

## **Overview of Microbial Enzymatic Production of Algal Oligosaccharides for Nutraceutical Applications**

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### **Abstract**

Global requirement for algal foods is increasing, as they are progressively consumed for its nutrition and health. Macroalgae is a proven source of metabolites, proteins, pigments, bioactive compounds and algal polysaccharides. The unique polysaccharides such as agar, carrageenan, porphyran, alginate, fucoidan, laminarin and ulvan are known for its wide range of bioactivities and extensively used for applications from tissue engineering to drug delivery. However, there are few limitations due to its high molecular size, low compatibility and hydrocolloid nature. Hence the enzymatically produced algal oligosaccharides has drawn tremendous attention due to its green synthesis, solubility and lower molecular size. They are reported to have bioactivities including antioxidant, antiglycemic, immunostimulatory, anti-inflammatory and prebiotic activities, which can be used in the healthcare and nutraceutical industry for the manufacture of functional foods and dietary supplements. However, identification of potential microorganisms, producing polysaccharide hydrolyzing enzymes remains a major bottle neck for efficient utilization of bioactive algal oligosaccharides. This review summarizes the recent developments in the identification and characterization of microbial enzymes for the production of bioactive algal oligosaccharides. This can improve our understanding of bioactive algal oligosaccharides and pave way for efficient utilization of macroalgae to prevent various chronic diseases.

**Keywords** Macroalgae · Microbial enzymes · Algal oligosaccharides · Nutraceutical applications

## **Introduction**

Macroalgae, also known as seaweeds are primitive, multicellular, aquatic plants. They usually occur along the coasts in the intertidal, sublittoral-to-littoral zones and grow on rocks or other hard substrata where photosynthetic light is available. They are a sustainable source of various bioactive molecules, which has diverse health benefits and wide applications as pharmaceutical and nutraceutical compounds (Holdt and Kraan 2011; Tanna and Mishra 2018, 2019). Their unique polysaccharide, pigment and polyphenolic content are largely known to be responsible for its bioactivity. Macroalgae is well accepted as a marine vegetable and consumed regularly in many South East Asian countries. Studies show that regular consumption of macroalgae can reduce the occurrence of chronic diseases including cancer, obesity, diabetes and it also reduces the mortality rate (Tanna and Mishra 2018; Nunes et al. 2020). However, the bioactive potential of macroalgae are not fully explored for its nutraceutical applications though there is a vast diversity, of about 7500 of red, 2000 of brown, and 1800 of green macroalgal species across the globe (Keith et al. 2014). Macroalgae does not require arable land, freshwater or fertilizers for its cultivation. They play a significant ecological role through carbon fixation and can sequester higher levels of carbon dioxide per unit area than terrestrial plants and microalgae (Mongin et al. 2016). The ability of these ecosystems to reduce the carbon foot print is widely recognized as an important process for mitigating the effects of climate change. Large scale cultivation of macroalgae can also contribute to the welfare of the community and associated industries by providing jobs and employment. This can improve the economic status of the coastal communities, fishermen and rural population by developing alternate employment opportunities. However, the benefits of this important, renewable and sustainable bioresource for generation of “Blue Economy” is not efficiently utilized (Buschmann et al. 2017).

## **Algal polysaccharides and its limitations for nutraceutical applications**

Macroalgae is classified into three major groups Red algae (Rhodophyta), Brown algae (Phaeophyta) and Green algae (Chlorophyta) based on their pigment and polysaccharide composition (Keith et al. 2014). Red algae are phylogenetically the oldest division and has the largest number of species. Cell wall of red algae is mainly composed of sulfated galactan such as agar, carrageenan and porphyran (Surget et al. 2017). Brown algae, which is the second largest macroalgal group, is rich in phytochemicals, polysaccharides, soluble fiber and iodine and is known to have enormous bioactive potential (Baghel et al. 2020). They are mainly composed of three unique polysaccharides alginate, fucoidan and laminarin. Among the macroalgae, green algae are the least explored division of macroalgae for their bioactive potential and in food application (Surget et al. 2017). The green algae of genera *Ulva* and *Enteromorpha* are mainly composed of sulfated polysaccharide ulvan. Ulvan is a

polydisperse hetero-polysaccharide, with rare sugars such as iduronic, glucuronic and sulfated rhamnose. The high molecular weight of ulvan which is approximately 189 to 8200 kDa, limits their applications in food and pharmaceutical industries. Though, it is reported to have anti-influenza, hepatotoxic, antioxidant, and anti-proliferative property (Lahaye and Robic 2007; Kidgell et al. 2019).

Algal polysaccharides from various macroalgae have enormous potential to be used as dietary fibers, hydrocolloids, anticancer, anticoagulant products, drug delivery and tissue engineering applications (Singh et al. 2011; Tanna and Mishra 2019). However, their utility in nutraceutical and functional food industries is restricted due to their high molecular weight, low biocompatibility and water solubility properties (Lovegrove et al. 2017; Gopinath et al. 2018). Hydrolysis of these polysaccharides lead to the production of low molecular weight, biocompatible and soluble oligosaccharides, which has far reaching applications in health care industries than the parent polysaccharide (Charoensiddhi et al. 2017; Cheong et al. 2018).

Complex polysaccharides of macroalgae can be hydrolysed into algal oligosaccharides (AOS) either by chemical or through enzymatic processes. In chemical hydrolysis, polysaccharides are treated with concentrated acid and heated at high temperature for several hours to get the final product (Lee and Lee 2016). This leads to the production of monomers and undesirable toxic by-products such as furfural, hydroxymethyl furfural and high amounts of reducing sugars like glucose, xylose, arabinose and galactose along with the oligosaccharides, which reduces the purity of oligosaccharides. These undesirable by-products limit the application of AOS in functional food and nutraceutical applications (Wang et al. 2019). On the contrary, during enzymatic hydrolysis, the polysaccharides are broken down by specific enzymes that cleaves or hydrolyzes the glycosidic bonds to produce stereo specific oligosaccharides (Xu et al. 2018a, b). The AOS produced through enzymatic hydrolysis are also shown to possess superior nutraceutical and health care applications than parent polysaccharide (Cheong et al. 2018). Hence polysaccharide hydrolysis using microbial enzymes is the best alternative approach to produce bioactive oligosaccharides for nutraceutical applications. However, identification of novel microbial enzymes is the bottle neck for efficient bioutilization of the unique complex algal polysaccharides.

Microbes especially those associated with the marine macroalgae and benthic organisms grazing on seaweeds are known to produce extracellular enzymes to break down the complex algal polysaccharides into oligosaccharides. An understanding of the microbial enzymes capable of converting algal polysaccharides to AOS through enzymatic hydrolysis is vital for efficient bio-prospection of macroalgae which is an underexplored marine bioresource.

## **Microbial enzymes for the production of algal oligosaccharides**

Research on the marine microorganisms, its physiology and adaptations has contributed for the growth of the biotechnology sector. Microbes are universally distributed and they are recognized as the most potential reservoir of diverse enzymes. The use of microbial enzymes in food fermentation is an age-old process and fermentation are still applied in many food preparations. The robust nature of microbial enzymes such as stability and tolerance to varied range of pH and temperature, multi-functionality, economic feasibility, consistency, easy product modification and optimization make them potential candidate for food industries than plant and animal based enzymes (Zhang and Kim 2010; Moreno et al. 2017). Microbial enzymes such as amylase, protease, lipases and xylanase from bacteria and fungi are widely used in various food applications to improve taste and texture of food (Raveendran et al. 2018).

Microbial enzymes involved in the hydrolysis or cleavage of the complex and unique algal polysaccharides can be grouped as glycoside hydrolases and polysaccharide lyases. The glycoside hydrolase (GH) are the group of enzymes which hydrolyze the glycosidic bond between the algal sugars in the polysaccharides. A large majority of the AOS producing enzymes agarase, carrageenase, porphyranase, fucoidanase and laminarinase belong to this group. Polysaccharide lyase (PL), the second group of enzymes which break down the polysaccharides as they cleave the (1→4) glycosidic bond between anionic sugars through  $\beta$ -elimination mechanism as seen in alginate lyase and ulvan lyase. These enzymes exhibit very high specific activity on the polysaccharide substrates and are known to have wide application in structural analysis, controlling rheological properties of polysaccharides, protoplast formation and mainly in the production of bioactive AOS (Moreno et al. 2017). In recent years, these enzymes are in much demand due to the market potential for algal oligosaccharides in functional foods, as they were accorded GRAS “Generally Recognized As Safe” status by United States Food and Drug Administration. Since then, the global requirement for these microbial polysaccharide degraders are increasing for the production of functional oligosaccharides and this is actively driving the research in this area; some examples of hydrolyzing enzymes are given in Table 1.

Studies have shown that microbes associated with live macroalgae (Oh et al. 2010, Gupta et al. 2013, Lin et al. 2017), decaying forms (Li et al. 2011b, Zhu et al. 2018a, He et al. 2018, Chen et al. 2018) and seaweed sludge (Huang et al. 2013) are the major sources of algal polysaccharide degrading enzymes (Table 1). Reports also show that microbes associated with marine molluscs are also capable to producing these enzymes (Miyaniishi et al. 2003, Lin et al. 2012, Kusaykin et al. 2017, Ulaganathan et al. 2018, Gao et al. 2019). Both bacteria and fungi isolated from seawater (Potin et al. 1993; Zhu et al. 2015; Zeng et al. 2016; Zhu et al. 2018a; Zhu et al. 2019) and sediment (Wu et al.

2011; Liao et al. 2011; Li et al. 2017; Qin et al. 2018; Zhang et al. 2018; Lee et al. 2019) both from coastal (Zhang et al. 2019b) and deep sea environment (McLean and Williamson 1979; Hatada et al. 2011; Chen et al. 2016) are shown to produce these enzymes to breakdown algal polysaccharides. Few reports show that microbes associated with terrestrial plants (Ribeiro et al. 2019), soil (Suzuki et al. 2003; Giese et al. 2006; Kawai et al. 2006; Wang et al. 2006; Lakshmikanth et al. 2009; Song et al. 2014; Kim et al. 2015; Boucelkha et al. 2017) and human gut (Stender et al. 2019) are also capable to produce these enzymes. Carrageenase followed by agarase and alginate lyase are the well-studied enzymes for the production of AOS. Porphyranase and ulvan lyase are the least explored enzymes and they are so far reported to be produced only by bacteria associated with marine algae and from marine sediment and seawater (Hehemann et al. 2010; Przybylski et al. 2015; Zhang et al. 2019).

### **Enzymatic production of algal oligosaccharides**

AOS are low molecular weight carbohydrates composed of 2-20 units of simple monomer residues. They are readily soluble in water, which makes it biocompatible and provides with a wide range of bioactivity. Though they have been commercialized since the 1980s as low-calorie bulking agents. It has gained much interest in food industry after research findings across the globe showed that non-digestible oligosaccharides can be included as functional food and were credited with the prebiotic status (Moreno et al. 2017; De et al. 2018). Among the non-digestible carbohydrates, functional oligosaccharides are gaining out-standing popularity owing to their physiological benefits to the consumers (Table 2). Recently, they have garnered much interest in food and pharmaceutical sector owing to overwhelming consumer preference for healthier food (Benwei et al. 2020). The complex nature of the algal polysaccharides and the different moieties present requires specific enzymes for the production of each AOS. The complex nature of the various algal polysaccharides and the different enzymes identified and characterized for the production of each AOS are summarized below.

#### **Agaro-oligosaccharides**

Agar is a linear polysaccharide mainly composed of basic alternating units of 3-O-linked- $\beta$ -D-galactose (G) and 4-O-linked 3, 6-anhydro- $\alpha$ -L galactose (AG) (Fig. 1). Agarases are the group of enzymes which belongs to the GH family that catalyze the hydrolysis of agar. They are classified into  $\alpha$ -agarase and  $\beta$ -agarase based on their cleavage mechanism (Gupta et al. 2013; Lin et al. 2017).  $\alpha$ -agarases (EC 3.2.1.158) belongs to the GH96 family which hydrolyses the  $\alpha$ -1, 3 linkage of agar and produces agaro-oligosaccharides with anhydro-galactose at the reducing end (Potin et al. 1993; Zhang et al. 2018; Lee et al. 2019).  $\beta$ -agarase (EC 3.2.1.81) hydrolyzes the  $\beta$ -1, 4 linkages, and the neoagaro-oligosaccharides with  $\beta$ -D-galactose at the reducing end are produced.  $\beta$ -agarase are mainly classified

into four GH families in Carbohydrate-Active Enzyme (CAZy) database which includes GH16, GH50, GH86 and GH118 based on their amino acid sequence (Lin et al. 2017).

Agarase activity has been reported in a wide range of bacteria isolated from seawater, associated with marine sediments, marine algae, marine molluscs and from freshwater and soil (Fu and Kim 2010). Only a few  $\alpha$ -agarases obtained from marine bacteria *Alteromonas agarilyticus*, *Thalassomonas* sp. and *Catenovulum sediminis* (Potin et al. 1993; Zhang et al. 2018; Lee et al. 2019) are known. However,  $\beta$ -agarase activity has been reported from various bacteria including *Zobellia* sp., *Bacillus* sp., *Alteromonas* sp., *Acinetobacter* sp., *Pseudoalteromonas* sp., *Vibrio* sp., *Agarivorans* sp., *Pseudomonas* sp., *Paenibacillus* sp., *Flammeovirga* sp., *Thalassospira* sp. and *Aquimarina* sp. which produces either extracellular or intracellular  $\beta$ -agarase (Table 1). The recombinant enzyme from cloning and expression of  $\beta$ -agarase gene in *Bacillus subtilis* showed production of neoagaro-oligosaccharides with 30 fold greater activity than the original strain (Ohta et al. 2004). Recently Xu et al. (2018a, b) have reported the production of neoagaro-octaose and neoagaro-decaose from enzymatic hydrolysis of agar extracted from *Gelidium amansii* using recombinant  $\beta$ -agarase. The neoagaro-oligosaccharides obtained using the recombinant  $\beta$ -agarase showed production of specific products tetra, hexa or octa oligosaccharides with antioxidant, anti-inflammatory and prebiotic activity (Wang et al. 2004; Chen et al. 2006; Hu et al. 2006; Wang et al. 2017; Xu et al. 2018a).

#### Carrageenan oligosaccharide

Carrageenan is a linear sulfated polysaccharide mainly composed of repeating disaccharide units of 3-O-linked- $\beta$ -D-galactose (G) and 4-O-linked 3, 6-anhydro- $\alpha$ -L galactose (AG). They are categorized as  $\kappa$ -,  $\iota$ - and  $\lambda$ -carrageenan based on the number of sulfate groups present per disaccharide unit (Yermak et al. 2017). The  $\kappa$ -carrageenan is shown to have only one sulfate group,  $\iota$ - carrageenan has two sulphate groups and  $\lambda$ -carrageenan has three sulfate groups per disaccharide (Fig. 2). Each of the carrageenan are hydrolyzed by their specific enzymes including  $\kappa$ -carrageenase (EC 3.2.1.83),  $\iota$ -carrageenase (EC 3.2.1.157) and  $\lambda$ -carrageenases (EC 3.2.1.162) which subsequently leads to the production of  $\kappa$ -,  $\iota$ - and  $\lambda$ -carrageenan oligosaccharides respectively (Kalitnik et al. 2016). They are all endohydrolases and belong to the GH family, which cleave the internal  $\beta$ -1, 4 linkage in  $\kappa$ -,  $\iota$ - and  $\lambda$ -carrageenan (Li et al. 2014; Shen et al. 2017). Studies have shown that  $\kappa$ -carrageenase belongs to the family of GH16 and  $\iota$ -carrageenase belongs to GH82 family and  $\lambda$ -carrageenases is classified within GH150 family in the CAZy database (Guibet et al. 2007; Gobet et al. 2018).

The  $\kappa$ -carrageenase is the well-studied enzyme class (Table 1) and reported from gram negative bacterial genera *Pseudomonas*, *Cytophaga*, *Tamlana*, *Pseudoalteromonas*, *Vibrio*, *Pedobacter*,

*Thalassospira* and *Zobellia* (Potin et al. 1991; Sun et al. 2010; Liu et al. 2011; Zhu and Ning 2016; Guo et al. 2018; Zhu et al. 2018a; Zhang et al. 2019).  $\kappa$ -carrageenase production was also reported in gram positive bacteria *Cellulosomicrobium cellulans* and *Bacillus* sp. (Youssef et al. 2012; Ghanbarzadeh et al. 2018). The  $\iota$ -carrageenase production are reported in bacteria *Alteromonas fortis*, *Microbulbifer thermotolerans*, *Cellulophaga* sp., *Flavobacterium* sp. (Michel et al. 2001; Hatada et al. 2011; Ma et al. 2013; Li et al. 2017) and  $\lambda$ -carrageenases from *Pseudolateromonas*, *Pseudomonas* and *Bacillus* sp. (Ohta and Hatada 2006; Guibet et al. 2007; Li et al. 2014; Gobet et al. 2018). Studies on recombinant  $\kappa$ -carrageenase from *Zobellia* sp. expressed in methylated yeast *Pichia pastoris* showed production of  $\kappa$ -carrageenan tetra- and hexa-oligosaccharides (Yu et al. 2017). Xu et al (2019) reported production of recombinant  $\iota$ -carrageenase in *Brevibacillus choshinensis* to form  $\iota$ -carrageenan oligomer. The recombinant  $\lambda$ -carrageenase from *Pseudolateromonas carrageenovora* used in the production of tetra- and hexa- $\lambda$ -carrageenan oligosaccharides (Shen et al. 2017). These enzymatically produced oligosaccharides showed potential antioxidant, immunoregulatory and antitumor activity than parent carrageenan (Table 2).

#### Porphyran oligosaccharide

Porphyran is a sulfated polysaccharide mainly composed of a linear backbone of alternating 3-linked methylated  $\beta$ -D-galactose (G) and sulfated 3, 6-anhydro- $\alpha$ -L-galactose (AG) units (Correc et al. 2011; Przybylski et al. 2015).  $\beta$ -porphyranase (EC 3.2.1.178) are the group of GH enzymes studied so far belonging to the GH16 and GH86 family which catalyzes the hydrolysis of porphyran at  $\beta$ -1, 4 glycosidic linkage (Gong et al. 2018).  $\beta$ -porphyranase specifically recognizes and cleaves  $\beta$ -1, 4 linkage between methylated G and sulfated AG to produce porphyran oligosaccharides with methylated G at the reducing end (Fig. 3).

Microbial  $\beta$ -porphyranase enzymes are reported so far only from marine habitats (Table 1), in bacteria associated with macroalgae and coastal waters such as *Zobellia galactanivorans*, *Pseudoalteromonas atlantica* and *Wenyngzhuangia funcanilytica* (Hehemann et al. 2010; Przybylski et al. 2015; Zhang et al. 2019a). Correc et al. (2011) used  $\beta$ -porphyranase isolated from *Zobellia galactanivorans* for the production of hexa-PO with G6S (1 $\rightarrow$ 3) G6M subunits. The  $\beta$ -methyl porphyranase isolated from *Pseudoalteromonas atlantica* hydrolyze the porphyran into porphyran oligomer of methylated disaccharide and dimethylated tetra-saccharides (Przybylski et al. 2015). Recently, GH16  $\beta$ -porphyranase was cloned from *Wenyngzhuangia funcanilytica* and expressed in *Escherichia coli*. This endo acting hydrolase gradually convert porphyran into low molecular weight porphyran oligosaccharides at wide pH range (pH 3.5-11) and its antitumor and antiobesity activity was confirmed through cell line studies (Osumi et al. 2002; Zhang et al. 2019a).

## Alginate Oligosaccharides

Alginate is a complex polymer of  $\alpha$ -L-glucuronic acid (GlcA) and its C5 epimer  $\beta$ -D-mannuronic acid (MnA). Alginate lyase are the group of polysaccharide lyases involved in the cleavage of alginate at  $\beta$  1 $\rightarrow$ 4 linkage to produce alginate oligosaccharides (Fig. 4). It largely belongs to polysaccharide lyase family PL5, PL6, PL7, PL14, PL15, PL17 or PL18. They are categorized into mannuronate lyase (EC 4.2.2.3) and glucuronate lyase (EC 4.2.2.11) based on their specificity to MnA- and GlcA-rich substrate respectively (Zhu et al. 2015; Zhu et al. 2018b). Few alginate lyase from PL17 and PL18 family exhibits bifunctional activity and can cleave both MnA- and GlcA-rich substrate and they are reported to hydrolyze alginate efficiently. Based on the mode of action, alginate lyase are grouped into endolytic and exolytic alginate lyase. Endolytic alginate lyase hydrolysis leads to the production of di-, tri-, tetra-, penta- and hexa-oligosaccharide as main product, while exolytic alginate lyase further degrade oligosaccharide into monomers.

Alginate lyase activity is reported from wide range of microorganisms isolated from seaweeds, seaweed factory sludge, seawater, terrestrial soil and human gut (Table 1). Various major bacterial genera produces alginate lyases which includes *Pseudoalteromonas*, *Pseudomonas*, *Flavobacterium*, *Vibrio* and *Bacillus* (Li et al. 2011a, b; Lundqvist et al. 2012; Huang et al. 2013; Zhu et al. 2018b). Alginate lyase was also reported from fungi *Aspergillus oryzae* isolated from marine macroalgae and also from the saltmarsh cordgrass associated marine bacterium *Saccharophagus degradans* (Table 1). Polymannuronate specific lyase activity was reported in *Pseudomonas* sp. (Zhu et al. 2015) and *Bacteroides cellulosilyticus* (Stender et al. 2019) microorganism isolated from degrading seaweed such as *Flavobacterium* sp. (Huang et al. 2013) *Microbulbifer* sp. Q7 (Yang et al. 2018) and *Cellulophaga algicola* (Fischer and Wefers 2019) showed polyglucuronate specific activity. Few microbial enzyme from *Pseudoalteromonas* sp. (Li et al. 2011b), *Vibrio furnissi* (Zhu et al. 2018b), *Gilvimirinus agarilyticus* (Huang et al. 2019) and *Flammeovirga* (Zhu et al. 2019) showed bifunctional activity. The gene from *Microbulbifer* sp. was expressed in *E. coli* to produce recombinant polyglucuronate specific alginate lyase and the enzyme was tested for production of alginate oligosaccharide with degree of polymerization 2-5 (Yang et al. 2018). Enzymatically produced alginate oligosaccharides exhibited antioxidant, immunoregulatory, anti-inflammatory and hypoglycemic properties (Zhou et al. 2015; Han et al. 2019; Tran et al. 2019; Xing et al. 2020).

## Fucoidan oligosaccharides

Fucoidan is a highly sulfated polysaccharide and is composed of  $\alpha$ -L-fucose units linked by (1 $\rightarrow$ 4) and (1 $\rightarrow$ 3) glycosidic bond with sulfation at C2, C3 or C4 which varies in each algal species (Yuan



and Macquarrie 2015). Fucoidanase (EC 3.2.1.44) are the enzymes of GH107 family that catalyze the hydrolysis of  $\alpha$ -L-fucoside linkage without removal of substitute groups and produce low molecular weight fucoidan oligosaccharides (Fig. 5) (Wang et al. 2007; Silchenko et al. 2013; Silchenko et al. 2018).

The isolation, purification and characterization of fucoidanase have been reported from bacteria *Pseudoalteromonas citrea* (Bakunina et al. 2002), *Fucobacter marina* (Sakai et al. 2003), *Formosa algae* (Silchenko et al. 2013), *Sphingomonas paucimobilis* (Kim et al. 2015), *Mariniflexile fucanivorans* (Cao et al. 2018) and also from marine fungi *Dendryphiella arenaria* (Wu et al. 2011) and *Fusarium* sp. LD8 (Shvetsova et al. 2014). So far only one study reported recombinant fucoidanase from *Formosa algae* to produce low molecular weight bioactive fucoidan oligosaccharide with immunoregulatory activity (Silchenko et al. 2013; Silchenko et al. 2018).

#### Laminarin oligosaccharides

Laminarin is a water soluble storage glucan found in brown macroalgae and it consists of glucose as monomeric units inter-connected through  $\beta$ -(1 $\rightarrow$ 3) and some  $\beta$ -(1 $\rightarrow$ 6) links (Kadam et al. 2015; Lee and Lee 2016). Laminarinase or glucanase are the group of GH enzymes involved in the hydrolysis of laminarin to produce laminarin oligosaccharides (Fig. 6) (Pang et al. 2005; Kim et al. 2006). They are classified as exo- $\beta$ -(1 $\rightarrow$ 3)-glucanase (EC 3.2.1.58) and endo- $\beta$ -(1 $\rightarrow$ 3)-glucanase (EC 3.2.1.6 and EC 3.2.1.39). The exo- $\beta$ -(1 $\rightarrow$ 3)-glucanase hydrolyze laminarin at terminal and formed monomer units and the endo- $\beta$ -(1 $\rightarrow$ 3)-glucanase hydrolyze the  $\beta$ -1 $\rightarrow$ 3 linkage randomly within the chain of laminarin into  $\beta$ -(1 $\rightarrow$ 6) linked laminarin oligosaccharide. The side chain in laminarin limits the activity of endo glucanase, owing to steric hindrance and limits complete hydrolysis. Endo- $\beta$ -(1 $\rightarrow$ 3)-glucanase belongs to different GH families including GH16, GH17, GH55, GH64 and GH81. Though  $\beta$ -(1 $\rightarrow$ 3)-glucanase is widely studied, few studies have also reported  $\beta$ -(1 $\rightarrow$ 6) glucanase enzyme production from marine bacterium *Formosa algae* and *Saccharophagus degradans* which belongs to the GH 30 and GH 5 family respectively (Becker et al. 2017; Badur et al. 2020).

The glucanase producing microorganisms were isolated from both marine and terrestrial niches associated with macroalgae, molluscs and terrestrial plants (Table 1). The  $\beta$ -(1 $\rightarrow$ 3) glucanase producing bacterium includes *Bacillus clausii* NM1, *Formosa algae* and *Vibrio breoganii* 1C10. Some fungi such as *Botryosphaeria* sp., *Phanerochaete chrysosporium*, *Pseudocardium sachalinensis* and *Trichoderma harzianum* are reported to produce laminarinase. Wang et al. (2017) first time reported both  $\beta$ -(1 $\rightarrow$ 3) and  $\beta$ -(1 $\rightarrow$ 6)-glucanase activity from marine bacterium *Saccharophagus degradans* isolated from decaying saltmarsh cordgrass belongs to GH5 family. Badur et al. (2020) has

investigated four laminarinase belonging to GH16 and GH17 family, isolated from *Vibrio breoganii* that can produce both low and high molecular weight laminarin oligosaccharide, with degree of polymerization 3-4 and >8 respectively. The endo- $\beta$ -(1 $\rightarrow$ 3)-glucanase isolated from *Bacillus circulans* and *Bacillus clausii* NM1 could hydrolyze laminarin into  $\beta$ -1 $\rightarrow$ 3 glucan oligomer with anti-inflammatory activity (Kim et al. 2006).

#### Ulvan oligosaccharides

Ulvan is a sulphated heteropolysaccharide mainly extracted from green algae and structurally constituted by alternating units of D-glucuronic acid (GlcA) or L-iduronic acid (IdoA), linked to sulfated  $\alpha$ -L-rhamnose (Rha3S) (Lahaye and Robic 2007; De et al. 2018). Ulvan lyase (EC 4.2.2.) are the least explored enzymes involved in the hydrolysis of ulvan, which belong to the polysaccharide lyase family PL24 or PL 25 or PL28 as described in the CAZy database (Ulakanathan et al. 2018).

Ulvan lyase activity have been reported from microorganisms isolated from green algal surface, marine molluscs grazing on alga and sediment including *Persicivirga ulvanivorans*, *Pseudoalteromonas* sp., *Formosa agriphila*, *Nonlabens ulvanivorans* and *Alteromonas* sp. (Qin et al. 2018; Gao et al. 2019; Reisky et al. 2018). Ulvan lyase cleave  $\beta$ -1, 4 glycosidic bond between Rha3S linked to either GlcA or IdoA by  $\beta$ -elimination mechanism with the release of ulvan oligosaccharides that have 4-deoxy-L-threo-hex-4-enopyranosiduronic acid (EPU) at the non-reducing end (Fig. 7) (Reisky et al. 2018). Ulvan lyase isolated from *Alteromonas* species produced low molecular weight oligosaccharides, mainly composed of unsaturated rhamnose 3-sulfate and uronic acid. Similarly, ulvan lyase isolated from *Pseudoalteromonas* sp. showed potential to hydrolyze ulvan into ulvan oligomer including disaccharide (EPU-Rha3S) and tetra-saccharides (EPU-Rha3S-Xyl-Rha3S) (Qin et al. 2018). Gao et al (2019) studied the cloning, expression and characterization of recombinant ulvan lyase from *Alteromonas* sp. A321 and showed production of even numbered ulvan oligosaccharide. As of now there are no studies on the bioactivity of enzymatically produced ulvan oligosaccharides.

#### **Bioactivity of enzymatically produced algal oligosaccharides**

In recent years, there has been a growing interest in natural biomolecules for their potential, preventive role in managing chronic diseases, especially those associated with oxidative stress. Hence, many researchers are driven to explore algal products as its nutritional importance are brought to light. Bioactive oligosaccharides, especially AOS is investigated in depth as a source of nutraceutical or functional food as they have multiple bioactivities. Agaro, carrageenan and alginate oligosaccharides are among the well-studied AOS for their bioactivity such as antioxidant, antiglycemic, anti-inflammatory, prebiotic along with hepatoprotective and antitumor activity (Table 2). Recent studies

are exploring the enzymatic production and bioactivity of other AOS such as porphyran, fucoidan, laminarin and ulvan oligosaccharides (Table 1).

#### Antioxidant activity

Biomolecules that scavenge reactive oxygen species (ROS), prevent a cascade of physiological stress as it is the key signalling molecule which elicit the metabolic processes that leads to the progression of numerous chronic diseases (Fig. 8). Almost all the AOS studied have reported radical scavenging activity based on 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2-azino-bis (3-ethylbenzotiazolin)-6-sulfonic acid (ABTS), hydroxyl or superoxide radical assays (Chen 2006; Xu et al. 2018) (Table 2). Studies have shown that AOS of carrageenan (Yuan et al. 2005), porphyran (Zhao et al. 2006) and agar (Wang et al. 2004) reported higher antioxidant activity than parent polysaccharides. The enzymatically hydrolysed AOS of agar (Xu et al. 2018) and carrageenan (Sun et al. 2015) showed higher inhibition of free radicals, compared to acid and hydrogen peroxide hydrolysed polysaccharide. Zhang et al. (2020) have been reported the antioxidant potential of enzymatically produced alginate oligosaccharide, shows good inhibitory activity of up to >88%, >81% and >61% towards hydroxyl, ABTS and DPPH radicals. The alginate oligosaccharide was shown to inhibit (89%) lipid oxidation along with hydroxyl, ABTS and superoxide free radical inhibition activity.

#### Antiglycemic activity

Oligosaccharides are able to inhibit the enzymes involved in the metabolism of carbohydrates including  $\alpha$ -amylase,  $\alpha$ -glucosidase and dipeptidyl peptidase-4. These are the target enzymes which can be inhibited for treatment or prevention of type 2 diabetes (T2D). So far only AOS of agar and alginate are reported to have antiglycemic activity mostly accompanied along with antioxidant activity (Hao et al. 2011; Han et al. 2019). *In vitro* antiglycemic studies on neoagaro-oligosaccharides showed significant inhibition of  $\alpha$ -glucosidase and relatively lower inhibition of  $\alpha$ -amylase enzymes (Hong et al. 2017). *In vivo* mice model studies of neoagaro-oligosaccharides showed stimulation of growth in mice suffering from T2D. Administration of different doses of neoagaro-oligosaccharides in T2D mice model showed lowering of blood glucose and improvement in glucose tolerance level. It also able to alleviate hepatocyte swelling and recovered pancreatic  $\beta$ -cells involved in insulin secretion (Lin et al. 2019). Alginate oligosaccharides showed antiglycemic activity as it can stimulate the expression of genes for GLUT4 and insulin receptor. Additionally, it also can enhance phosphorylation of proteins involved in insulin signaling pathways to prevent T2D (Hao et al. 2011). Antiglycemic activity is also reported from crude algal extracts of brown and red algae and studies show that antioxidant along with antiglycemic activity can aid to control diabetes more efficiently (Holdt and Kraan 2011).

### Anti-inflammatory activity

The oxidative stress lead to the generation of lipopolysaccharide induced inflammation through elevation of nitric oxide and prostaglandin level. The agaro, neoagaro and alginate oligosaccharides suppress the elevated level of nitric oxide, prostaglandin E2 and pro-inflammatory cytokines including tumor necrosis factor- $\alpha$ , interleukin-1 $\beta$ , interleukin 6 in lipopolysaccharide induced murine macrophage and human monocytes (Enoki et al. 2010; Zhou et al. 2015). Xu et al (2012) reported significant correlation between  $\kappa$ -carrageenan oligosaccharide concentration and inhibition of viability and content of nitric oxide, tumor necrosis factor- $\alpha$  and interleukin 10 released by lipopolysaccharide activated microglial cells. Agaro-oligosaccharide administration in colitis induced mice showed the upregulation in expression of heme oxygenase-1 in colonic mucosa. Irrespective to the length of oligosaccharides, the 3, 6-anhydro- $\alpha$ -L-galactose at the reducing end, showed similar induction of heme oxygenase expression (Enoki et al. 2010; Higashimura et al. 2013). *In vivo* studies on neoagaro-oligosaccharides showed significant inhibition of lipopolysaccharide induced inflammatory response in mouse macrophage cells. The inhibition may be due to the reduction of lipopolysaccharide, induces nitric oxide synthase and interleukin-1 $\beta$  expression through downregulation of both pathways including mitogen activated protein kinase and nuclear factor- $\kappa$ B signaling pathways (Wang et al. 2017). This suggest that the degree of polymerization and number of sulfate group of oligosaccharides play an important role on their anti-inflammatory activity (Guo et al. 2018).

### Immunoregulatory activity

Prolonged oxidative stress leads to inflammation which leads to excessive production of cytokines and macrophages which can suppress the immune response of the individual. The effect of laminarin oligosaccharides on the expression of genes involved in the regulation of immune function showed upregulation of genes for interleukin 7, 15 and interleukin 2 receptor, T-cell receptor beta, which is linked with immunity improvement (Kim et al. 2006). Studies found that the low molecular weight AOS, especially the carrageenan oligosaccharides (1.7kDa) showed increased secretion of tumor necrosis factor  $\alpha$ , involved in immune modulation. Treatment of murine macrophage cell line with carrageenan oligomer results in the upregulation of expression of toll-like receptor 4, cluster of differentiation 14 and myeloid differentiation 2 (Yuan et al. 2011). Sulfated fucoidan oligosaccharides produced after enzymatic hydrolysis of fucoidan, showed induction in the expression level of antigen activation involved in innate and adaptive immunity (Silchenko et al. 2013; Yao et al. 2014). The unsaturated alginate oligosaccharide induced nitric oxide production, nitric oxide synthase and stimulated tumor necrosis factor- $\alpha$  production. It also induces macrophage activation to release nitric oxide and tumor necrosis factor- $\alpha$  involved in nuclear factor- $\kappa$ B and mitogen activated protein kinase

signaling pathways (Xu et al. 2014). The carrageenan oligosaccharide showed higher induction of interleukin 10 and the cytokine level in both human cell lines and mice blood cells which improves its immune function (Kalitnik et al. 2016).

### Prebiotic Activity

Beneficial gut microbes like *Lactobacillus* sp., *Bifidobacterium* sp. etc. play an important role as they inhibit the growth of pathogenic bacteria including *Salmonella* sp. or *Escherichia coli*, *Shigella* and *Peptoniphilus* (Han et al. 2019). Any change in the composition of the gut microflora due to oxidative or physiological stress can destabilise the metabolic activity of the individual. Some of the AOS are categorized as prebiotic compounds as they are not digested by gastrointestinal enzymes and helps in the proliferation of beneficial gut microbes. The neoagaro-oligosaccharides found to have high inhibition to enzymes of upper gastrointestinal tract, so it can reach to the colon and serve as substrate for intestinal microflora (Hong et al. 2017). The administration of neoagaro (Hu et al. 2006) and alginate oligosaccharides (Wang et al. 2006) is shown to significantly proliferate the number of *Bifidobacterium* and *Lactobacillus* anaerobically with no side effect during *in vivo* and *in vitro* studies. These AOS were able to stimulate better growth of beneficial bacteria in comparison to fructooligosaccharides (FOS) or galactooligosaccharides (Hu et al. 2006). In addition to this, reports show that these AOS are safe for consumption as they do not cause any adverse effects like belching and abdominal bloating and improves the general physiological well-being of the individual (Wang et al. 2006).

Most of the AOS from red and brown algal polysaccharides shows very good bioactivity which has wide therapeutic applications. However, so far there are no studies on the bioactivity of green algal AOS, which is the least explored group of AOS (Table 2). The antioxidant, antiglycemic, anti-inflammatory, immunoregulatory and prebiotic properties are essential criteria for an efficient nutraceutical or functional food (Fig. 8) which is in high demand globally.

### **Multiple bioactive potentials of enzymatically produced algal oligosaccharides for nutraceutical application**

The present generation is greatly affected due to the changes in lifestyle and frequent consumption of carbohydrate rich diet and decreased physical exercise. This leads to accumulation of high glucose in blood for a prolonged period of time causing hyperglycemia. Increased glucose accumulation in the blood leads to its autoxidation, oxidative phosphorylation and formation of advanced glycation end products which lead to generation of ROS (Fig. 8). In an individual with a healthy physiological condition, ROS are effectively eliminated by antioxidant enzymes produced by the defense system of

the body. However, excessive ROS production overwhelms the natural detoxification system and leads to oxidative stress. The induction of cellular pathways by oxidative stress leading to the onset of various chronic diseases including diabetes due to pancreatic  $\beta$ -cell dysfunction, lack of insulin production and malfunction of insulin receptors. It is one of the major chronic ailments and after its onset, the first-line of treatment is through the inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase enzyme activity. These are the primary enzymes which are involved in the metabolism of dietary carbohydrates into glucose. Inhibition of these enzymes can significantly decrease the blood glucose level. Therefore, it is the most common therapeutic approach to delay glucose release and absorption (Lin et al. 2019). However, preventing the oxidative stress using AOS with antioxidant property in combination with antiglycemic activity could be a much effective strategy for diabetes management. Hence, there is a huge demand for natural sources of bioactive compounds with ROS scavenging and antiglycemic activity.

Oxidative stress also causes immune diseases, inflammatory disorders and alteration in gut microbiota (Chen et al. 2006). AOS are known to prevent all of these chronic disorders as they play a significant role to inhibit the lipopolysaccharide induced response and activate cytokine to prevent inflammatory and immune diseases respectively. It also can proliferate the beneficial gut microbiota and inhibit the growth of pathogenic bacteria (Fig. 8). Hence algal oligosaccharides have gained a lot of attention in food industry as it can greatly aid in preventing and managing chronic diseases and to improve the general health of the individuals. An understanding of the various enzymes and AOS produced through enzymatic hydrolysis from diverse algal polysaccharides is of utmost importance. This can facilitate its potential to be widely explored for functional food applications which is the need of the hour as there are increasing reports of chronic diseases among individuals of age groups starting from toddlers to aging adults globally.

## **Conclusions**

Algal sugars and bioactive products of macroalgae has been studied and well known for its nutraceutical and pharmaceutical properties. However, there are a few limitations for efficient utilisation of macroalgal biomass globally. Lack of efficient processing methods and low yield limits the complete utilisation of algal based products. Identification of efficient microbial enzymes for production of bioactive algal oligosaccharides can greatly improve the bio utilisation efficiency of algal products. This can increase the commercial value of the algal biomass collected from the wild or from aquaculture practices and pave way for sustainable utilization of macroalgae and its bio-prospection which is an underexplored marine bioresource.

## **Acknowledgements**

Ashok S Jagtap is thankful to Council of Scientific and Industrial Research, India, for the award of Junior Research Fellowship (Grant number 31/026/(0300)/2017-EMR-I). This is NIO contribution number XXXX (number to be included after acceptance of the manuscript).

## **Declarations**

### **Funding**

Ashok S Jagtap received Junior Research Fellowship, Grant number 31/026/(0300)/2017-EMR-I from Council of Scientific and Industrial Research, India.

### **Conflict of Interest/Competing interests**

On behalf of all authors, the corresponding author states that there is no conflict of interest.

### **Ethics approval**

Not applicable

### **Consent to participate**

Not applicable

### **Consent for publication**

Not applicable

### **Availability of data and material**

Not applicable

### **Code availability**

Not applicable

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## Figure legends

**Fig. 1** Structure of agar and its enzymatic hydrolysis to produce agaro-oligosaccharide and neoagaro-oligosaccharide, where G: galactose, AG: 3, 6 anhydro-L-galactose

**Fig. 2** Structure of carrageenan and its enzymatic hydrolysis to produce  $\kappa$ -,  $\iota$ - or  $\lambda$ - carrageenan oligosaccharides, where G: galactose, AG: 3, 6 anhydro-L-galactose

**Fig. 3** Structure of porphyran and its enzymatic hydrolysis to produce porphyran oligosaccharide, where G: galactose

**Fig. 4** Structure of alginate and its enzymatic hydrolysis to produce alginate oligosaccharide, where GlcA: glucuronic acid, MnA: mannuronic acid

**Fig. 5** Structure of fucoidan and its enzymatic hydrolysis to produce fucoidan oligosaccharide

**Fig. 6** Structure of laminarin and its enzymatic hydrolysis to produce  $\beta$ 1-3 or  $\beta$ 1-6 laminarin oligosaccharide

**Fig. 7** Structure of ulvan and its enzymatic hydrolysis to produce ulvan oligosaccharide (Xyl= xylose, Rha3S=  $\alpha$ -L-rhamnose 3-sulphate, GlcA= glucuronic acid, IdoA=iduronic acid, EPU=4-deoxy-L-threo-hex-4-enopyranosiduronic acid)

**Fig. 8** Schematic representation of bioactive potential of algal oligosaccharides in nutraceutical applications



**Table 1** Microbial enzymes identified for the production of algal oligosaccharides

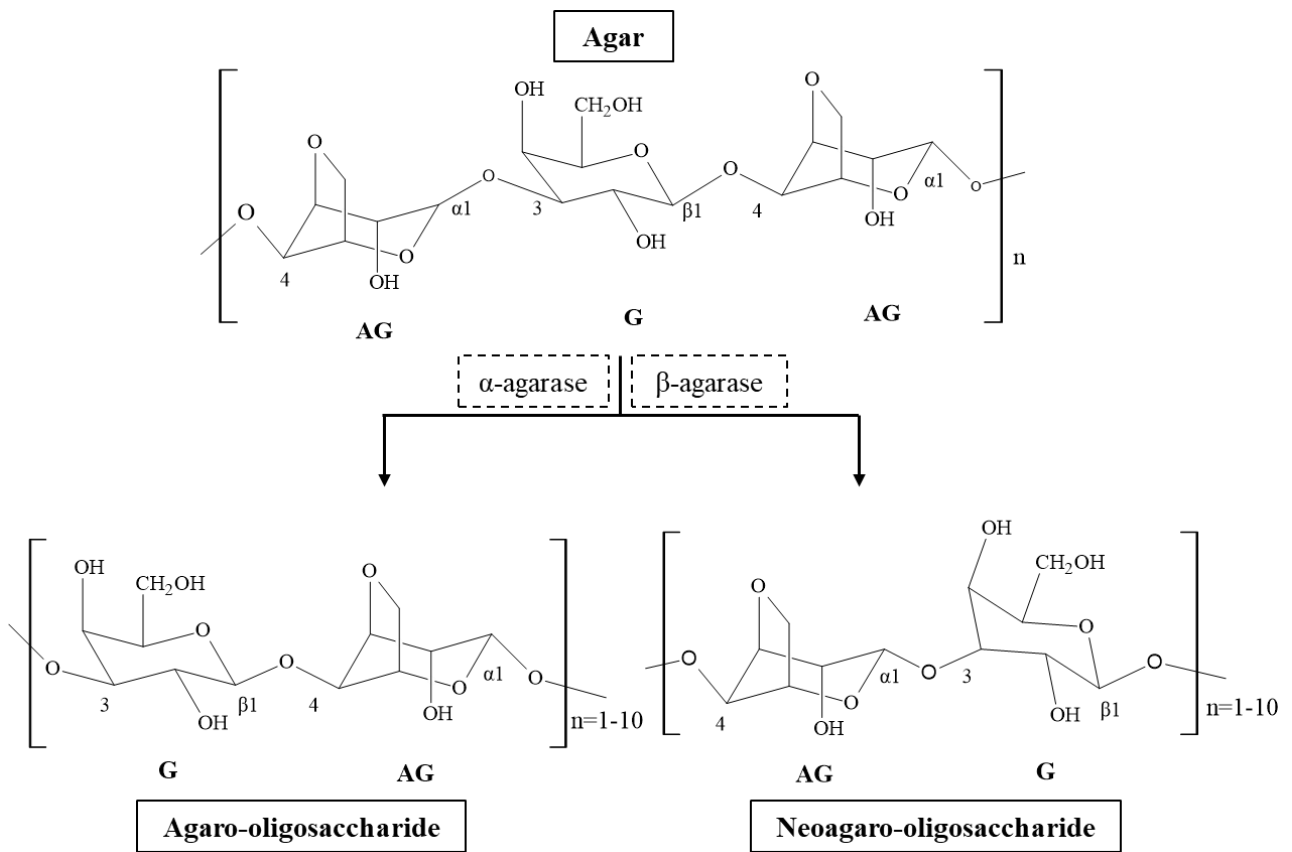
<b>Enzymes</b>	<b>Microbial source</b>	<b>Habitat</b>	<b>Reference</b>
$\alpha$ -agarase	<i>Alteromonas agarolyticus</i>	Seawater	Potin et al. (1993)
EC 3.2.1.158	<i>Thalassomonas</i> sp. LD5, <i>Catenovulum sediminis</i> WS1-A	Marine sediment	Zhang et al. (2018); Lee et al. (2019)
$\beta$ -agarase	<i>Zobellia galactanivorans</i>	Marine algae	Jam et al. (2005);
EC 3.2.1.81	<i>Pseudoalteromonas</i> sp. AG4, <i>Pseudomonas</i> sp., <i>Aquimarina agarilytica</i> ZC1		Oh et al. (2010); Gupta et al. (2013); Lin et al. (2017)
	<i>Agarivorans</i> sp. HZ105	Marine molluscs	Lin et al. (2012)
	<i>Flammeovirga</i> sp. OC-4, <i>Thalassospira profundimonas</i>	Seawater	Chen et al. (2016); Zeng et al. (2016)
	<i>Vibrio</i> sp. strain CN41	Marine sediment	Liao et al. (2011)
	<i>Bacillus</i> sp. MK03, <i>Alteromonas</i> sp. SY37-12, <i>Acinetobacter</i> sp. AG LSL-1 <i>Paenibacillus</i> sp. SSG-1	Terrestrial soil	Suzuki et al. (2003); Wang et al. (2006); Lakshmikanth et al. (2009); Song et al. (2014)
$\kappa$ -carrageenase	<i>Cytophaga</i> sp., <i>Tamlana</i> sp. HC4, <i>Pseudoalteromonas porphyrae</i> , <i>Cellulosimicrobium cellulans</i> , <i>Vibrio</i> sp. NJ-2, <i>Thalassospira</i> sp. Fjfst-32, <i>Zobellia</i> sp. ZL-4	Marine algae	Potin et al. (1991); Sun et al. (2010); Liu et al. (2011); Youssef et al. (2012); Zhu and Ning (2016); Guo et al. (2018); Zhang et al. (2019)
	<i>Pseudomonas carrageenovora</i> , <i>Pedobacter hainanensis</i> NJ-02	Seawater	McLean and Williamson (1979); Zhu et al. (2018a)
	<i>Bacillus</i> sp. HT19	Terrestrial soil	Li et al. (2019)
$\iota$ -carrageenase	<i>Alteromonas fortis</i> , <i>Cellulophaga</i> sp. QY3	Marine algae	Michel et al. (2001); Ma et al. (2013)

	<i>Microbulbifer thermotolerans</i> ,	Marine sediment	Hatada et al. (2011);
	<i>Flavobacterium</i> sp. YS-80-122		Li et al. (2017)
$\lambda$ -carrageenase	<i>Pseudomonas carrageenovora</i>	Marine algae	Guibet et al. (2007)
EC 3.2.1.162	<i>Pseudoalteromonas</i> CL9	Marine sediment	Ohta and Hatada (2006)
	<i>Bacillus</i> sp.	Terrestrial soil	Li et al. (2014)
$\beta$ -porphyranase	<i>Zobellia galactanivorans</i> ,	Marine algae	Hehemann et al.
EC 3.2.1.178	<i>Pseudoalteromonas atlantica</i>		(2010); Przybylski et al. (2015)
	<i>Wenyngzhuangia fucanilytica</i>	Seawater	Zhang et al. (2019a)
Alginate lyase	<i>Pseudoalteromonas</i> sp.	Marine algae	Li et al. (2011b);
EC 4.2.2.3 / EC 4.2.2.11	<i>Pseudomonas alginovora</i> , <i>Formosa algae</i> <i>Flavobacterium</i> sp. S20, <i>Sphingobacterium</i> sp. <i>Microbulbifer</i> sp., <i>Vibrio furnissii</i> , <i>Sphingomonas</i> sp. ZH0, <i>Bacillus</i> sp. Alg07, <i>Cellulophaga algicola</i> , <i>Aspergillus oryzae</i> <i>Gilvimarinus agarilyticus</i>		Lundqvist et al. (2012); Huang et al. (2013); Falkeborg et al. (2014); Yang et al. (2018); Zhu et al. (2018b); He et al. (2018); Chen et al. (2018); Fischer and Wefers (2019); Huang et al. (2019); Belik et al. (2020); Singh et al. (2011)
	<i>Pseudomonas</i> sp. E03,	Seawater	Zhu et al. (2015);
	<i>Flammeovirga</i> sp.		Zhu et al. (2019)
	<i>Flavobacterium multivorum</i>	Terrestrial soil	Boucelkha et al. (2017)
	<i>Bacteroides cellulosilyticus</i>	Human gut	Stender et al. (2019)
	<i>Saccharophagus degradans</i>	Saltmarsh cordgrass	Kim et al. (2012)
Fucoidanase	<i>Fucobacter marina</i> ,	Marine algae	(Sakai et al. 2003);
EC 3.2.1.44	<i>Mariniflexile fucanivorans</i> , <i>Formosa algae</i>		(Cao et al. 2018); Silchenko et al. (2013)

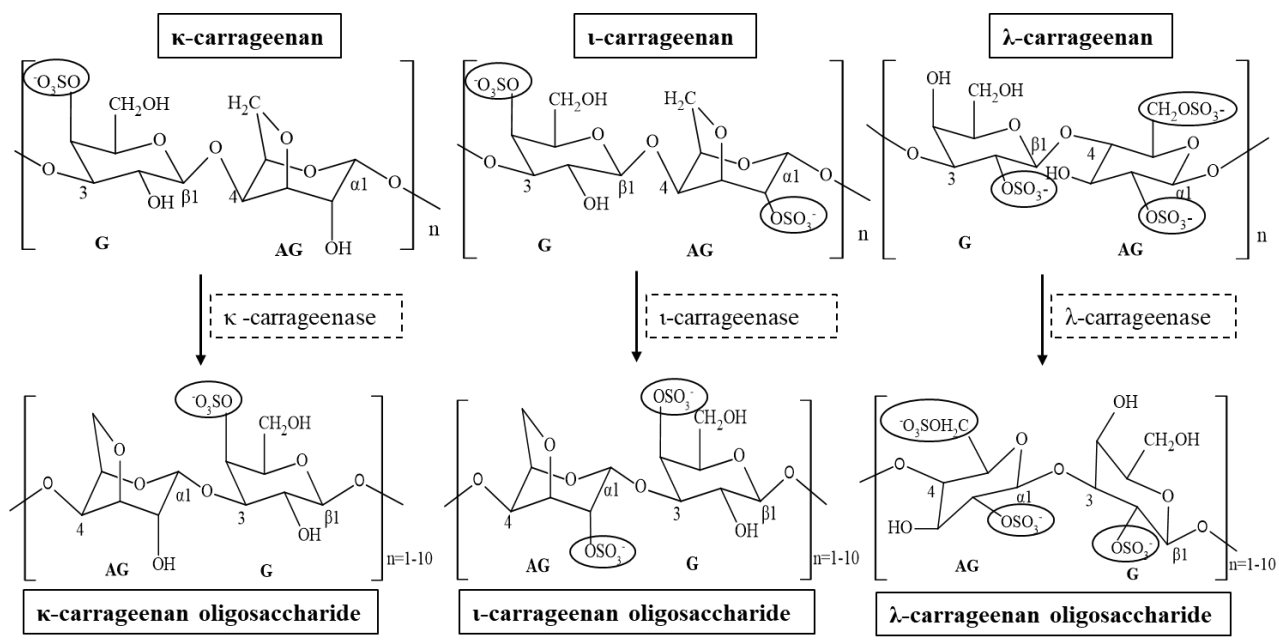
	<i>Pseudoalteromonas citrea</i>	Seawater	Bakunina et al. (2002)
	<i>Fusarium</i> sp. LD8, <i>Dendryphiella arenaria</i>	Marine sediment	Shvetsova et al. (2014); Wu et al. (2011)
	<i>Sphingomonas paucimobilis</i>	Terrestrial soil	Kim et al. (2015)
Glucanase/ Laminarinase	<i>Formosa algae</i> KMM 3553, <i>Vibrio breoganii</i> 1C10	Marine algae	Becker et al. (2017); Badur et al. (2020)
EC 3.2.1.6/ EC 3.2.1.39	<i>Bacillus clausii</i> NM-1, <i>Pseudocardium sachalinensis</i>	Marine molluscans	Miyanishi et al. (2003); Kusaykin et al. (2017)
	<i>Trichoderma harzianum</i>	Terrestrial plant	Ribeiro et al. (2019)
	<i>Botryosphaeria</i> sp., <i>Phanerochaete chrysosporium</i>	Terrestrial soil	Giese et al. (2006); Kawai et al. (2006)
	<i>Saccharophagus degradans</i>	Saltmarsh cordgrass	Wang et al. (2017)
Ulvan lyase EC 4.2.2.-	<i>Persicivirga ulvanivorans</i> , <i>Formosa agariphila</i>	Marine algae	Collen et al. (2011); Reisky et al. (2018)
	<i>Nonlabens ulvanivorans</i> , <i>Alteromonas</i> sp.	Marine molluscans	Ulaganathan et al. (2018); Gao et al. (2019)
	<i>Pseudoalteromonas</i> sp.	Marine sediment	Qin et al. (2018)

**Table 2** Bioactive potential of enzymatically produced algal oligosaccharides

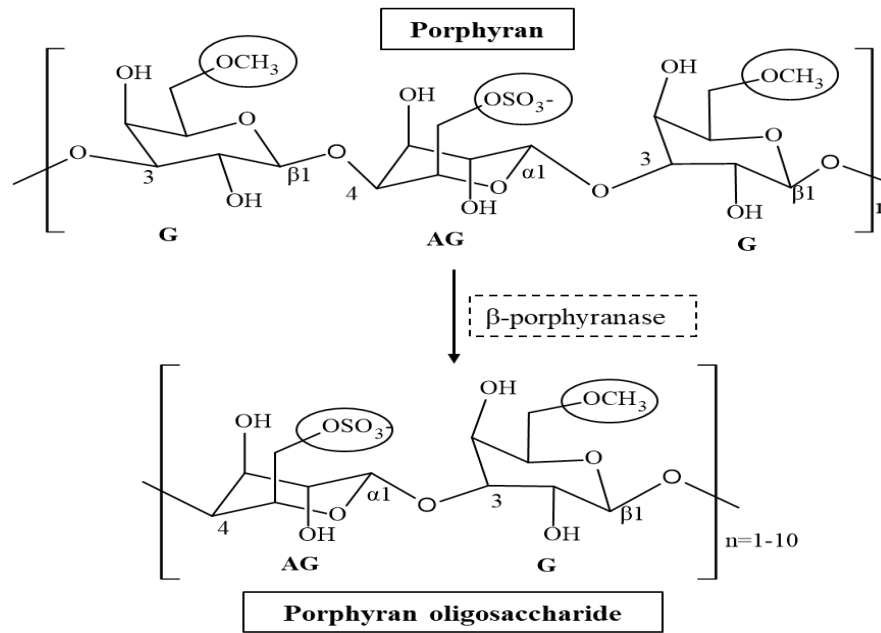
<b>Algal oligosaccharides</b>	<b>Bioactivity reported</b>	<b>References</b>
Agaro-oligosaccharides	Antioxidant, hepatoprotective, antiglycemic, prebiotic and anti-inflammatory activity	Wang et al. (2004); Chen et al. (2006); Hu et al. (2006); Wang et al. (2017); Xu et al. (2018a)
Carrageenan oligosaccharides	Antioxidant, prebiotic, anti-inflammatory and immunoregulatory	Yuan et al. (2005); Yuan et al. (2011); Xu et al. (2012); Yao et al. (2014); Han et al. (2019)
Porphyran oligosaccharides	Antioxidant, antitumor and antiobesity activity	Osumi et al. (2002); Zhao et al. (2006); Gong et al. (2018)
Alginate oligosaccharides	Antioxidant, antiglycemic, prebiotic, anti-inflammatory, immunoregulatory and antiobesity activity	Wang et al. (2006); Hao et al. (2011); Falkeborg et al. (2014); Xu et al. (2014); Zhou et al. (2015); Han et al. (2019); Tran et al. (2019); Zhang et al. (2020)
Fucoidan oligosaccharides	Antioxidant immunoregulatory and antitumor activity	Wang et al. (2007); Silchenko et al. (2013); Silchenko et al. (2018)
Laminarin oligosaccharides	Antitumor and immunoregulatory activity	Pang et al. (2005); Kim et al. (2006)
Ulvan oligosaccharides	No Study	



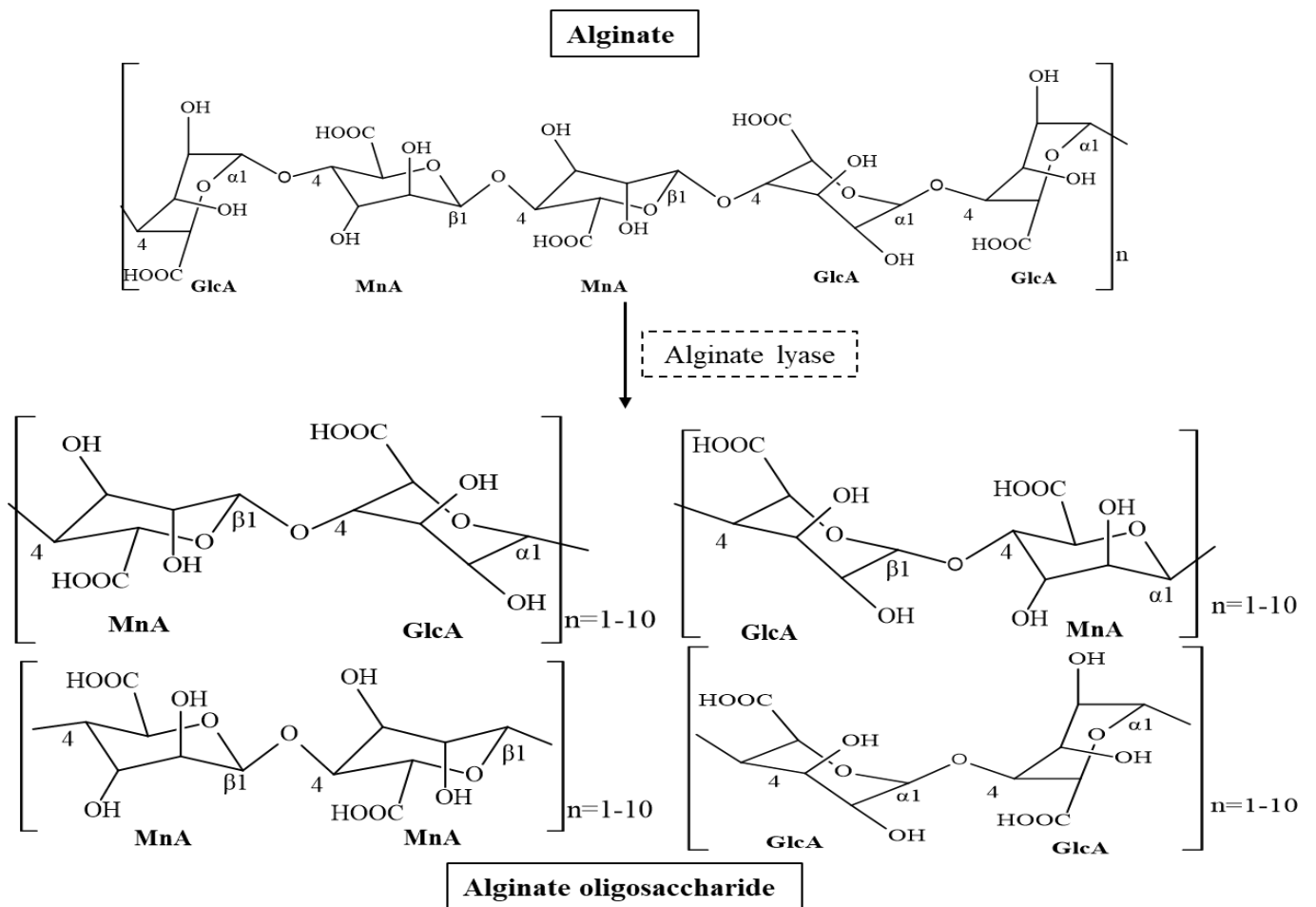
**Fig. 1** Structure of agar and its enzymatic hydrolysis to produce agaro-oligosaccharide and neoagaro-oligosaccharide, where G: galactose, AG: 3, 6 anhydro-L-galactose



**Fig. 2** Structure of carrageenan and its enzymatic hydrolysis to produce  $\kappa$ -,  $\iota$ - or  $\lambda$ - carrageenan oligosaccharides, where G: galactose, AG: 3, 6 anhydro-L-galactose

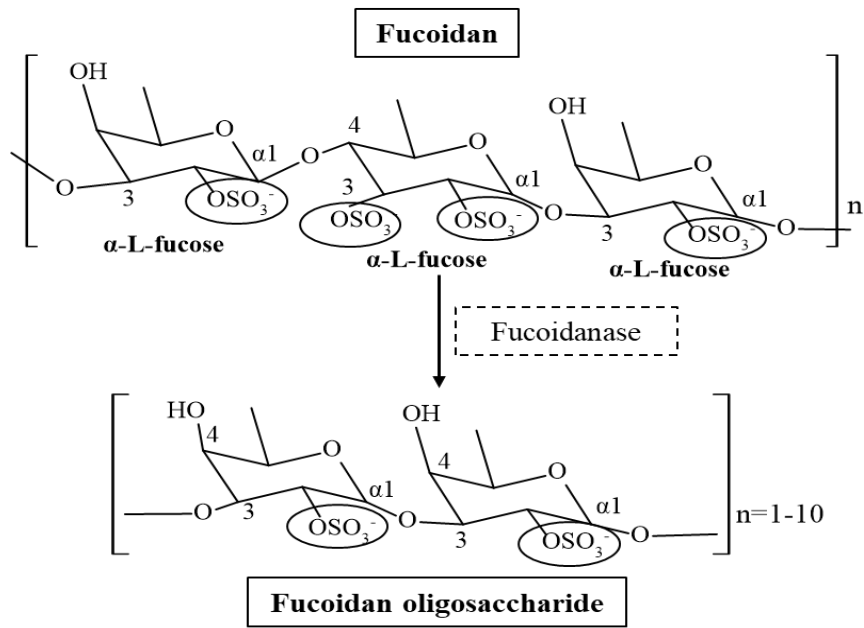


**Fig. 3** Structure of porphyran and its enzymatic hydrolysis to produce porphyran oligosaccharide, where G: galactose

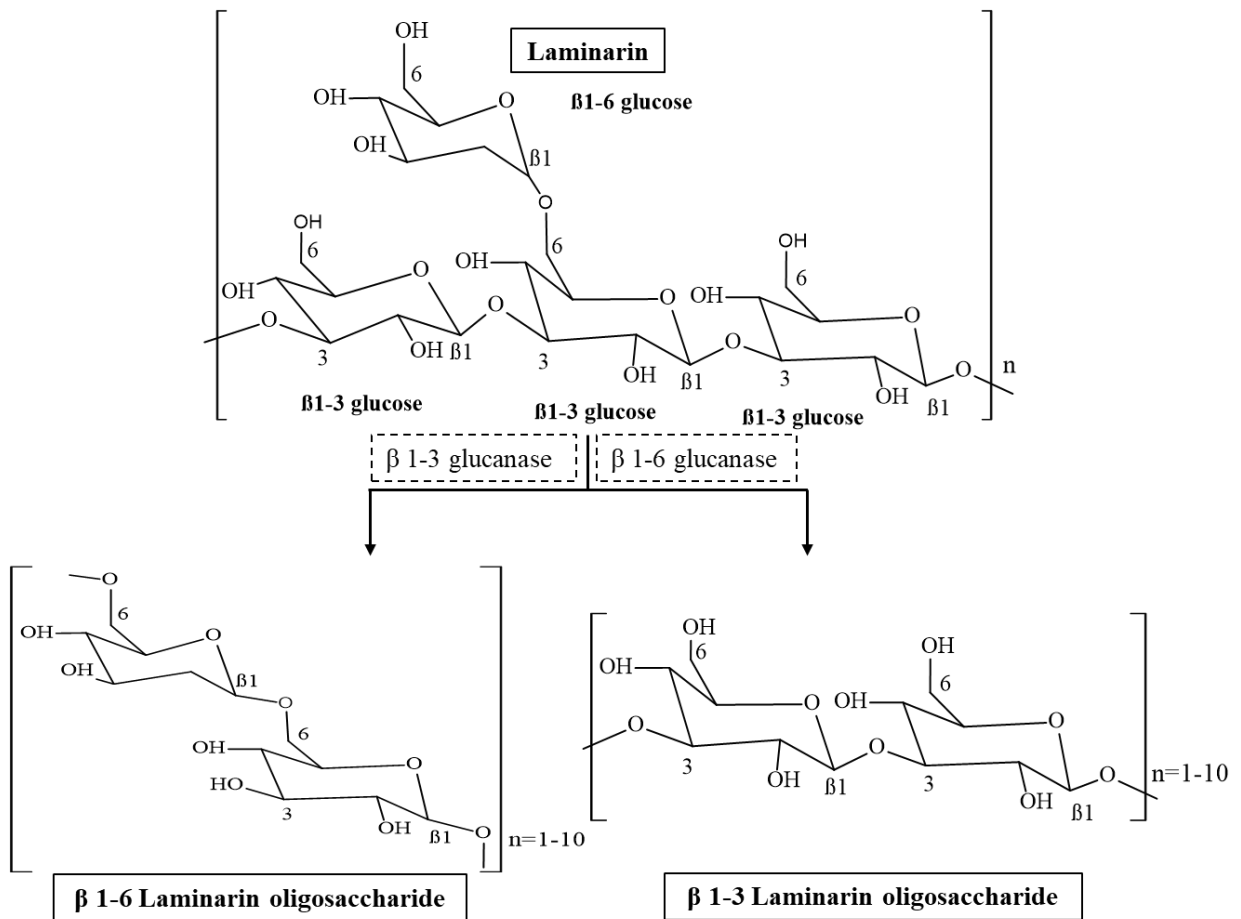


**Fig. 4** Structure of alginate and its enzymatic hydrolysis to produce alginate oligosaccharide, where GlcA: glucuronic acid, MnA: mannuronic acid

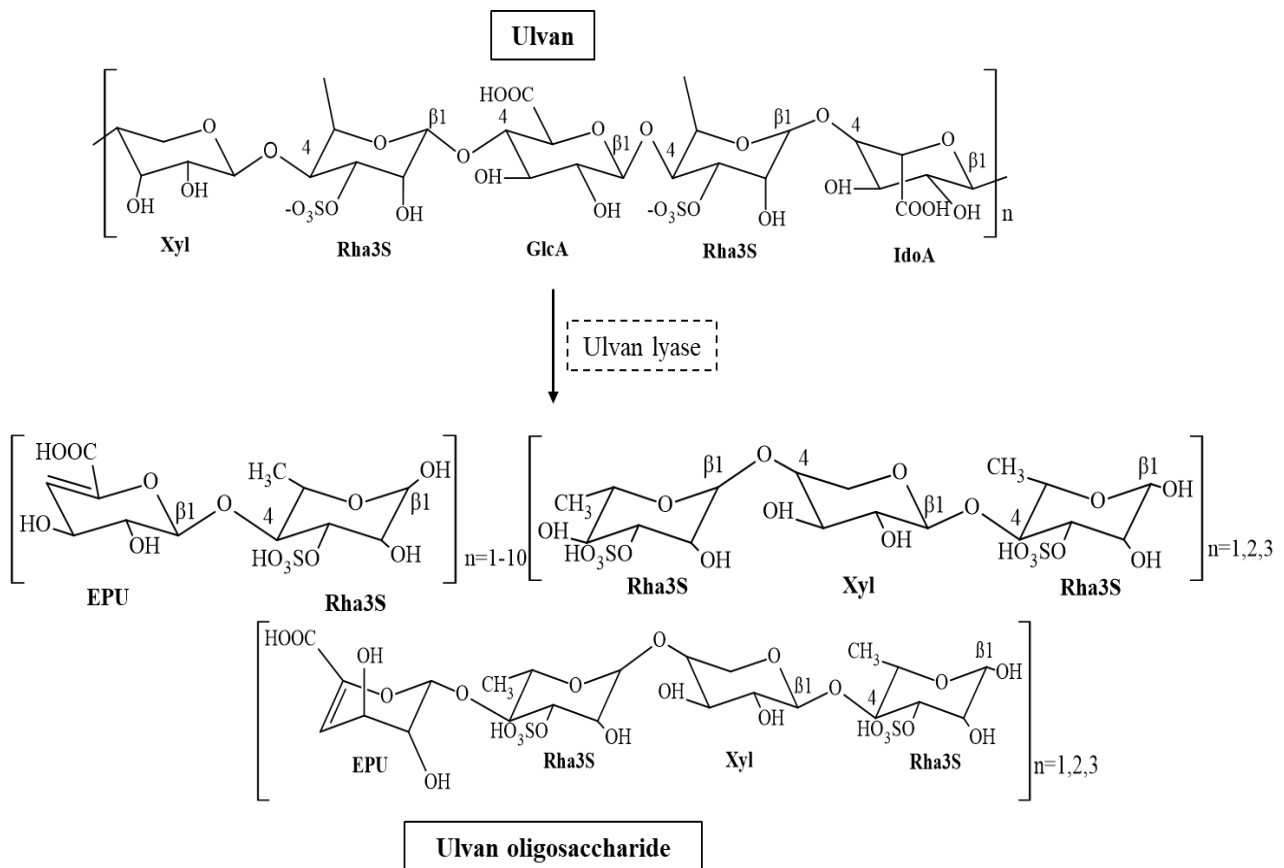




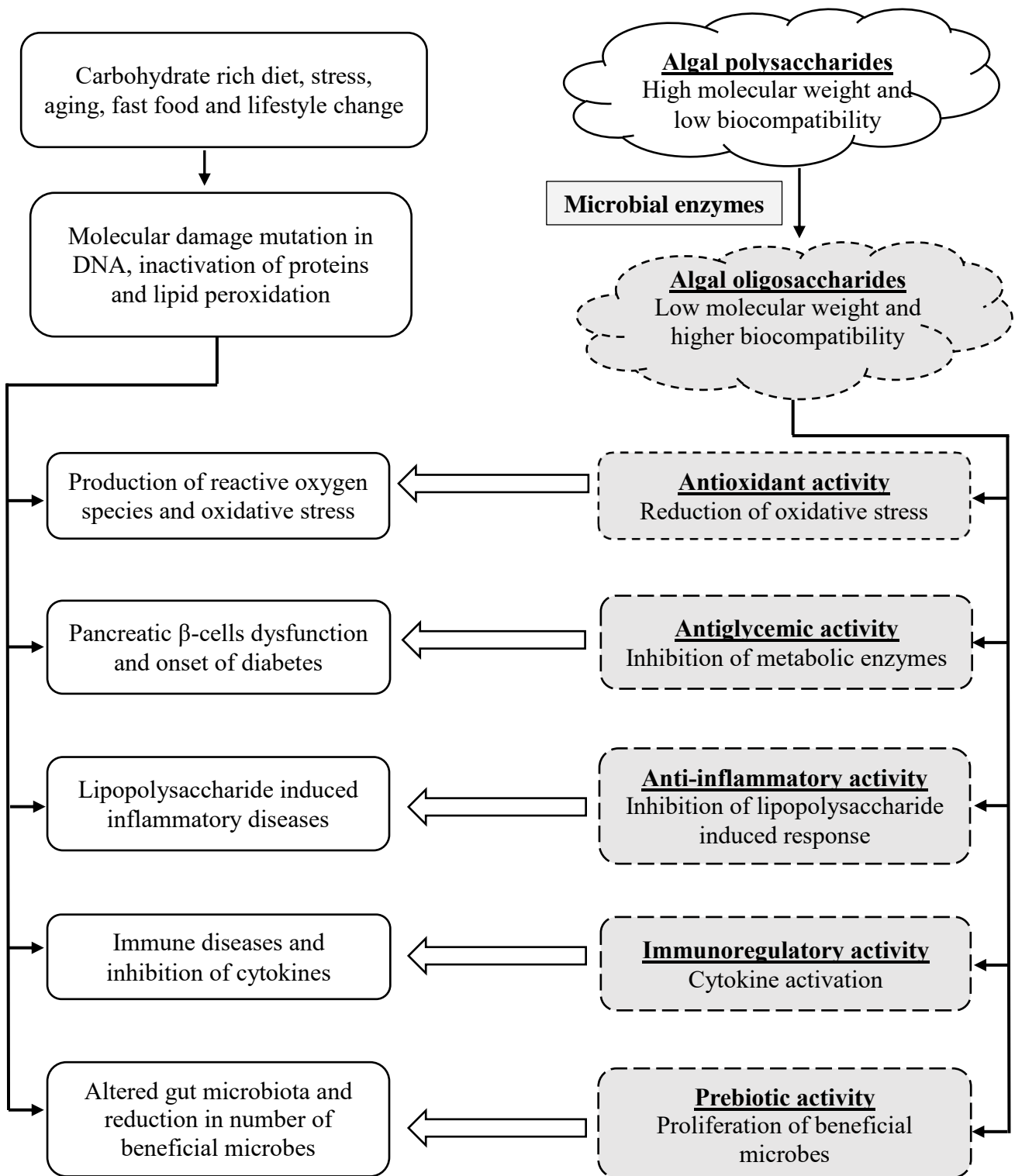
**Fig. 5** Structure of fucoidan and its enzymatic hydrolysis to produce fucoidan oligosaccharide



**Fig. 6** Structure of laminarin and its enzymatic hydrolysis to produce  $\beta$ 1-3 or  $\beta$ 1-6 laminarin oligosaccharide



**Fig. 7** Structure of ulvan and its enzymatic hydrolysis to produce ulvan oligosaccharide (Xyl= xylose, Rha3S=  $\alpha$ -L-rhamnose 3-sulphate, GlcA= glucuronic acid, IdoA=iduronic acid, EPU=4-deoxy-L-threo-hex-4-enopyranosiduronic acid)



**Fig. 8** Schematic representation of bioactive potential of algal oligosaccharides in nutraceutical application