Se-PM (seleno-polymannuronate). In-vitro results have shown that Se-PM inhibits the accumulation of $A\beta_{1-42}$ oligomer. Furthermore, in N2a-sw cells, Se-PM significantly decreased BACE1, APP, cytochrome c, & Bax/Bcl-2 and increased the mitochondrial membrane potential. In summary, Se-PM stimulates the survival of N2a-sw cells and, therefore, can be therapeutically used to prevent neurodegeneration (Bi et al. 2020). Similarly, in lipopolysaccharide (LPS)-treated primary microglia and astrocytes, Bi and coworkers (2018) revealed that Se-PM decreased the production of PGE_2 & NO, expression of COX-2 & iNOS, and secretion of IL-6, IL-1, & TNF-α. They also documented that Se-PM has an attenuating effect on LPS-induced over-activation of MAPK and NF-κB signaling. This study concludes that Se-PM may help reduce neuro-inflammation (Bi et al. 2018).

Anti-hypertensive and cardio-protective properties

The anti-hypertensive effect was assessed with sodium alginate oligosaccharides on Wistar-Kyoto and hypersensitive rats for seven weeks (Ueno et al. 2012). The studied AOS mainly comprised of unsaturated uronic acids (dU) and α-L-guluronate (G), and/or β-D-mannuronate (M). The Dahl salt-sensitive (Dahl S) rats were treated with AOS at 4 or 8% (w/w) for seven weeks in order to assess the antihypertensive effect and found that AOS significantly reduces salt-induced hypertension in Dahl S rats (Terakado et al. 2012). Other researchers reported similar results where AOS treatment decreases blood pressure elevation in Dahl S rats (Hiura, Chaki, and Ogawa 2001; Kimura et al. 2005). They also suggest that the possible effects of AOS may help to prevent the early stages of kidney injury.

The molecular mechanism of anti-hypersensitivity of alginate oligosaccharides derived from seaweed alginate on Dahl salt-sensitive (Dahl S) rats was investigated (Moriya et al. 2013). Initially, rats were fed with 4% NaCl, and AOS was administrated subcutaneously at 60 mg/day for 14 days. The AOS increased systolic blood pressure in untreated Dahl S rats, and it was age-dependent. They also found that administration of AOS via subcutaneous completely abolished salt-induced hypertension in treated rats, but there was no difference in the level of fecal or urinary sodium excretion during the treatment. They concluded that the AOS diminishes salt-induced hypertension in rats fed with AOS through direct action on vascular vessels and not by reducing salt absorption.

Recently, AOS has reported possessing a protective effect against acute doxorubicin cardiotoxicity mainly due to inhibition of ER (endoplasmic reticulum) and oxidative stress-mediated apoptosis. In the mice model, Guo et al. (2017) demonstrated that pretreatment of AOS helps in protecting against myocardial I/R injury. Further, this pretreatment ameliorated the dysfunctionality of the cardiac system by reducing infarct size, cardiac injury, I/R-induced myocardial apoptosis, and concentration of cardiac troponin-I. Pretreated AOS inhibited the ER stress-mediated apoptotic pathways reflected by upregulation of B-cell lymphoma-2 and suppression of C/EBP homologous protein (Guo et al. 2017). Pulmonary arterial hypertension (PAH) is an acute cardiovascular disease associated with an elevation in progressive pulmonary vascular re-modelling and pulmonary vascular resistance. AOS formed due to depolymerization of alginate extracted from brown algae alleviates pulmonary hypertension. Accordingly, monocrotaline administrated to experimental rats at a rate of 60 mg/kg resulted in induction of pulmonary hypertension. Afterwards, AOS (5, 10, & 20 mg/kg/day) was intraperitoneally injected into these rats for a time of 3-weeks. Results revealed prevention in the development of pulmonary hypertension in a concentration-

dependent manner. AOS inhibited the TGF-β1/p-Smad2-signaling pathway and, therefore, helped prevent MCT-induced pulmonary vascular re-modelling (Feng et al. 2020). In acute myocardial ischemia injured experimental mice, pretreatment with enzymatically degraded alginate oligosaccharide (dose: 200 mg kg^{-1} day^{-1} for one week) inhibited the upregulation of 4-HNE & NOX2 induce oxidative stress conditions in such subjects (Guo et al. 2017).

Anti-diabetic properties

Seaweeds or seaweed derived compounds are frequently reported to have potent antidiabetic effects, but AOS are not well documented for their anti-diabetic activity. Ying et al. (2015) studied the Alpha-glucosidase inhibitory activity of alginate hydrolysates poly-mannuronates (PM), poly-guluronates (PG), oligo-mannuronate (OM) and oligo-guluronates (OG) prepared by acid hydrolysis of alginate. They noticed PM and PG possessed higher AGI activity against rat intestinal alpha-glucosidase. In differentiated 3T3-L1 adipocytes, oligomannuronate and chromium (III) derivatives have been found to upregulate the AMPK-PGC1α signaling pathway (Hao et al. 2011). Further, in skeletal muscle cell lines, insulin sensitivity also increased due to oligomannuronate and chromium (III) complexes (Hao et al. 2015). Purposely, AOS resulted in upregulation of GLUT-4 and insulin receptor expression due to stimulation of AMPK and insulin (PI3K/Akt) signaling pathways. Among all the examined three complexes of oligomannuronate, the one containing 2% chromium (III) revealed the highest anti-diabetic potential compared to other derivatives and un-derivatized AOS (Hao et al. 2015).

Likewise, PGS (Polyguluronate sulfate) enhanced FGF19/FGFR1c and FGF1/FGFR1c pathways (Lan et al. 2017). This study revealed that PGS and its derived oligosaccharides stimulated both these pathways better than non-sulfated PGs. AOS administration enhanced the concentration of beneficial microorganisms (*L. gasseri* & *L reuteri*) that have the potential to elevate glucose tolerance and insulin secretion (Wang et al. 2020). They further stated that AOS increased the production of SCFAs (butyric acid, acetic acid, & propionic acid) (Wang et al. 2020). Traditionally, *Sargassum confusum* (Brown seaweed) has been prescribed for the treatment of various metabolic syndromes. Oligosaccharide derived from *Sargassum confusum* (SCO) has been investigated for its anti-diabetic properties. Characterization of SCO revealed the presence of units of sulfated anhydrogalactose and methyl sulfated galactoside. SCO administration reduced the levels of fasting blood glucose. Furthermore, SCO regulated IRS1/PI3K (insulin receptor substrate 1/phosphatidylinositol 3-kinase) and JNK (c-Jun N-terminal kinase) pathways. This study highlights the role of SCO as a functional product against diabetes (Yang et al. 2019).

Miscellaneous health beneficial effects

The low molecular weight polyguluronate sulfate (PGS) was prepared by the chemical sulfation of PG (Zhao et al. 2007), and the structure was further reported as 2,3-O-desulfated-1,4-poly-L-guluronic acid (PG) with about 1.5 sulfate per sugar residue (Wu, Wang, et al. 2016). The degree of polymerization was about 20–30, and molecular weight of about 9 kDa with 33% of sulfate content. The prepared PGS was assayed for anti-hepatitis B virus (HBV). The results suggest that PGS possessed dose- and time-dependent inhibitory effects on HBsAg and HBeAg, both expression and secretion with a low level of cytotoxicity. This activity's principle is an association between PGS and activation of NF-kB and Raf/MEK/ERK signaling pathways that lead to enhancing the cellular interferon system to accelerate HBV clearance.It is well documented that seaweed biomolecules including seaweed biopolymers have radioprotective effects and are extensively used in cosmeceutical industries. Many reports demonstrated that alginate extracted from seaweeds has homeostatic properties and alginate dressing significantly accelerated wound healing of experimental burn wounds (Blaine 1947; Barnett and Varley 1987; Attwood 1989). This was further confirmed by Tajima et al. (1999), where AOS derived from enzymatic hydrolysis of sodium alginate by *Alteromonas sp.* alginate lyase. In vitro, AOS modulates cell morphology, cell proliferation and collagen expression in human skin fibroblast, but they reported that the mechanism behind this activity was unclear. These results agreed with earlier reports (Schmidt et al. 1993; Rosdy and Clauss 1990) where the proliferation of mouse L929 fibroblasts is initially inhibited up to 25% by calcium alginate recovers to control levels within five days of treatment.

Series of alginate derived oligosaccharides (ADO) with molecular weight ranging from 373–571 Da were prepared from alginate through enzymatic degradation using alginate lyase and tested for radioprotective effect (He et al. 2013). In order to investigate the UV stress response, cellular uptake, anti-UVR activity, extracellular antioxidant capacity, hemolysis inhibition and antioxidant activities on several biological model systems such as *C. albicans*, *E. coli* and *B. subtilis* spores were evaluated with prepared alginate oligosaccharide, and the results were compared to chitooligosaccharides COS). The ADO prepared through enzymatic hydrolysis was nontoxic and potent anti-UVR and antioxidant activities, including preventing radical scavengers and lipid peroxidation in cellular systems.

OligoG CF-5/20 is a low molecular weight alginate oligosaccharide (2600 Da) mainly consist of >85% guluronic acid (Khan et al. 2012). OligoG CF-5/20 has already entered preclinical animal and human studies and reported to be safe for delivery via an inhaled route in the diseased lung through the examination conducted in healthy human Phase I and CF patients in Phase II studies (Pritchard et al. 2016). Many osteoporotic patients suffering from degenerative lumbar disease (DLD) experiences compression fractures, back pains, and abnormal spinal curvature. PLIFC (Posterior lumbar intervertebral fusion with cages) is an effective intervention for osteoporosis patients with degenerative lumber disease. Nevertheless, certain complications (neurological problems, infection, pain, etc.) are associated with cages in this surgical procedure. Owing to the anti-inflammatory and

antioxidative properties of AOS, they are significant in treating infections. Ninety-six osteoporosis patients suffering from DLD receiving PLIFC were randomly divided into two groups, i.e., ONAS (orally administrated oligosaccharide Nano-medicine of alginate sodium)-group (100 mg daily) and control (pluronic nanoparticles)-group (100 mg). 1-month post-treatment showed reduced infection rate and side effects in AOS treated group compared to the control group. Similarly, compared to the control group, Japanese orthopedic scores and fusion rates were noticed more in experimental groups. As a result, the alginate oligosaccharide treated group demonstrated a significant reduction in complication and enhanced therapeutic effects in patients suffering from DLD due to regulation of serum miR-155 (Qu et al. 2017).

Agricultural applications

Plant growth promotion

Organic fertilizers or natural-based biomolecules for plant growth and development are gaining much attention among farmers. In this context, seaweeds and seaweed extract have been extensively studied for their plant growth regulation effect. Various seaweed biomolecules including growth hormones (auxin, cytokinin and gibberellins) (Stirk et al. 2004; Stirk and Van Staden 2014), betaines (Stirk et al. 2014), brassinosteroids (Stirk et al. 2014), polyphenols (Rengasamy et al. 2015), polysaccharides and oligosaccharides have been reported for plant growth promotion. Alginate oligosaccharides are reported to be potent plant growth stimulant which enhances seed germination and seedling growth. They are involved in nitrogen metabolism and are effective against various stress responses.

Nowadays, interest in the use of bio-polymers for bio-stimulating the growth of plants. One of this biopolymer is oligo-alginate, which helps regulate biological processes and could be employed as a plant growth stimulator. Bio-stimulators enhance the tolerance of the plant to abiotic stress conditions such as salinity. AOS with varied molecular weights were examined to assess the effect on physiological activities and growth of *Eucomis autumnalis* under salinity. In this study, *Eucomis autumnalis* bulbs were coated with depolymerized Na-alginates having different molecular mass, i.e., 32,000, 42,000, & 64,000 g/mol. Overall, all these AOS proved to be plant growth stimulator while AOS with low molecular mass (32000 g/mol) showed the best effect (Salachna et al. 2018). The effect of AOS on seed germination, seedling growth and stress responses were investigated in Chinese cabbage *Brassica campestris* (Zhang, Yin, et al. 2013). AOS at 30 mg.L^{-1} remarkably increased net photosynthetic rate, water use efficiency, CO_2 carboxylation efficiency and light saturation point. It also enhanced the production of growth hormones GA3 and IAA and increased the yield by 14.9% compared to control after eight days of AOS application. Alginate oligosaccharide treated with radiation (Co-60) in combination with $K_2S_2O_8$ decreased the cost of production and promoted the seed germination rate & plant growth. Faba bean seeds were soaked, and the plant was sprayed with alginate oligosaccharide (Mw: 1.25–1900 kDa) solutions. Results revealed that low molecular weight AOS had the highest seed germination and plant growth (Abd El-Mohdy 2017).

The plant growth-promoting effect of AOS were observed by An et al. (2009). The AOS were prepared using alginate lyase from newly isolated *Flavobacterium sp*. LXA displayed elicitor activity by stimulating accretion of phytoalexin and inducing phenylalanine ammonia-lyase (PAL) in soybean cotyledon and antimicrobial activity against *Pseudomonas aeruginosa*. The observed activity might be due to the induction of the PAL enzyme, involved in the synthesis of phenolic compounds in plants. They also found maximum bioactivity of AOS with DP 6.8. In another study, the positive effect of AOS on elicitation and accumulation of phytoalexin glyceollins in soybean was reported (Jia et al. 2012). They found that 4% AOS significantly induces glyceollins production in soybean seeds at optimal conditions. Similar effects were reported by Xian-Zhen et al. (2009) that AOS with DP 6.8 prepared from AGN12 bacterial alginate lyase showed higher elicitor activity on soy cotyledon. The AOS has also shown a remarkable growth-promoting effect on microalgae *Nannochloropsis oculata* at 20 mg/ml, where the growth was five times higher than the control without AOS (Yokose et al. 2009). AOS was reported to promote the germination and shoot elongation of certain plants (Tomoda et al. 1992; Yonemoto et al. 1993). The average molecular weight of alginate (MW 25700) from *Eisenia bicyclis* depolymerized into low molecular weight AOS (MW 1800) using alginate lyase, which promotes germination and shoots elongation of certain plants (Tomoda et al. 1992; Yonemoto et al. 1993). In contrast, the plant growth-promoting efficacy of AOS was investigated on tobacco cells by a time-resolved fluorometric method using two Eu^{3+} complexes as luminescence probe (Guo, Yin, et al. 2012), and the results were compared with chitosan oligosaccharide (COS). The activity of COS was higher than AOS with the faster binding rate of tobacco cells and had substantial effects on IAA generation in the cells.

The alginate oligosaccharides with different DP's (11.4, 9.2, 7.9, 6.5, 5.3 and 4.2) were obtained through alginate lyase degradation and further fractionated with ethanol (Zhang et al. 2015). The resulted derivatives were subjected to elicitor activity with rice and to study the anti-pathogenic effect. Among the fractionated derivatives, DP 7.9 and 6.5 showed maximum germination and significantly induced the PAL, catalase, and peroxidase to protect rice seedling against the pathogenic attack of *Magnaporthe grisea* and decreased the disease index from 17.74 to 10.81% and also recorded 39.06% of protection efficacy. An et al. (2008) reported that phytoalexin production was concentration-dependent, and there was no elicitor activity found with AOS less than 0.003%. Similarly, Park et al. (2004) reported that DP of AOS well correlates with the phytoalexin accumulation in soybean cotyledon.

Although alginate oligosaccharides are reported to potential plant growth promotors, the mechanism behind this stimulatory effect was unclear. Recently, Zhang et al. (2014)

explained the mechanism of AOS on root growth of rice (*Oryza sativa* L) with emphasis on auxin metabolism. The expression of auxin-related genes such as OsYUCCA1, OsYUCCA5, OsIAA11, and OsPIN1 in rice tissues was screened with different AOS concentrations (10–80 mg/ml). They concluded that AOS accelerates the IAA biosynthesis through an increased level of OsIA11 and OsPIN1 genes, and it significantly minimizes the activity of IAA oxidase in rice roots and increases the calcium signaling.

Nitrogen metabolism plays a vital role in plant physiology as it is essential to synthesis amino acids, proteins and nucleic acids. Nitrogen metabolism is directly linked with Ca2+ ions, which induces the various enzymes involved in nitrogen metabolism (O'Neal and Joy 1973; Srivastava and Singh 1987; Lam et al. 1996; Zhang et al. 2011). It was already reported that the calcium-alginate complex initiates the signal transduction pathways in plants, but the mechanism is not yet fully explored (Farmer et al. 1991; Yokose et al. 2009). This was further confirmed by Zhang et al. (2011), where calcium and alginate oligosaccharides in the nitrogen metabolism of Chinese cabbage were studied in the hydroponic culture. The increased amount of nitrate reductase, glutamine synthase, glutamate dehydrogenase, and endopeptidase was well correlated with increased total protein and total nitrogen and decreased nitrate content in shoots with AOS treated cabbage plants.

Similarly, Zhang, Liu et al. (2013) found that the exogenous application of AOS significantly induced Ca2+ ions, which regulates nitrogen metabolism in the flowering of Chinese cabbage. In another study, the role of alginate oligosaccharide in root development was evaluated (Zhang, Liu et al., 2013). AOS at a 10–80 mg L^{-1} was effective for inducing nitric oxide generation in wheat roots in a dose-dependent manner. These results revealed that Ca2+ signaling might be a downstream messenger of nitric oxide in AOS persuaded root formation facilitated by auxin.

The alginate oligomers produced from *Eisenia bicyclis* alginate by enzymatic hydrolysis using alginate lyase was tested for seed germination and shoot elongation (Yanemoto et al. 1993). The prepared oligomer had an average molecular weight of about 1800. They noticed a higher seed germination rate and enhanced proliferation with alginate oligomers, but the germination effect was the same as alginate polymers. Yamasaki et al. (2012) compared the effect of alginate oligosaccharide mixtures (AOMs) prepared using enzymatic and acid hydrolysis on green alga's growth and fatty acid composition *Chlamydomonas reinhardtii*. They noticed higher growth and production of C16:0, C18:2cis, C18:3 n-3 fatty acids in the green alga treated with enzymatically hydrolyzed AOM, then the acid hydrolyzed AOM.

Alginate oligosaccharides with a molecular weight of 1445 Da were prepared from alginate degradation using alginate lyase and used to test its effect on maize seed germination, an enzyme involved in the germination process (Hu et al. 2004b). All the AOS treatment influenced higher seed viability, and germination with the highest activity was observed at 0.75%. Treatment between 0.5% and 1.25% AOS reported as being most effective in the case of α- and β-amylase. Increased root and shoot growth was noticed after seven days with 34 and 46% growth rates, respectively.

Sodium alginate was enzymatically digested with *Psuedoalteromonas sp.* strain 272 alginate lyase to obtain polymannuronate and polyguluronate oligomers, and further, these PM and PG were digested with the same enzyme in order to get PM and PG oligomer mixtures (Xu et al. 2003). These oligomers and oligomer mixtures were subjected for the root elongation activity, and the results revealed that PM and PG showed no activity, but the enzyme digested PG possessed root growth-promoting activity at 0.5 mg/ml in carrot and rice plants with pentamers was most active.

The alginate oligomers produced using enzymatic hydrolysis of sodium alginate using *Alteromonas macleodii* alginate lyase promoted the elongation of barley roots, especially radicle growth with increased elongation rate from 209 mm/h to 5.3 mm/h (Tomoda et al., 1994). Also, AOS oligomers significantly increased the activity of alcohol dehydrogenase activity where the activity was 2-fold greater than control under hypoxic conditions. Similarly, oligomer mixtures (AOS triglycuronates 6 and 15) prepared from polymannuronate, and polyguluronate digested with *Alteromonas macleodii* showed significantly higher root elongation in barley seedlings (Natsume et al. 1994).

Enzymatically hydrolyzed sodium alginate using *Corynebacterium* sp. alginate lyase and the resulting hydrolysates were tested for plant growth-promoting activity on lettuce seedlings (Iwasaki and Matsubara 2000). All the oligosaccharide mixtures exhibited increased root elongation at a concentration ranging from 200 to 3000 µg/ml. Among these, tri-, tetra- and penta- and hexasaccharide reported excellent root growth promoters in lettuce bioassay and suggested that the root elongation depended on the degree of polymerization.

Alginate oligosaccharides not only promote plant growth but also stimulates metabolite content. Recently, Khan et al. (2011) investigated the effect of AOS on the growth, yield and alkaloid production of the opium poppy (*Papaver somniferum* L). The results demonstrate that 120 ppm of AOS obtained through irradiation significantly enhances plants' physiological process and overall growth. The presence of alkaloids such as morphine, codeine, thebaine and noscapine was detected in AOS treated plants with significant variations between treated and control plants.

Effect of AOS on stress response

Alginate oligosaccharides with varying degrees of polymerization (DP) were obtained from sodium alginate using Gracilibacillus A7 alginase hydrolysis and found that the DP values decreased with increased reaction time. The final reaction alginate lysates were tested for plant growth-promoting efficacy under stress conditions (Tang et al. 2011). The effect of hydrolyzed AOS on root elongation of salt-stressed *Brassica campestris* L was evaluated. The anti-stress effect was increased with decreased DP value, AOS with lower DP value significantly attenuated the salt stress, and antioxidant enzymes' determination confirmed it. They also

suggested that the AOS may act as an osmo-protective agent during seed germination.

Enzymatically prepared alginate oligosaccharides were tested for plant elicitor activity on *Triticum aestivum* under polyethylene glycol induced drought stress (Liu et al. 2013). AOS was applied alone at 1000 mg L^{-1}, and in combination with PEG at 150 mg L^{-1} PEG + 1000 mg L^{-1} AOS, results were compared with water control and PEG control. AOS treatment improved seedling growth (18%), root length (26%), fresh weight (43%) and relative water content (33) compared to PEG-treated plants. The increased antioxidant enzymes and reduced malondialdehyde content were observed with AOS-treated plants. The proposed mechanism for the observed drought resistance of wheat during the growth period might be due to the regulation ABA-dependent signal pathway by AOS. Alginate oligosaccharides are obtained by degrading alginate. Purposely, Li et al. (2018) conducted a study to understand the mechanism of AOS involved in improving water stress resistance in cucumber. They reported that AOS treatment reduced the growth of drought-stressed cucumbers and significantly increased the fresh weight, stomatal conductance, transpiration rate, and photosynthesis rate.

Furthermore, enhanced antioxidative properties and upregulation of genes responsible for ABA (abscisic) signaling were noticed due to AOS application. This study concludes that AOS treatment improved drought resistance status in cucumber due to the expression of antioxidative enzymes genes responsible for the ABA-signaling pathway (Li et al. 2018). As mentioned earlier, bio-stimulators perform well for plant growth promotion under abiotic stress conditions such as salinity. The varied molecular mass of AOS significantly enhanced *Eucomis autumnalis* in the first phase of the study. While in the second phase of the study, plants were exposed to salt stress conditions, and it was reported that AOS coatings curtailed the adverse effects of salinity. Compared to non-AOS treated plants, AOS treated plants subjected to water having NaCl showed less weight reduction in different parts (bulbs, pigment content, & antioxidative properties) of this plant (Salachna et al. 2018).

Concluding remarks and prospects

Natural substances from seaweeds or marine macroalgae always give immense opportunities for the development of new valued products with a wide range of applications, including food, pharmaceutical, and agriculture. Recently, substantial consideration has directed toward the seaweed polysaccharides and their oligosaccharides. Alginate oligosaccharides are the degradation products of anionic polysaccharide alginate and frequently studied for their excellent food, biomedical and plant growth properties. This review will provide a comprehensive knowledge regarding importance of alginate oligosaccharides in food, agriculture, and pharmaceutical industries due to their preservative, plant growth promotor, and biological role respectively. Moreover, information on health promoting benefits and their potential role as functional ingredients in preparation of nutraceuticals, cosmeceuticals, complementary and alternative medicines for broad readership. Oligo G, an alginate oligomer, has entered in clinical trials phase IIb for cystic fibrosis, and it is a second alginate-derived marine drug after propylene glycol alginate sodium sulfate (PSS), which is a commercially available heparinoid drug used to treat heart and brain diseases with a production value of about US$140 million per year. To the best of our knowledge, there are no algal saccharides, and its oligosaccharides are reported which are in the clinical pipeline except for PSS and Oligo G. The other AOS derivatives listed in this review possessed a wide range of pharmacological properties and further researched are needed among the marine pharmacologists to explore the AOS to develop new drugs. Apart from its pharmacological value, AOS has also been listed as immune booster and has wide opening in the aquaculture industry by increasing production costs. Various alginate oligosaccharides reported in this review have excellent plant growth-promoting abilities and play a significant role against abiotic and biotic stresses. This dual role of AOS might be interesting to develop new plant biostimulants or growth regulators. Thus, there is an urgent need to update the status on the production and application of alginate oligosaccharides in detail to realize the potential of AOS and to develop new products for the benefit of food, pharmaceutical and agricultural industries.

Conflict of interest

The authors declare that they have no competing interests.

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