Bacterial carrageenases: an overview of production and biotechnological applications

Prakram Singh Chauhan · Arunika Saxena

Received: 9 May 2016 / Accepted: 10 June 2016
© The Author(s) 2016. This article is published with open access at Springerlink.com

Abstract Carrageenan, one of the phycocolloids is a sulfated galactan made up of linear chains of galactose and 3,6-anhydrogalactose with alternating α-(1→3) and β-(1→4) linkages and further classified based on the number and the position of sulfated ester(s); κ-, τ- and λ-carrageenan. Enzymes which degrade carrageenans are called κ-, τ-, and λ-carrageenases. They all are endohydrolases that cleave the internal β-(1→4) linkages of carrageenans yielding products of the oligo-carrageenans. These enzymes are produced only by bacteria specifically gram negative bacteria. Majority of the marine bacteria produce these enzymes extracellularly and their activity is in wide range of temperature. They have found potential applications in biomedical field, bioethanol production, textile industry, as a detergent additive and for isolation of protoplast of algae etc. A comprehensive information shall be helpful for the effective understanding and application of these enzymes. In this review exhaustive information of bacterial carrageenases reported till date has been done. All the aspects like sources, production conditions, characterization, cloning and biotechnological applications are summarized.

Keywords Carrageenan · Bacterial carrageenase · Glycoside hydrolase · Biotechnological applications

Introduction

Hydrocolloids can be defined as substances that interact with water to form colloid systems. Hydrocolloid polysaccharides (agar, alginates and carrageenans) have significant importance, both technologically and economically, since they are used in the various biotechnological industries due to their distinct physico-chemical properties (Knudsen et al. 2015; Zhu and Ning 2016; Xiao et al. 2016).

Carrageenans are commercially important hydrophilic colloids (water soluble gums) which occur as matrix material in numerous species of red seaweeds [comprise up to 50% dry weight (Rhodophyta)] wherein they serve a structural function analogous to that of cellulose in land plants. They are a group of biomolecules composed of linear polysaccharide chains with sulphate half-esters attached to the sugar unit. It exists in different forms depending on the number of sulphate substituents per disaccharide unit: one in κ-carrageenan, two in τ-carrageenan and three in λ-carrageenan. These properties allow carrageenans to dissolve in water, form highly viscous solutions, and remain stable over a wide pH range (Ruitter and Rudolph 1997; Yao et al. 2014; Liu et al. 2016).

Microbial enzymes which hydrolyze hydrocolloids have drawn considerable interest recently because enzymatic degraded products of carrageenan is still in infancy compared to that of other hydrocolloids such as agar, alginate etc. Enzymes which degrade carrageenans are called κ-, τ-, and λ-carrageenases. They all are endohydrolases that cleave the internal β-(1→4) linkages of carrageenans yielding products of the oligo-carrageenans. Oligo-carrageenan produced by the action of microbial enzymes can be more advantageous than produced by acid hydrolysis because enzymes are highly specific to their substrates and...
they generate oligo-derivatives are uniform in molecular weights (Yao et al. 2014).

The oligosaccharides derived from carrageenan have been shown to exhibit antitumor, anti viral activity which indicated that oligo-carrageenan could possess significant potential for biomedical and physiological applications (Li et al. 2014a, b). In addition to this carrageenase have a various important application such as bioethanol production, textile industry, as a detergent additive and for isolation of protoplast of algae etc.

Due to their versatile industrial applicability, interest has greatly increased in carrageenases during the past decade (Necas and Bartosikova 2013). A number of carrageenases from various organisms are being reported, cloned and studied at molecular level. In this review, information on carrageenases from most of the microorganisms currently reported have been compiled in terms of the production conditions, enzyme properties, gene cloning and potential industrial applications.

**Carrageenan occurrence and structure**

Majority of the seaweeds that produce carrageenan as their main cell-wall material belong to the red algae, or Rhodophyta. The carrageenans are extracted from the carrageenophyte red seaweed genera *Kappaphycus*, *Gigartina*, *Eucheuma*, *Chondrus*, and *Hypnea*, in which the carrageenans comprise up to 50 % of the dry weight (Knudsen et al. 2015). Κ-Carrageenan is mostly extracted from *Kappaphycus alvarezi*, known in the trade as *Eucheuma cottonii*, while 1-carrageenan is predominantly produced from *Eucheuma denticulatum*, also known as *Eucheuma spinosum*. λ-Carrageenan is obtained from seaweeds within the *Gigartina* and *Chondrus* genera, which as sporophytic plants produce λ-carrageenan while they make a κ/λ-hybrid as gametophytic plants (Van De Velde et al. 2001). These κ/λ-hybrid type carrageenans, also known as “kappa-2” or “weak-gelling kappa carrageenans”, consist of mixed polysaccharide chains containing both κ- and λ-units and range from almost pure 1-carrageenan to almost pure κ-carrageenan (Table 1).

The seaweed is dried quickly to prevent degradation, and is then baled for shipment to processing facilities. The seaweed is repeatedly washed to remove gross impurities such as sand, salt, and marine life, and then undergoes a hot alkali extraction process, releasing the CG from the cell. Once CG is in a hot solution, it undergoes clarification and then is converted to powder (Rowe et al. 2009). Meanwhile, extraction parameters (such as temperature, pH, duration) and alkaline pre-treatment duration have important effects on the chemical structure and gelling properties (Hilliou et al. 2006).

Carrageenan is a high molecular mass material (>100 kDa) with a high degree of polydispersity. Carrageenans are hydrophilic sulfated linear galactans that mainly consist of D-galactose and 3,6-Anhydro-D-galactose (3,6-AG) units bound together with alternating α-1,3 and β-1,4 linkages. The 3-linked units occur as the 2-and 4-sulfate, or unsulfated, while the 4-linked units occur as the 2-sulfate, the 2,6-disulfate, the 3,6-anhydride, and the 3,6-anhydride-2-sulfate. Sulfation at C3 apparently never occurs. Except the galactose and sulfate, other carbohydrate residues (for example xylose, glucose and uronic acids) and substituents (for example methyl ethers and pyruvate groups) are present in carrageenans (Michel et al. 1997; Knudsen et al. 2015) (Fig. 1).

This base structure is consistent in the three main commercially used carrageenans, κ-, λ-, and ι-carrageenan. κ-Carrageenan has one sulfate ester, while 1- and λ-carrageenan contain two and three sulfates per dimer, respectively (Fig. 1). The number and position of ester sulfate groups as well as the content of 3,6-AG influence the properties of different carrageenans. Higher levels of ester sulfate mean lower solubility temperature and lower gel strength. Kappa type carrageenan has an ester sulfate content of about 25–30 % and a 3,6-AG content of about 28–35 %. Iota type carrageenan has an ester sulfate content of about 28–30 % and a 3,6-AG content of about 25–30 %. Lambda type carrageenan has an ester sulfate content of about 32–39 % and no content of 3,6-AG. Considering that each natural carrageenan is a complex galactose-based polysaccharide that has different quantities of sulphate esters at different positions and with different distributions, the term disaccharide repeating unit refers to the idealized structure (Campo et al. 2009).

**Microbial carrageenanolytic system**

**Enzymatic hydrolysis of carrageenan**

Due to the complex chemical structure of carrageenan and specific cleavage of linkages in the backbones of carrageenan polymers, specific enzymes are required for the respective structures without the risk of modification of the native structure. Enzymes, which degrade carrageenans, are called κ-carrageenases (EC 3.2.1.83), 1-carrageenases (EC 3.2.1.162), and λ-carrageenases (EC 3.2.1.167). They all are endohydrolases that cleave the internal β-(1-4) linkages of carrageenans yielding oligogalactans of either neocarboabiose or neoagarobiose series (Fig. 2) (Barbeyron et al. 2000). It was reported that κ-carrageenases and 1-carrageenases degrades carrageenan by hydrolyzing the β-(1-4) [breaking internal linkages rather than hydrolyzing units from the ends] linkages to a series of homologous,
even-numbered oligosaccharides. Although, both enzymes are processive, in which the enzyme does not dissociate from the substrate and instead slides along the polysaccharide, cleaving all possible bonds (DP4s and DP2s). In contrast, \( \kappa \)-carrageenase cleaves internal linkages more randomly manner, resulting in higher amounts of DP6s (and possible other higher DPs as products) compared to the products from \( \iota \) - and \( \iota \)-carrageenase hydrolysis. Since these hydrolases display strict substrate specificity, they obviously recognize the sulfation pattern on the digalactose repeating unit. Digestion by carrageenases generates oligogalactans of various sizes, most likely carbohydrates with a degree of polymerization (DP) of 2, 4, and 6. The reason for the production of different DPs is a result of the heterogenous carrageenan structure and the mechanisms that the enzymes follow. The alternating \( \alpha \)-1,3 and \( \beta \)-1,4 linkages in the carrageenans results in successive \( \beta \)-1,4 linkages to be in opposite orientations and hence only every second disaccharide is in the right position for cleavage (Michel et al. 2001a; Guibet et al. 2007; Lemoine

| Polysaccharide | Sources | Backbone made up of alternating units | No. of sulfate ester | Properties | Gel formation | Viscosity |
|----------------|---------|---------------------------------------|---------------------|------------|--------------|----------|
| \( \kappa \)-Carrageenan | *Kappaphycus alvarezii* | 3,6-Anhydro-D-galactose, D-Galactose 4-sulfate | One | Hot solution | KCl promote gelling | Low |
| \( \iota \)-Carrageenan | *Eucheuma denticulatum* | 3,6-Anhydro-D-galactose-2-sulfate, D-Galactose 4-sulfate | Two | Soluble in cold or hot aqueous solution | Ca\(^{2+}\) promote gelling | High |
| \( \lambda \)-Carrageenan | *Gigartina* and *Chondrus* genera | D-galactose-2,6-disulfate, D-galactose, D-galactose-2-sulfate | Three | Soluble in cold or hot aqueous solution | Non-gelling | High/medium |

**Fig. 1** Structure of kappa, iota and lambda carrageenans

**Fig. 2** Schematic representation of two disaccharide-repeating units of \( \kappa \)-carrageenan having reducing as well as non reducing ends. Carrageenan cleavage site are indicated by *arrow* (Michel et al. 2001a)
et al. 2009). A chain length of four sugar residues is required for the binding of carrageenases to ensure hydrolysis. The substrate binding surface can be split into different subsites. Subsites are numbered from \(-n\) to \(+n\) (\(n\) being an integer) from nonreducing to reducing ends of the mannan substrate, respectively (Davies et al. 1997). Cleavage of the glycosidic bond occurs between subsite \(+1\) and \(-1\) (Fig. 2). Oligo-carrageenan produced by the action of microbial enzymes can be more advantageous than produced by acid hydrolysis because enzymes are highly specific to their substrates and they generate oligo-derivatives are uniform in molecular weights (Yao et al. 2014).

**Reaction mechanism of carrageenan degrading enzymes**

Different carrageenases exhibit different mechanism for the hydrolysis polymer. \(\kappa\)-Carrageenases hydrolyse their substrates by a retaining mechanism which occurs via double-displacement reaction (Fig. 3) (Chauhan et al. 2012, 2014a; Chauhan and Gupta 2016). The key amino acids involved in the retaining mechanism reaction are two catalytic carboxylate residues that exist at opposite sides of sugar plane. The enzymes in this mechanism works in tandem involving two steps i.e. glycosylation and deglycosylation (Chauhan et al. 2015). In the first step of double displacement mechanism, it facilitates the departure of leaving group by donating proton to the glycosyl oxygen atom. Simultaneously a second carboxylate moiety facilitates the nucleophilic attack on the anomeric carbon to form glycosyl-enzyme intermediate (Fig. 3). In the second step, the deprotonated acid/base functions as a general base which activates nucleophile. This activated nucleophile then cleaves the glycosyl enzyme complex (Fig. 3). In spite of wide array of GH, hydrolysis of glycosidic bond is generally catalyzed by either glutamate (Glu) or Aspartate (Asp).

\(\iota\)-Carrageenases hydrolyse their substrate by inverting mechanism (Fig. 4). In this, there is an inversion of the anomeric configuration of the starting material. Here, the two crucial carboxylic residues act as general acid and base catalysts and these groups are *circa* 10.5 \(\AA\) apart from each other. It this specific case, this distance is larger than in retaining GHs because the substrate and the water...
molecule must be present simultaneously in the active site of the enzyme during the hydrolytic process. Figure 4 shows the proposed mechanism of action for inverting GHs, which occurs via a single-displacement type of mechanism. In this case, one of the carboxylate residues protonates the scissile glycosidic oxygen atom while the other coordinates the nucleophile (i.e., the water molecule) to assist its deprotonation and in this way completes the hydrolysis reaction (Michel et al. 2001b, 2003).

**Assay methods for carrageenases**

Numerous screening methods exist for detecting carrageenanolytic activity in microorganisms. A solid medium giving fast assays is useful for the direct measurement and isolation of carrageenan degrading organisms from natural substrates. Common screening techniques used for the detection of carrageenan degrading enzymes involve plate assays where the individual polymer, is added into the basal growth medium. The formation of analogous hydrolyses is indicated by the plate depression-forming activity or liquefaction or clearing of the opaque medium as the substrate is hydrolyzed and in this way completes the basal growth medium. The formation of analogous hydrolyses is indicated by the plate depression-forming activity or liquefaction or clearing of the opaque medium as the substrate is hydrolyzed and in this way completes the hydrolysis reaction (Michel et al. 2001b, 2003).

In liquid assay method carrageenase activity is determined using carrageenan as a substrate. In viscometric method, flow time of the digest (enzyme + substrate) was determined at once and also at regular intervals after putting the viscometer in water bath at particular temperature. One viscometric unit of enzyme activity was defined as the amount of enzyme which would reduce the specific viscosity of the substrate by 50 % (Mclean and Williamson 1979).

In spectrophotometric method reducing sugar released from the substrate by the action of enzyme was determined by Somogyi–Nelson procedure (Somogyi 1952). Here addition of alkaline copper sulfate was added which helpful to stop the reaction between enzyme and substrate. In Dinitrosalicylic acid (DNSA) method reducing sugar released by the action of enzyme on substrate was quantified with Dinitrosalicylic acid (DNSA) reagent (Miller 1959) by taking the absorbance at 560 nm whereas in Kidby method reaction mix were incubate with Kidby solution (1 % Na₂CO₃ and 0.03 % potassium hexacyanoferrate III). Color development was achieved by placing tubes in a water bath 100 °C. Finally, the spectral absorbance of samples at 420 nm was measured (Kidby and Davidson 1973). One unit of κ-carrageenase activity was defined as the amount of enzyme needed to release 1 μmol reducing sugars (D-galactose equivalent) per min.

In neocuproine method, assay mixture was incubated for particular time and temperature followed by the addition of 1 ml of alkaline copper reagent (4 % Na₂CO₃, 1.6 % calipers and recorded. Width is converted to enzyme activity using a dilution of the enzyme and plotting zone width with respect to the log of enzyme concentration (Kang and Kim 2015).

Fig. 4 Inverting mechanism for the degradation of carrageenan by λ-carrageenases via single displacement reaction (Bras et al. 2012)
Glycine and 0.045 % CuSO₄) to stop the reaction. After that 1 ml of 5 mM Neocuprine–HCl reagent was added to the tube and was kept in a boiling water bath. It was necessary to add ethanol (50 % v/v) to the reaction mixture before measuring the absorbance. One unit of the enzyme activity was defined as the amount which liberated 1 mmol galactose equivalent per minute under the assay conditions (Dygert et al. 1965).

**Carrageenases classification and structural characteristics**

Based on amino acid sequences, these enzymes belong to distinct glycoside hydrolase (GH) families. Although the various carrageenans are chemically rather close, but the respective GHs that degrade different types of carrageenans do not belong to the same family. κ-Carrageenases belong to GH16 (family 16 of the GHs) (Barbeyron et al. 1994, 1998), a polyspecific family which encompasses at least eight different enzymatic activities, including β-agarases ([http://www.cazy.org/) (Coutinho and Henrissat 1999; Cantarel et al. 2009). Phylogenetic analysis investigations demonstrated that family GH16 enzymes have evolved from a common ancestor and that κ-carrageenases have likely emerged from the β-agarase branch (Jam et al. 2005) and they belong to clan GH-B. κ-Carrageenases hydrolyse β-(1 → 4) glycosidic linkages with retention of the anomeric configuration (Potin et al. 1995). τ-Carrageenases define the unrelated family GH82 (Michel et al. 2006; Rebuffet et al. 2010), while λ-carrageenases constitute a new GH family, although it has not been yet classified in the carbohydrate-active enzymes (CAZy) database (Barbeyron et al. 1998). The κ-carrageenase belongs to this family adopts a typical β-jellyroll fold (polypeptide chain is wrapped around a barrel core like a jelly roll) whereas τ-carrageenases carry β-helix in 3D-structure (Guibet et al. 2007).

Carrageenases have been poorly characterized from a structural point of view. Till now three dimensional structure of only two carrageenase i.e., κ-carrageenase (CgkA) from *Pseudoalteromonas carrageenovora* and τ-carrageenase (CgiA_Af) from *Alteromonas fortis* has been solved. Both of the enzymes display a tunnel-shaped active site suggesting a processive mode of action in which the enzyme does not dissociate from the substrate and instead slides along the polysaccharide, cleaving all possible bonds. The κ-carrageenase chain is composed of alternating neutral and negatively charged sugars (DA and G4S, respectively). To accommodate the dual nature of its substrate, κ-carrageenase features in its active site both conserved aromatic and basic residues which are predicted to interact with DA and G4S moieties, respectively (Michel et al. 2001b) whereas carrageenan which consists of only negatively charged sugars (DA2S and G4S) is recognized by κ- and τ-carrageenase essentially through ionic interactions between its sulfate groups and several conserved arginine residues of the protein (Michel et al. 2003). Studies on κ- and τ-carrageenases have provided some insight into sulfated polysaccharide–protein interactions; but the chemical complexity of sulfated polysaccharides is a patent obstacle to such analyses and researcher looking for better structural characterization (Michel et al. 2001a).

However, they all hydrolyze carrageenan substrates, but these carrageenases do not share significant sequence homology. Although they share some common binding site for ions which helpful in stabilizing the enzyme. Here the three dimensional structure of τ-carrageenase (CgiA_Af) from *Alteromonas fortis* is shown in Fig. 5. The enzyme folds into a right-handed parallel β-helix of 10 complete turns with flanked by two domains (A and B) in the C-terminal region. Glu245, Asp247, or Glu310, in the cleft of the enzyme, are proposed as candidate catalytic residues. The protein contains one sodium and one chloride binding site and three calcium binding sites shown to be involved in stabilizing the enzyme structure. The crystallographic structure of this enzyme was also solved in the presence of substrate oligocarrageenans, where a tetrasaccharide and a disaccharide have been located in subsites +4 to +1 and −3 to −4, respectively (Michel et al. 2001b). The *A. fortis* iotase hydrolyzes the β-(1–4) bond by an inverting mechanism and produces neo-τ-carratetraose and neo-τ-carractetraose as end products. Like the τ-carrageenases, κ-
carrageenases are endohydrolases, breaking internal linkages rather than hydrolyzing units from the ends. Also, both enzymes are processive, hydrolyzing several units in succession. In contrast, λ-carrageenase cleaves internal linkages randomly (Michel et al. 2003; Guibet et al. 2007; Knudsen et al. 2015).

**Sources of carrageenases**

Carrageenases have been produced from marine bacteria that belong to two distantly related lineages, *Proteobacteria* and *Bacteroidetes*, although most of the isolates belong to the former group. Carrageenases appears to carry out different functions, depending on the producing organism. Carrageenases from bacteria are often employed in the degradation of polysaccharides particularly carrageenan from marine rhodophytes, are major raw materials for a number of industries worldwide. In this regard, studies on important carrageenan degraders reported in the recent years are listed in Table 2. Among bacteria, degradation is mostly confined to Gram negative, mainly various *Pseu- doalteromonas*, *Cellulophaga*, *Pseudomonas*, *Cytophaga*, *Tamlana*, *Vibrio*, *Catenovulum*, *Microbulbifer*, *Zobellia*, *Alderson*doalteromonas, *Cellulophaga*, *Pseudomonas*, *Cytophaga*, *Tamlana*, *Vibrio*, *Catenovulum*, *Microbulbifer*, *Zobellia*, *Alteromonas* (Shangyong et al. 2013; Yao et al. 2013; Ziayoddin et al. 2014; Mou et al. 2004; Feixue et al. 2010; Li et al. 2015; Hatada et al. 2011; Liu et al. 2013; Michel et al. 2001b; Zhu and Ning 2016). However, some Gram positive bacteria like *Bacillus* sp. and *Cellulosimicrobium* have also been reported to produce carrageenase (Kang and Kim 2015; Youssef et al. 2012). Till date no fungal species is identified which are able to produce carrageenase which is going to be very hot area in near future for exploring the microbial diversity.

**Production conditions and properties**

A number of bacterial species are capable of degrading carrageenan with the help of carrageenases enzyme which are mainly inducible and extracellular, however, some intracellular report are also available (Potin et al. 1995; Dyrset et al. 1997; Liu et al. 2011, 2014; Beltagy et al. 2012). It is further proposed that major and minor repeat units (dimeric units) produced from the natural polysaccharide by the action of carrageenase can act as inducer for λ-, κ-, and possibly other carrageenases (Bellion et al. 1982). Other substrates like, nutrient broth and red sea weed powder have also been practiced for the same purpose, since they offer significant benefit due to their cheaper cost and abundant availability (Li et al. 2014a, b; Kang and Kim 2015). Interestingly, it was found that various simple sugars (fructose, glucose, galactose, lactose, sucrose, mannose, maltose) did not induce carrageenase production in medium and they shows catabolite repression effect. Infact co-supplementation of simple sugars with carrageenan found to decrease the production slightly. Although the growth of bacteria was increased in the medium co-supplemented with glucose, fructose or maltose. This may be due to rapid utilization of these simple sugars by the bacteria resulting in an increase in cell mass by vigorous growth (Ziayoddin et al. 2014; George et al. 2014a, b; Sondhi et al. 2015).

The production of carrageenases are greatly influenced by nutritional and physicochemical factors, such as time, optimum temperature, optimum pH, carbon and nitrogen sources, inorganic salts, agitation and dissolved oxygen concentration (Ziayoddin et al. 2012). Various microbes require different incubation times for maximum carrageenases production. It ranges from 12 h in *Bacterium 1* (Greer and Yaphe 1984) to 14 days in *Bacillus* sp. SYR4. (Kang and Kim 2015). Temperature and pH, giving maximum yield, correspond with the optimal conditions for the growth of the organism. Similar to other metabolic enzymes, optimum carrageenase production in bacteria is in the neutral temperature (Zhou et al. 2008; Feixue et al. 2010; Ma et al. 2013) with the exception of *Bacillus* sp. Lc50-1 which require 55 °C for production (Li et al. 2014a, b).

Carrageenases have been produced by submerged fermentation in most of the studies (Sarwar et al. 1983a, b, 1987; Youssef et al. 2012; Ziayoddin et al. 2014). However, few attempts have been made for the production of mannosidases by solid state fermentation (SSF). Ziayoddin et al. (2012) have used agro wastes for the enhanced production of carrageenases by SSF. Among them wheat bran induced the production of this enzyme more with enzyme activity of 7.44 Ug⁻¹. The production of carrageenase has been increased many fold by optimization of the parameters using response surface methodology such as in case of *Cellulosimicrobium cellulans* κ-carrageenase activity to about 2.3 times higher than that obtained from the basal medium (Youssef et al. 2012) whereas in *Pseu- domonas elongate*, 32-fold increase in κ-carrageenase production was achieved as compared to initial by statistical optimization method (Kambhaty et al. 2007a). Ostgaard et al. (1993) successfully grow *Pseudomonas carrageenovora* in stirred-tank fermentor (14 L) for scale-up. Interestingly improved production (630 Uml⁻¹) of the enzyme carrageenase was obtained by starting bacterial growth on a cheap carbon source (lactose) and adding small amounts of carrageenan (0.15 %) to initiate enzyme production. Similar to this Dyrset et al. (1997) showed the role of casamino acid (3.5 gl⁻¹) in fed batch fermentation of *Pseudomonas carrageenovora* NUMB 302 and significantly achieved increase in enzyme activity by 2.6 times (84,000 Uml⁻¹).
| S. no. | Name of organism | Carbon source/fermentation conditions | Temp. optima (°C) of activity | Temp. stability | pH optima of activity | pH stability | Molecular weight of protein (KDa) | References |
|-------|------------------|--------------------------------------|-------------------------------|----------------|----------------------|-------------|-------------------------------|------------|
| 1     | ALAB-001         | YEa and tryptone in seawater/30 °C/8 days | NRa                        | NRa            | NRa                  | NRa         | 37 kDa                       | Tayco et al. (2013) |
| 2     | Bacterium 1      | WYb medium having Iota-carrageenan/22 °C/150 rpm/12 h | 40                         | NRa            | 8.0                  | NRa         | NRa                          | Greer and Yaphe (1984) |
| 3     | Bacterium 1      | MMc having carrageenan/22 °C/48 h/pH 7.5 | 30                         | NRa            | 7.2                  | NRa         | NRa                          | Bellion et al. (1982) |
| 4     | Bacillus sp. Lc50-1 | NBd/55 °C | 75                        | 50 %/75 °C/45 min | 8.0             | 70 %/pH 6–9/15 min/75 °C | 37 kDa | Li et al. (2014b) |
| 5     | Bacillus sp. SYR4 | RSPM/37 °C/180 rpm/14 days/pH 7.4 | 30                        | NRa            | 7.5                  | NRa         | NRa                          | Kang and Kim (2015) |
| 6     | Catenovulum sp. LP | LM/30 °C/48 h/pH 7.5 | 35                        | 50 %/35 °C/36 h | 6.0             | >50 %/pH 5.0–9.0/35 °C/1 h | 75.5 kDa | Li et al. (2015) |
| 7     | Cellulosimicrobium cellulans | Kappa-carrageenan/37 °C/250 rpm/24 h/pH 7.5 | 30                        | NRa            | 6.0                  | NRa         | NRa                          | Beltagy et al. (2012) |
| 8     | Cellulosimicrobium cellulans | ZMP/37 °C/250 rpm/24 h/pH 7.5 | 37                        | NRa            | 7.5                  | NRa         | NRa                          | Youssef et al. (2012) |
| 9     | Cellulophaga lytica strain N5-2 | Kappa-carrageenan/35 °C/20 h | 35                        | >85 %/40 °C/pH 7.0/150 min | 7.0             | >80 %/pH 7.0/360 min/35 °C | 40.8 | Yao et al. (2013) |
| 10    | Cellulophaga sp. QY3 | Iota-carrageenan/25 °C/150 rpm/20 h/pH 7.0 | 50                        | >80 %/50 °C/pH 7.0/60 min | 7.0             | >70 %/pH 5.0–10.6/720 min/4 °C | 48.3 kDa | Ma et al. (2013) |
| 11    | Cytophaga lk-C783 | ZMP having carrageenan/25 °C/150 rpm/72 h | 25 °C                     | 50 %/50 °C/pH 7.0/10 min | 7.6             | NRa         | 100 kDa                       | Sarwar et al. (1983a, b, 1987) |
| 12    | Cytophaga MCA-2 | ZMP having carrageenan/32 °C/150 rpm/36 h/pH 7.5 | 28                        | 0 %/55 °C/30 min | 7.2             | 32 %/pH 10.83/24 h | 30 kDa | Mou et al. (2004) |
| 13    | Dsij strain     | ZMP/22 °C/250 rpm/24–40 h | 40                        | 100 %/30 °C/pH 7.2/210 min | 7.2             | 100 %/7.0/60 min/40 °C | 40 kDa | Potin et al. (1991) |
| 14    | Marine bacteria | Carrageenan/20 °C–25 °C/100 rpm/72–96 h | 40                        | NRa            | 7.5                  | NRa         | NRa                          | Yappe and Baxter (1955) |
| 15    | Pseudoalteromonas sp. QY203 | Kappa-carrageenan/25 °C/150 rpm/48 h | 45                        | 70 %/35 °C/pH 7.2/48 h | 7.2             | >70 %/6.5–9.06 h/4 °C | 34 | Shangyong et al. (2013) |
| 16    | Pseudomonas aeruginosa ZSL-2 | Kappa-carrageenan + MMSM/37 °C/180 rpm/24 h | 28                        | NRa            | 8.0                  | NRa         | NRa                          | Ziyoddin et al. (2014) |
| 17    | Pseudoalteromonas strain CL19 | Kappa-carrageenan/20 °C/72 h | 35                        | NRa            | 7.0                  | NRa         | 100 kDa                       | Ohta and Hatada (2006) |
Table 2 continued

| S. no. | Name of organism | Carbon source/fermentation conditions | Temp. optima (°C) of activity | Temp. stability | pH optima of activity | pH stability | Molecular weight of protein (KDa) | References |
|--------|------------------|--------------------------------------|-----------------------------|----------------|----------------------|-------------|----------------------------------|------------|
| 18     | *Pseudomonas carrageenovora* HLX | Carrageenan/25 °C/60-66 h/pH 7.02 | 30 | 100 %/40 °C/ 210 min | 7.5 | NR<sup>a</sup> | NR<sup>a</sup> | Weigl and Yaphe (1966) |
| 19     | *Pseudoalteromonas carrageenovora* IFO 12985 | BM<sup>1</sup> having kappa or Iota-carrageenan/27 °C/48 h | NR<sup>a</sup> | NR<sup>a</sup> | NR<sup>a</sup> | NR<sup>a</sup> | 1.4 kDa | Henares et al. 2010) |
| 20     | *Pseudomonas elongata* MTCC 5168 | LM<sup>1</sup> having carrageenan/37 °C/180 rpm/24–32 h | 40 | >90 %/−20 °C/ 25 days | 5.67.7 | NR<sup>a</sup> | 128 kDa | Kambhaty et al. 2007b) |
| 21     | *Pseudoalteromonas porphyrae* LL-1 | 2216E medium having carrageenan/28 °C/160 rpm/28 h/pH 7.5 | 55 | >95 %/30 °C/1 h | 8.0 | 100 %/pH 8.0/ 6 h/4 °C | 40 kDa | Liu et al. 2011) |
| 22     | *Pseudoalteromonas* WZUC10 | BM/25 °C/200 rpm/48 h/pH 7.5 | 30 | 90 %/40 °C/pH 7.1/ 2 h | 7.5 | NR<sup>a</sup> | 45 kDa | Zhou et al. 2008) |
| 23     | *Pseudoalteromonas* carageenovora ATCC-43555 | Y2MM<sup>4</sup> having carrageenan/ 20 °C/48 h/250 rpm | 30 | NR<sup>a</sup> | 7.5 | NR<sup>a</sup> | 97 kDa | Guibet et al. 2007) |
| 24     | *Pseudomonas carrageenovora* NCMB no. 302 | LM<sup>1</sup> having carrageenan/25 °C/100 rpm/60–80 h | 40 | NR<sup>a</sup> | 8.0 | NR<sup>a</sup> | 35 | Mclean and Williamson 1979; Ostgaard et al., (1993); Dyset et al. (1997) |
| 25     | *Pseudomonas elongata* | IM<sup>4</sup> having carrageenan/37 °C/180 rpm/32 h/pH 7.0 | 37 | NR<sup>a</sup> | 7.5 | NR<sup>a</sup> | NR<sup>a</sup> | Kambhaty et al. (2007a) |
| 26     | *Pseudoalteromonas* sp. AJ5-13 | FMB<sup>h</sup> having carrageenan/ 28 °C/150 rpm/36 h/pH 7.6 | 55 | 100 %/28 °C/ 30 min | 8.0 | >50 %/pH 7.2–8.6/ 12 h/4 °C | 35 kDa | Ma et al. (2010) |
| 27     | *Pseudomonas aeruginosa* ZSL-2 | MMS<sup>h</sup> having carrageenan/ 30 °C/170 rpm/24 h | 40 | NR<sup>a</sup> | 8.0 | NR<sup>a</sup> | NR<sup>a</sup> | Ziayoddin et al. (2012) |
| 28     | *Pseudoalteromonas* sp. ASY5 | NR<sup>a</sup> | 60 | 100 %/50 °C/7.5 | 7.5 | 100 %/pH 7–9 | 30 kDa | Xu et al. (2015) |
| 29     | *Tamlana* sp. HC4 | FMB<sup>h</sup> having carrageenan/ 28 °C/150 rpm/pH 7.5 | 30 | >91 %≤<45 °C/2 h | 8.0 | 100 %/pH 7.2–8.6/ 4/11 °C | 66.4 kDa | Feixue et al. (2010) |
| 30     | *Vibrio* sp. CA-1004 | MM<sup>h</sup> having carrageenan/25 °C/100 rpm/5 days | 40 | >90 %/40 °C/ 10 min | 8.0 | >80 %/pH 5–11/ 4 °C/24 h | 35 kDa | Araki et al. (1999) |
| S. no. | Name of organism | Carbon source/fermentation conditions | Temp. optima (°C) of activity | Temp. stability | pH optima of activity | pH stability | Molecular weight of protein (KDa) | References |
|-------|------------------|--------------------------------------|------------------------------|----------------|----------------------|-------------|----------------------------------|------------|
| 31    | *Vibrio* sp. NJ-2 | MM\(^c\) having carrageenan/30 °C/ 150 rpm/48 h | 40                           | 90 %/40 °C/30 min | 8.0                  | >70 %/pH 6–10/24 h/4 °C | 33 kDa           | Zhu and Ning (2016)          |

\(^a\) Yeast extract  
\(^b\) Weigl and Yaphe medium  
\(^c\) Minimal medium  
\(^d\) Nutrient broth  
\(^e\) Red seed powder medium  
\(^f\) Liquid medium  
\(^g\) Zobell medium  
\(^h\) Minimal mineral salts medium  
\(^i\) Bellion's medium  
\(^j\) Basal medium  
\(^k\) Y-2 modified medium  
\(^l\) Improved medium  
\(^m\) Fermentation medium B  
\(^n\) Not reported
A comprehensive comparison of the characteristics of bacterial carrageenases (Table 2) it was observed that they can operate in a wide temperature range (25–75 °C) (Sarwar et al. 1983a, b; Mou et al. 2004; Li et al. 2014a, b). Most of them have temperature optima from 30 to 40 °C. Optimum pH for their activity varies from 5.6 to 7.5 (Khamphatay et al. 2007b; Guibet et al. 2007; Kang and Kim 2015) with carrageenases from genus of Pseudomonas having their optimal activity in mild alkaline conditions pH 8.0 (Ostgaard et al. 1993; Ziayoddin et al. 2012, 2014). Carrageenase from Bacillus sp. Lc50-1 has an optimal activity at 75 °C and a pH of 8.0 is useful for applications where high concentration of carrageenan is required because at high temperature it is more soluble as well as more oligosaccharides production (Li et al. 2014b).

Various metal ions and reagents affect carrageenase activities differently in bacteria. A common trend has been recognized, in most of the cases enzyme activity was negatively affected by heavy metals like Hg2+, Co2+, Zn2+, Cu2+, Ag+, Pb2+ which indicated that carrageenase activity is cation independent and they were able to alter the enzyme conformation (Araki et al. 1999; Ma et al. 2010; Shangyong et al. 2013; Li et al. 2015). The inhibition by mercuric ions may indicate the importance of thiol containing amino acid residues in the carrageenase function (Li et al. 2015). However, metals like Na+, K+, Ca2+, Mg2+ are known to increase the carrageenase activity (Ma et al. 2010; Shangyong et al. 2013; Li et al. 2014b) especially Na+ at high concentration (up to 500 mmol L−1) significantly increase the activity (Ma et al. 2013). It was hypothesized that at high concentration it changes the physical condition of carrageenan would have an effect on enzyme reaction. Nevertheless, carrageenan decomposition around the colony was visible on the carrageenan plate which contained 3 % NaCl as a component of artificial sea water medium [ASW] (Sarwar et al. 1983a, b). Reagents such as EDTA, Iodoacetic acid and Tween-80 did not affect too much to the enzyme activity (Sarwar et al. 1983a; Li et al. 2015).

Kinetics of mannan depolymerization, i.e., Michaelis–Menten constant (Km) and the maximal reaction velocity (Vmax) values have been reported for different bacterial carrageenases (Potin et al. 1991; Feixue et al. 2010; Ma et al. 2010). Km and Vmax values reported for Pseudoalteromonas porphyrae LL-1 using k-carrageenan as a substrate were 4.4 mgml−1 and 0.1 mgmin−1 ml, respectively (Liu et al. 2011) and for Pseudomonas elongata MTCC 5168 were 6.7 mgml−1 and 4 μmol min−1 mg with same substrate (Khamphatay et al. 2007b). Studies on the production conditions and properties of important carrageenan degraders reported in the recent years have been summarized in Table 2.

Cloning and expression of the carrageenase gene

Till the advent of recombinant DNA technology, enzymes were produced by fermentation of the microorganisms that express the enzymes. Purification of target enzymes from a pool of proteins requires tedious purification steps thereby increasing their costs. Recombinant DNA technology allows large scale expression of carrageenases in heterologous protein expression hosts. In recent years, a number of studies have been published on the cloning and manipulation of bacterial carrageenases genes from new and previously reported organisms with the aim of enzyme overexpression, analyzing the primary structure of the protein and protein engineering for the alteration of the enzyme properties to suit its commercial applications (Chauhan et al. 2014b, c, d, e) (Table 3). The gene encoding carrageenase enzyme cloned from various bacteria has been expressed in E. coli in majority of the report available in literature (Guibet et al. 2007; Kobayashi et al. 2012; Liu et al. 2013). However, some genes have been expressed into other hosts also like Bacillus subtilis (Hatada et al. 2011).

Carrageenase production has been increased through heterologous expression in a number of cases (Michel et al. 2001b). High levels of expression has been achieved by cloning the Microbulbifer thermotolerans JAMB-A94T carrageenase gene in the heterologous host (Bacillus subtilis) yielding activity of 105 U l−1 which is about 200 fold higher (Hatada et al. 2011). Some genetic engineering has also been done by researcher to scale up the production of carrageenase. The production of carrageenase from ZoBellia sp. ZM-2 was increased up to 9 times using the natural signal peptide of native strain as well as removing the amino acids of about 20 kDa from C-terminal end of the gene (post translational modification) and expressing it in E. coli (Liu et al. 2013). In addition to this specific activity of carrageenase was also increased to some extent when they were expressed in heterologous hosts (Hatada et al. 2011; Liu et al. 2013). Cloning and expression strategies for different carrageenases have been summarized in Table 3 which might be helpful for planning future strategies for studying the carrageenases at the molecular level.

Application of carrageenases

The broad substrate specificities of carrageenases have attracted a great deal of attention in the last decade because of their biotechnological potential in various industrial processes. The following section will discuss some of the
| S. no. | Origin | Host | Gene size (bp)/enzyme (aa/kDa) | Carbon source/fermentation conditions | Temp. optima (°C) of activity | Temp. stability | pH optima of activity | pH stability | Family | Reference |
|-------|--------|------|-------------------------------|--------------------------------------|-------------------------------|----------------|----------------------|-------------|--------|-----------|
| 1     | Alteromonas carrageenovora ATCC 43555 | E. coli | 1191 bp/397 aa/44.4 kDa | LB M^a/25 °C/24 h | NR^e | NR^e | NR^e | NR^e | 16 | Barbeyron et al. (1994) |
| 2     | Alteromonas fortis | E. coli | 1425 bp/475 aa/53.3 kDa | M9 M^b/12 °C/15 h | 40 | NR^e | 7.2 | NR^e | 82 | Michel et al. (2000); Michel et al. (2001a, b) |
| 3     | Cellulophaga sp. QY3 | E. coli | 1479 bp/492 aa/53.8 kDa | LB M^c/37 °C/36 h/100 rpm | 50 | NR^e | 7.0 | NR^e | 82 | Ma et al. (2013) |
| 4     | Cytophaga drobachiensis | E. coli | 1635 bp/545 aa/61.6 kDa | LB M^d/22 °C/pH 7.2 | NR | NR^e | NR^e | NR^e | 16 | Barbeyron et al. (1998) |
| 5     | Pseudoalteromonas carrageenovora ATCC-43555 | E. coli | 943 bp/314 aa/105 kDa | LB M^e & M9 M^f/37 °C/6 h | 30 | NR | 7.5 | NR | 16 | Guibet et al. (2007) |
| 6     | Pseudoalteromonas carrageenovora | E. coli | 32.9 kDa | M9 M^g/12 °C/15 h | NR | NR | 7.0 | NR | 16 | Michel et al. (1999) |
| 7     | Pseudoalteromonas tetraodonis JAM-K142 | E. coli | 1194 bp/397 aa/85 kDa (dimer) | LB M^h/30 °C/24 h/150 rpm | 30 | >28.1 %/50 °C/8.8 | >20 %/pH 2.1–1.6/4 °C/15 min | NR^e | Kobayashi et al. (2012) |
| 8     | Microbulbifer thermotolerans JAMB-A94^T | Bacillus subtilis | 1707 bp/569 aa/55 kDa | CL TM^i/30 °C/72 h/250 rpm | 50 | NR^e | 7.5 | >80 %/pH 7.0–10.0/25 °C/30 min | 82 | Hatada et al. (2011) |
| 9     | Zobellia sp. ZM-2 | E. coli | 1638 bp/545 aa/45 kDa | LB M^j/23 °C/24 h,120 rpm | 39 | >95 %/35 °C/6.0 | >85 %/pH 6.0–8.0/120 min/20 °C | NR | Liu et al. (2013) |
| 10    | Zobellia galactanovorans | E. coli | 1473 bp/491 aa/51.9 kDa | LB M^k/22 °C/pH 7.2 | 40 | NR | 7.2 | NR^e | 16 | Barbeyron et al. (2000) |

^a Luria Bertani medium
^b M9 Medium
^c CaCl2 maltose tetracycline medium
^d Luria Bertani medium supplemented with carrageenan
^e Not reported
most promising and newly explored applications of carrageenases (Fig. 6).

**In medical as antitumor agent**

To improve the physical properties of polysaccharides, conversion to oligosaccharides may be the best choice. Hydrolysis products of carrageenan i.e., carrageeno-oligosaccharides have potential anti-tumor properties (Yuan et al. 2006). As discussed in the section of “Enzymatic hydrolysis of carrageenan”, oligosaccharides generated by the enzymatic hydrolysis of the carrageenan are more uniform in size rather than chemical hydrolysis which further enhance their anti tumor property. It was reported that the low-molecular-weight carrageenan was effective on the anti-tumor activity, suggesting the molecular weight of oligosaccharides may play an important role (Haijin et al. 2003; Yuan and Song 2005). In addition to this bioactivity of oligosaccharides depends on several other structural features such as the degree of sulfation (DS), the sulfation position, type of sugar, and glycosidic branching. It was hypothesized that the carrageenan oligosaccharides destabilize the interaction between the glucosaminoglycan portion of proteoglycans and the extracellular matrices proteins, thus eliminating the adhesion of cancer cells to matrices, which is necessary in metastasis spread (Yuan et al. 2011).

Haijin et al. (2003) showed that carrageenan oligosaccharide with a molecular weight of 1726, administered orally at a dose of 100 mg kg$^{-1}$ in mouse markedly inhibited tumor formation. However, the anti-tumor activity of high-sulfonated carrageenan was much less than that of the non-sulfonated or light-sulfonated preparation. The activities of the latter products on superoxide dismutase and catalase were enhanced considerably, which suggests that carrageenan oligosaccharide was effective in promoting the antioxidation ability and eliminating danger from free radicals.
Recycling of sea weed waste into bioethanol production

Seaweeds are suitable for consumption by human beings and animals, and are a favorite food in Asian countries in particular. They are often used in fertilizers, fungicides, herbicides, and phycocolloids such as alginate, carrageenan, and agar (Kim et al. 2011). Worldwide consumption of seafood including seaweeds has increased steadily because of the associated health benefits. In recent years, the amount of seaweed waste has increased because of its use as an industrial resource and as a depolluting plant for cleaning inland sea areas and eutrophied seawater (Tang et al. 2011). Accordingly, the disposal and reuse of seaweed waste has become essential for the preservation of the marine environment and recycling of organic substances. The major species were brown seaweed and red seaweed, such as Porphyra tenera and Porphyra yezoensis. The carbohydrate content of red seaweed is 30–60 %, consisting mostly of agar and carrageenan. Unused seaweed waste is customarily discarded via landfill, incineration, or by dumping into the sea which also create environmental pollution. Kang and Kim (2015) isolated a Bacillus sp. SYR4 which were able to utilize sea weed waste as a carbon source by degrading both agar and carrageenan and produced reducing sugars which serves as a substrate for bioethanol production leading to 7–10 wt % of ethanol could be produced by the isolate.

Use as a detergent additive

Carrageenans are used in dairy food products such as ice cream, yogurt, flavored milks, whipped toppings, puddings, cheeses, sour cream, juices, ready to spread icings, jams, jellies, salad dressing, candies where it use as a texture modification. In most of the food products, kappa carrageenan presents either, alone or in combination with another type of carrageenan which can cause laundry stains (Aronson et al. 2001). As carrageenans have a high affinity for cellulose fibers, therefore, they adsorb these stains to the fabric very tightly because they are not easy to remove. Polysaccharide degrading enzymes, like carrageenase, can be used as laundry additives to hydrolyze the gums present in these food stains. McDonald and Schmidt (2009) have formulated detergent composition (fabric cleaning compositions, surface cleaning compositions, oral cleaning compositions, contact lens cleaning compositions, dish cleaning compositions) containing combination of carrageenase (about 1–80 %) and surfactant (cationic, anionic or mixture thereof) which are able to hydrolyze polysaccharides efficiently hence removing the gum containing stains and giving excellent cleaning properties.

Removal of excess printing paste after textile printing

In printing of textiles, it is common to use a printing paste containing a dye and a thickener. Among the commonly used thickeners are biological polymers (alginate, starch or modified starch, locust bean gum, galactomannan or modified galactomannan and carboxymethyl cellulose). With most printing methods, the polymer and excess dye must be removed by washing with water after the fixation of the print. Generally, a large amount of water is required for complete removal due to the high viscosity and low water solubility of the printing paste. Insufficient removal leads to unsatisfactory quality of the finished textile for the following reasons: (1) dye may be transferred to other parts of the printed textile or to other garments during laundering by the consumer. (2) Residual thickener will make printed areas stiff (Salem et al. 2008).

Pedersen et al. (1995) reported that composition comprising carrageenase along with other polysaccharide degrading enzymes will decrease process time as well as the amount of energy and water needed to achieve a satisfactory quality of the textile by hydrolyzing these polymers.

For isolation of protoplast of algae

Carrageenases can be used for the isolation of protoplast of algae along with other cell wall degrading enzymes such as Cellulase and Macerozyme. This isolated protoplast can be used for genetic engineering experiments for the production of improved algal strains yielding better quality carrageenan of commercial value (Chen et al. 1994).

Khambhaty et al. (2007b) showed the role of bacterial carrageenase (Pseudomonas elongata MTCC 5168) in isolation of protoplast of red algae Kappaphycus alvarezi. They demonstrated that carrageenase in combination with commercially available Cellulase and Macerozyme yielded protoplast from K. alvarezi where in absence of κ-carrageenase, did not yield protoplasts. Morover presence or absence of individual enzymes, the composition of the osmoticum and the age of tissue were instrumental for protoplast yields (Zablackis et al. 1993).
Other applications

Prevent red algal bloom

The enzymes produced by marine bacteria could effectively control red algal bloom contamination. Thus, it prevents bio fouling of submerged marine surface or pipes by acting on complex polysaccharide layers (Kambhatya et al. 2007c).

Used in structural functional studies

Carrageenases provide the opportunity to investigate the structure–function relationships of the hydrolases that degrade self-associating sulfated polysaccharides (Michel et al. 2001a).

Protein extraction form the cell wall

Most red seaweed possesses high level of proteins (10–30% dry weight) (Morgan et al. 1980). These proteins can be extracted by hydrolytic enzymes like carrageenase (Fleurence et al. 1995). For example, the degradation of cell Wall polysaccharides by hydrolytic enzymes is used for the isolation of extensin, a protein linked to cell wall polysaccharide of higher plants (Lamport 1969).

Conclusion

Microbial carrageenases have attracted great attention in near past because of their useful applications. Such enzyme systems are not only of academic interest since they have potential biotech applications in a wide range of industrial enzyme markets. Exploitation of biodiversity to provide microorganisms that produce carrageenases well suited for their diverse applications is considered to be one of the most promising future alternatives. The knowledge summarized in this review, regarding the known sources of carrageenases, and their properties would be a great help to study these enzymes, so that they can be effectively utilized for various biotechnological processes.

Acknowledgments This study is funded by Department of Education, Employment and Workplace Relations, (DEEWR) Australian government (Grant No. ERF_PDR_128381_2014/4478_2015). Prakram Singh Chauhan is highly thankful to Australian Government for providing Endeavour Post Doctoral Research Fellowship.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights and informed consent Research involving Human Participants and/or Animals/Informed consent: not applicable for this study.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

Araki T, Higashimoto Y, Morishita T (1999) Purification and characterization of kappa carrageenase from a marine bacterium, Vibrio CA-1004. Fish Sci 65(6):937–942
Aronson MP, Brown CP, Chatfield RJ, Willis E (2001) Aqueous detergent compositions thickened using carrageenan. US Patent 6265368B1
Barbeyron T, Henрисsат B, Kloareg B (1994) The gene encoding the κ-carrageenase of Alteromonas carrageenovora is related to β-1,3-1,4-glucanases. Gene 139:105–109
Barbeyron T, Gerard A, Potin P, Henрисsат B, Kloareg B (1998) The κ-carrageenase of the marine bacterium Cytophaga drobachiensis. Structural and phylogenetic relationships within family-16 glycoside hydrolases. Mol Biol Evol 15:528–537
Barbeyron T, Michel G, Potin P, Henрисsат B, Kloareg B (2000) η-Carrageenases constitute a Novel Family of glycoside hydrolases, unrelated to that of κ-Carrageenases. J Biol Chem 275(45):35499–35505
Bellion C, Hamar GK, Yape W (1982) The degradation of Eucheuma spinosum and Eucheuma cottonii carrageenans by η-carrageenases and κ-carrageenases from marine bacteria. Can J Microbiol 28:874–884
Beltyay EA, Yousef AS, El-Shenaway MA, El-Assar SA (2012) Purification of kappa (κ)-carrageenase from locally isolated Cellulosimicrobium cellulans. Afr J Biotechnol 11(52):11438–11446
Bras FN, Fernandes PA, Ramos MJ, Cerqueira NMFSA (2012) Glycosidases-A Mechanistic Overview. In: Chang CF (ed) Comprehensive studies on Glycobiology and Glycotechnology. INTECH, pp 117–134
Campo VL, Kawano DF, Silva DBD, Carvalho I (2009) Carrageenans: biological properties, chemical modifications and structural analysis—a review. Carbohydr Polym 77:167–180
Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henрисsат B (2009) The carbohydrate-active enzymes database (CAZy): an expert resource for Glycogenomics. Nucleic Acids Res 37:D233–D238
Chauhan PS, Gupta N (2016) Insight into microbial mannosidase: a review. Cri Rev Biotechnol 8:1–12
Chauhan PS, Puri N, Sharma P, Gupta N (2012) Mannanases: microbial sources, production, properties and potential biotechnological applications. Appl Microbiol Biotechnol 93:1817–1830
Chauhan PS, George N, Sondhi S, Puri N, Gupta N (2014a) An overview of purification strategies for microbial mannanases. Int J Pharma Bio Sci 5:176–192
Chauhan PS, Sharma P, Puri N, Gupta N (2014b) Purification and characterization of an alkali-thermostable β-mannanase from Bacillus nealsorni PN-11 and its application in manno-oligosaccharides preparation having prebiotic potential. Eur Food Res Technol 238:927–936
Chauhan PS, Sharma P, Puri N, Gupta N (2014c) A process for reduction in viscosity of coffee extract by enzymatic hydrolysis of mannan. Bioproc Biosyst Eng 37:1459–1467
Chauhan PS, Soni SK, Sharma P, Saini A, Gupta N (2014d) A mannanase from Bacillus nealsomi PN-11: statistical optimization of production and application in biobleaching of pulp in combination with xylanase. Int J Pharma Sci Technol 5:237–251
Chauhan PS, Bharadwaj A, Puri N, Gupta N (2014e) Optimization of medium composition for alkali-thermostable mannanase production by Bacillus nealsomi PN-11 in submerged fermentation. Int J Curr Microbiol Appl Sci 3(10):1033–1045
Chauhan PS, Tripathi SP, Sangamwar AT, Puri N, Sharma P, Gupta N (2015) Cloning, molecular modeling and docking analysis of alkali-thermostable β-mannanase from Bacillus nealsomi PN-11. Appl Microbiol Biotechnol 99:8917–8925
Chen LCM, Craigie JS, Xie ZK (1994) Protoplast production from Porphyra linearis using a simplified agarase procedure capable of commercial application. J Appl Phycol 6:35–39
Coutinho PM, Henrissat B (1999) An integrated databases approach. In: Gilbert HJ, Davies G, Henrissat B, Svensson B (eds) Recent advances in carbohydrate bioengineering. The Royal Society of Chemistry, Cambridge, pp 3–12
Davies GJ, Wilson KS, Henrissat B (1997) Nomenclature for sugar-binding subsites in glycosyl hydrolases. Biochem J 321:557–559
Dyrgert S, Li LH, Florida D, Thoma JA (1965) Determination of reducing sugar with improved precision. Anal Biochem 13(3):367–374
Dysert N, Lystad KQ, Levine DW (1997) Development of a fermentation process for production of a κ-carrageenase from Pseudomonas carrageenovora. Enzyme Microb Technol 20:418–423
Feixue S, Yuxin MA, Ying W, Qian L (2010) Purification and characterization of novel κ-carrageenase from marine Tamalna sp. HC4. Chin J Ocean Limnol 28:1139–1145
Fleurence J, Massiani L, Guyader O, Mabeau S (1995) Use of enzymatic cell Wall degradation for improvement of protein extraction from Chondrus crispus, Gracilaria verrucosa and Palmaria palmate. J Appl Phycol 7:393–397
George N, Chauhan PS, Kumar V, Puri N, Gupta N (2014a) An approach to ecofriendly leather: lime and sulphide free dehairing of skin and hide using bacterial alkaline protease at industrial scale. J Clean Prod 79:249–257
George N, Chauhan PS, Puri N, Gupta N (2014b) Statistical optimization of process parameters for production of alkaline protease from Vibrio metchnikovi NG155 having application in leather industry. Int J Pharma Sci Technol 5:509–517
Greer CW, Yaph W (1984) Purification and properties of iota-carrageenase from a marine bacterium. Can J Microbiol 30:1500–1506
Guibet M, Barbeyron T, Genicot S, Kloareg B, Michel G (2007) Degradation of ι-carrageenan by Pseudoalteromonas carrageenovora λ-carrageenase: a new family of glycoside hydrolases unrelated to κ- and ι-carrageenases. Biochim J 414:105–114
Hajim N, Xiaolu J, Huashi G (2003) A κ-carrageenase derived oligosaccharide prepared by enzymatic degrading containing anti-tumor activity. J Appl Phycol 15:297–303
Hatada Y, Mizuno M, Li Z, Ohta Y (2011) Hyper-production and characterization of the κ-carrageenase useful for κ-carrageenan oligosaccharide production from a deep-sea bacterium, Microbulbifer thermotolerans IAM-B-94T, and insight into the unusual catalytic mechanism. Mar Biotechnol 13:411–422
Henares BM, Enriquez EP, Dayrit FM, Rojas N (2010) Iota-carrageenan hydrolysis by Pseudoalteromonas carrageenovora IFO12985. Philipp J Sci 139(2):131–138
Hilliou L, Larotonda FDS, Abreu P, Ramos AM, Sereno AM, Goncalves MP (2006) Effect of extraction parameters on the chemical structure and gel properties of κ/θ-hybrid carrageenans obtained from Mastocarpus stellatus. Biomol Eng 23:201–208
Jam M, Flament D, Alloch J, Potin P, Thion L, Kloareg B, Czjzek M, Helbert W, Michel G, Barbeyron T (2005) The endo-β-agarases AgaA and AgaB from the marine bacterium Zobellia galactanivorans: two paralogue enzymes with different molecular organizations and catalytic behaviours. Biochem J 385:703–713
Kang S, Kim JK (2015) Reuse of seaweed waste by a novel bacterium, Bacillus sp. SYR4 isolated from a sandbar. World J Microbiol Biotechnol 31(1):209–217
Khamphay Y, Mody K, Jha B (2007a) Purification and characterization of κ-carrageenase from a novel γ-proteobacterium, Pseudomonas elongata (MTCC 5261) syn. Microbulbifer elongatus comb. Nov. Biotechnol Bioprocess Eng 12:668–675
Khamphay YN, Mody KH, Jha B (2007c) Method for the preparation of κ-carrageenan. US 2007/0231866A1
Khamphay Y, Mody K, Jha B, Gohel V (2007) Statistical optimization of medium components for κ-carrageenase production by Pseudomonas elongate. Enzyme Microb Technol 40:813–822
Kidby DK, Davidson DJ (1973) A convenient ferricyanide estimation of reducing sugars in the nanomole range. Anal Biochem 55(1):321–325
Kim JH, Kim YH, Kim SK, Kim BW, Nam SW (2011) Properties and industrial applications of seaweed polysaccharides-degrading enzymes from the marine microorganisms. Korean J Microbiol Biotechnol 39:189–199
Knudsen NR, Ale MT, Meyer AS (2015) Seaweed hydrocolloid production: an update on enzyme assisted extraction and modification technologies. Mar Drugs 13:3340–3359
Kobayashi T, Uchimura K, Koida O, Deguchi S, Horikoshi K (2012) Genetic and biochemical characterization of Pseudoalteromonas tetradonidis alkaline κ-carrageenase. Biosci Biotechnol Biochem 76(3):506–511
Lamport DTA (1969) The isolation and partial characterization of hydroxyproline rich glycolipides obtained by enzymic degradation on primary cell walls. Biochemistry 3:1155–1163
Lemoine M, Nyvall Collen P, Helbert W (2009) Physical state of kappa-carrageenan modulates the mode of action of kappa-carrageenan from Pseudomonas carrageenovora carrageenase. Biochim J 419:545–553
Li J, Hu Q, Sewsita-Zilda D (2014a) Purification and characterization of a thermostable λ-carrageenase from a hot spring bacterium, Bacillus sp. Biotechnol Lett 36(8):1669–1674
Li L, Ni R, Shao Y, Mao S (2014b) Carrageenan and its applications for hydrolysis of κ-carrageenase gene from marine bacterium Zobellia sp. ZM-2. Appl Microbiol Biotechnol 97:10057–10067
Li Y, Huang Z, Qiao L, Gao Y, Guan H, Hwang H, Aker WG, Wang P (2015) Purification and characterization of a novel enzyme produced by Catenovulum sp. LP and its application in the pretreatment to Ulva prolifera for bio-ethanol production. Process Biochem 50(5):799–806
Liu GL, Li Y, Chi Z, Chi ZM (2011) Purification and characterization of κ-carrageenase from the marine bacterium Pseudoalteromonas porphyrae for hydrolysis of κ-carrageenan. Process Biochem 46:265–271
Liu Z, Li G, Mo Z, Mou H (2013) Molecular cloning, characterization, and heterologous expression of a new κ-carrageenase gene from marine bacterium Zobellia sp. ZM-2. Appl Microbiol Biotechnol 97:10057–10067
Liu Z, Tian L, Chen Y, Mou H (2014) Efficient extracellular production of κ-carrageenase in Escherichia coli: effects of wild type signal sequence and process conditions on extracellular secretion. J Biotechnol 185:8–14
Liu G, Wu S, Jin W, Sun C (2016) Amy63, a novel type of marine bacterial multifunctional enzyme possessing amylase, agarase and carrageenase activities. Sci Rep. doi:10.1038/srep18726
Ma YX, Dong SL, Jiang XL, Li J, Mou HJ (2010) Purification and characterization of κ-carrageenase from marine bacterium mutant strain Pseudoalteromonas sp. AJ5-13 and its degraded products. J Food Biochem 34:661–678

Ma S, Duan G, Chai W, Geng C, Tan Y, Wang L, Sourd F, Michel G, Yu W, Han F (2013) Purification, cloning, characterization and essential amino acid residues analysis of a New τ-carrageenase from Cellulophaga sp. QY3. PlosOne 8(5):e64666

McDonald HC, Schmidt B (2009) Kappa-carrageenase and kappa-carrageenase containing compositions. US20090048136A1

Mclean MW, Williamson FB (1979) Kappacarrageenase from Pseudomonas carrageenovora. Eur J Biochem 93(3):553–558

Michel AS, Mestdagh MM, Axelos MA (1997) Physico-chemical properties of carrageenan gels in presence of various cations. Int J Biol Macro 21:195–200

Michel G, Barbeyrion T, Flament D, Vernet T, Kloareg B, Dideberg O (1999) Expression, purification, crystallization and preliminary X-ray analysis of the κ-carrageenase from Pseudoalteromonas carrageenovora. Acta Cryst D55:918–920

Michel G, Flament D, Barbeyrion T, Vernet T, Kloareg B, Dideberg O (2000) Expression, purification, crystallization and preliminary X-ray analysis of the iota-carrageenase from Alteromonas fortis. Acta Crystallogr Sect D Biol Crystallogr 56:766–768

Michel G, Chantalat L, Duc E, Barbeyrion T, Henrissat B, Kloareg B, Dideberg O (2001a) The kappa-carrageenase of P. carrageenovora features a tunnel-shaped active site: a novel insight in the evolution of Clan-B glycoside hydrolases. Structure 9:513–525

Michel G, Chantalat L, Fanchon E, Henrissat B, Kloareg B, Dideberg O (2001b) The τ-carrageenase of Alteromonas fortis. A β-helix fold-containing enzyme for the degradation of a highly polyanionic polysaccharide. J Biol Chem 276:40202–40209

Michel G, Helbert W, Kahn R, Dideberg O, Kloareg B (2003) The structural bases of the processive degradation of κ-carrageenase, a main cell wall polysaccharide of red algae. J Mol Biol 334:421–433

Michel G, Nyval-Collen P, Barbeyrion T, Czajek M, Helbert W (2006) Bioconversion of red seaweed galactans: a focus on bacterial agarases and carrageenases. Appl Microbiol Biotechnol 71:23–33

Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal Chem 31:426–428

Morgan C, Wright JLC, Simpson J (1980) Review of chemical constituents of the red alga Palmaria palmale (dulse). Econ Bot 34:27–50

Mou H, Jiang X, Liv Z, Guan H (2004) Structural analysis of κ-carrageenase containing compositions. Carbohydr Res 331:271–283

Necas J, Bartosikova L (2013) Carrageenan: a review. Vet Med 58(4):187–205

Ohta Y, Hatada Y (2006) A novel enzyme, lambda-carrageenase isolated from a deep sea bacterium. J Biochem 140(4):475–481

Ostgaard K, Wangen BF, Knutsen SH, Asen IM (1993) Large-scale production and purification of a-carrageenase from Pseudomonas carrageenovora for application in seaweed biotechnology. Enzyme Microb Technol 15:326–333

Pedersen G, Hagen HA, Asfjord L, Sorensen E (1995) Removal of printing paste thicker and excess dye after textile printing. US5405414A

Potin P, Sansneau A, Le Gall Y, Rochas C, Kloareg B (1991) Purification and characterization of a new κ-carrageenase from a marine Cytophaga-like bacterium. Eur J Biochem 201:241–247

Potin P, Richard C, Barbeyrion T, Henrissat B, Gey C, Petillot Y, Forest E, Dideberg O, Rochas C, Kloareg B (1995) Processing and hydrolytic mechanism of the cgkA-encoded κ-carrageenase of Alteromonas carrageenovora. Eur J Biochem 228:971–997

Rebuffet E, Barbeyrion T, Jeudy A, Jam M, Czajek M, Michel G (2010) Identification of catalytic residues and mechanistic analysis of family GH82 iota-carrageenases. Biochemistry 49:7590–7599

Rowe RC, Sheskey PJ, Quinn ME (2009) Handbook of Pharmaceutical Excipients. Am Pharm Assoc Pharm Press, London

Ruiter GAD, Rudolph B (1997) Carrageenan biotechnology. Trends Food Sci Technol 8:389–395

Salem AA, Nassar SH, Rekaby MM (2008) Improving the removal of printing paste thickener and excess dye by enzymatic washing of printed cotton fabrics. J Nat Fibers 5(1):1–18

Sarwar G, Sakata T, Kakirriotom D (1983a) Isolation and characterization of carrageenan-decomposing bacteria from marine environment. J Gen Appl Microbiol 29:145–155

Sarwar G, Sakata T, Kasimoto D (1983b) The production and characteristic of carrageenase from Cytophaga. Bull Jpn Soc Sci Fish 49:1689–1694

Sarwar G, Matayoshi S, Oda H (1987) Purification of κ-carrageenase from marine Cytophaga species. Microbiol Immunol 31:869–877

Shangyong LI, Lanpan JIA, Linna W, Wengong YU, Feng H (2013) Purification and characterization of a new thermostable κ-carrageenase from the marine bacterium Pseudoalteromonas sp. QY203. J Ocean Univ China 12(1):155–159

Somogyi M (1952) Notes on sugar determination. J Biol Chem 195:19–23

Sondhi S, Sharma P, George N, Chauhan PS, Puri N, Gupta N (2015) A Novel Extracellular Thermal-Alkalil-Stable Laccase from Bacillus tequilensis SN4 having potential to biobleach softwood pulp. 3Biotech 5:175–185

Tang J, Wang M, Zhou Q, Nagata S (2011) Improved composting of Undaria pinnatifida seaweed by inoculation with Halomonas and Gracilibacillus sp. isolated from marine habitats. Biosour Technol 102:2925–2930

Tayco CC, Tablizo FA, Regalia RS, Lluisma AO (2013) Characterization of a κ-carrageenate-producing marine bacterium, Isolate ALAB-001. Philipp J Sci 142(1):45–54

Van De Velde F, Peppelman HA, Rollemma HS, Hans R (2001) On the structure of κ/τ-hybrid carrageenans. Carbohydr Res 331:271–283

Weigl J, Yapho W (1966) The enzymatic hydrolysis of carrageenan by Pseudomonas carrageenovora: purification of a κ-carrageenate. Can J Microbiol 12:939–947

Xiao A, Xu C, Lin Y, Ni H, Zhu Y, Cai H (2016) Preparation and characterization of κ-carrageenase immobilized onto magnetic iron oxide nanoparticles. Electron J Biotechnol 19:1–7

Xu C, Zhu Y, Ni H, Cai H, Li L, Xiao A (2015) Isolation, identification of a κ-carrageenate-producing bacterium and κ-carrageenate characterization. Wei Sheng Wu Xue Bao 55(2):140–148

Yao Z, Wang F, Gao Z, Jin L, Wu H (2013) Characterization of a κ-carrageenate from marine Cellulophaga lytica strain N5-2 and analysis of its degradation products. Int J Mol Sci 14:24592–24602

Yao Z, Wu H, Zhang S, Du Y (2014) Enzymatic preparation of κ-carrageenan oligosaccharides and their anti-angiogenic activity. Carbohydr Polym 100(1):359–367

Yapho W, Baxter B (1955) The enzymic hydrolysis of carrageenan. Appl Microbiol 3:987–993

Youssef AS, Beltagy EA, El-Shenawy MA, El-Assar SA (2012) Production of κ-carrageenate by Cellulosimicrobium cellulosi isolated from Egyptian Mediterranean coast. Afr J Microbiol Res 6(37):6618–6628

Yuan HM, Song JM (2005) Preparation, structural characterization and in vitro antitumor activity of kappa-carrageenan oligosaccharide fraction from Kappaphycus striatus. J Appl Phycol 17:7–13

Yuan HM, Song JM, Li XG, Li N, Dai JC (2006) Immunomodulation and antitumor activity of κ-carrageenan oligosaccharides. Cancer Lett 243:228–234
Yuan H, Song J, Li X, Li N (2011) Enhanced immunostimulatory and antitumor activity of different derivatives of \(\kappa\)-carrageenan oligosaccharides from *Kappaphycus striatum*. J Appl Phycol 23:59–65

Zablackis E, Vreeland V, Kloareg B (1993) Isolation of protoplasts from *Kappaphycus alvarezii var. tambalang* (Rhodophyta) and secretion of \(\kappa\)-carrageenan fragments by cultured cells. J Exp Bot 44:1515–1522

Zhou MH, Ma JS, Li J, Ye H, Huang K, Zhao X (2008) A kappa-carrageenase from a newly isolated *Pseudoalteromonas*-like bacterium, WZUC10. Biotechnol Bioprocess Eng 13:545–551

Zhu B, Ning L (2016) Purification and characterization of a New \(\kappa\)-carrageenase from the marine bacterium *Vibrio* sp. NJ-2. J Microbiol Biotechnol 26(2):255–262

Ziayoddin M, Shinde M, Lalitha J (2012) Orthogonal array approach for optimization of carrageenase production by solid state fermentation of *Pseudomonas aeruginosa* ZSL-2. J Microb Biochem Technol 4:4

Ziayoddin M, Lalitha J, Shinde M (2014) Increased production of carrageenase by *Pseudomonas aeruginosa* ZSL-2 using Taguchi experimental design. Int Lett Nat Sci 17:194–207