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REVIEW ARTICLE

Recombinant β-agarases: insights into molecular, biochemical, and physiochemical characteristics

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Abstract

Agarases (agarose 4-glycanohydrolase; EC 3.2.1.81) are class of enzymes that belong to glycoside hydrolase (GH) family capable of hydrolyzing agar. Their classification depends on hydrolysis pattern and product formation. Among all the agarases, β-agarases and the oligosaccharides formed by its action have fascinated quite a lot of industries. Ample of β-agarase genes have been endowed from marine sources such as algae, sea water, and marine sediments, and the expression of these genes into suitable host gives rise to recombinant β-agarases. These recombinant β-agarases have wide range of industrial applications due to its improved catalytic efficiency and stability in tough environments with ease of production on large scale. In this review, we have perused different types of recombinant β-agarases in consort with their molecular, physiochemical, and kinetic properties in detail and the significant features of those agarases are spotlighted. From the literature reviewed after 2010, we have found that the recombinant β-agarases belonged to the families GH16, GH39, GH50, GH86, and GH118. Among that, GH39, GH50, and GH86 belonged to clan GH-A, while the GH16 family belonged to clan GH-B. It was observed that GH16 is the largest polyspecific glycoside hydrolase family with ample number of β-agarases and the families GH50 and GH118 were found to be monospecific with only β-agarase activity. And, out of 84 non-catalytic carbohydrate-binding modules (CBMs), only CBM6 and CBM13 were professed in β-agarases. We witnessed a larger heterogeneity in molecular, physiochemical, and catalytic characteristics of the recombinant β-agarases including molecular mass: 32–132 kDa, optimum pH: 4.5–9, optimum temperature 16–60 °C, K_M: 0.68–59.8 mg/ml, and V_{max}: 0.781–11,400 U/mg. Owing to this extensive range of heterogeneity, they have lion's share in the multibillion dollar enzyme market. This review provides a holistic insight to a few aspects of recombinant β-agarases which can be referred by the upcoming explorers to this area.

Keywords Recombinant β-agarases · Glycoside hydrolase · Molecular characteristics · Physiochemical properties · Kinetic properties

Introduction

Agarases (EC 3.2.1.81) are the glycoside hydrolases (GH) that belong to the Carbohydrate Active Enzymes (CAZy) (2018). The glycoside hydrolases of β-agarases catalyzes the breakdown or modification of carbohydrates and glycoconjugates, whereas the carbohydrate-binding domains are non-catalytic modules with carbohydrate-binding activity that are attached to the glycoside hydrolases. These carbohydrate-binding domains facilitate the catalytic action by binding to the substrates, but they do not play crucial in agarolytic activity (Ohta et al. 2004). A few glycoside hydrolases those contain catalytic domains of different GH families are multifunctional enzymes. Depending upon the product profile and specificity to its substrate (Temuujin et al. 2012), agarases can be classified into endotype agarase and exotype agarase where they yield neoagarotetraose and neoagarobiose as their end products, respectively (Liang et al. 2014). Agarases perform a vital role in the breakdown of agar into simple sugars. Agar is considered to be one of the imperative hydrophilic polysaccharides that resides in the cell wall of red sea weeds which possess the gelling property. Agar is primarily composed of agarose and agaropectin (Fu et al. 2009). Almost 70% of agar is comprised

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with agarose and the remaining is of agaropectin. Agarose is embodied with (l-4)-linked 3, 6-anhydro-α-L-galactose and (l-3)-linked β-D-galactose alternatively (Carlsson and Malmqvist 1977). Agaropectin is embodied with 3, 6-anhydro-L-galactose repeating units along with sulfoxy/methoxy and pyruvate residues in its galactose units (Zhu et al. 2016). The hydrolysis of agar can be done by chemical and enzymatic methods. When compared to chemical hydrolysis of agar, the hydrolysis of agar enzymatically is believed to be efficient in terms of energy, effective in terms of cost, and friendly in terms of environment (Xiao et al. 2017). The enzyme that is involved in the breakdown of agar is synthesized by a number of microorganisms. To metabolize this agar, the microbes ought to have β-agarase as the predominant enzyme that breaks down the (l-3)-linked β-D-galactose of agar eclectically and produces neoagarobiose oligosaccharides with D-galactose at the reducing end (AGBO and MOSS 1979). Excluding that, the microbes also require other agar-degrading enzymes such as α-agarase and α-neoagarobiose hydrolase (NABH) (Ha et al. 2011).

Agarases have a wide range of biotechnological and industrial applications. Agarases directly have been applied for the protoplast isolation from algae (Lakshmikanth et al. 2006), DNA isolation from agarose gels (Sugano et al. 1993), and for the extraction of various substances from marine algae by breaking down their cell walls (Lakshmikanth et al. 2006). Moreover, not only the agarase, but also the hydrolysis products formed by agarase by means of its action on agarose have far-reaching applications in food industry, pharmaceutical industry, and cosmetic industry (Lu et al. 2009). These agarooligosaccharides have physiological activities such as anti-inflammatory, skin whitening effect (Jang et al. 2009), moisturizing effect (Yun et al. 2017), macrophage stimulating activity, anti-oxidative activity, immunopotentiating activity (Lakshmikanth et al. 2006), anti-obesity, and anti-diabetic effects (Hong et al. 2017), and providentially, they can also scavenge reactive free radicals and superoxide anion radicals, and inhibit lipid peroxidation (Lakshmikanth et al. 2006) and can serve as prebiotics (Yun et al. 2017).

The recombinant β-agarases belong to the glycoside hydrolase (GH) family such as GH16, GH39, GH50, GH86, and GH118. Predominantly, the recombinant β-agarases were from marine microbes and their genera include *Agarivorans* (Liu et al. 2014a, b; Lee et al. 2012; Lin et al. 2012), *Alteromonas* (Seo et al. 2014; Chi et al. 2014), *Aquimarina* (Lin et al. 2017), *Catenovulum* (An et al. 2018; Cui et al. 2014; Xie et al. 2013), *Cellulophaga* (Ramos et al. 2018), *Flammeovirga* (Dong et al. 2016; Hou et al. 2015; Yang et al. 2011; Chen et al. 2016; Di et al. 2018), *Gayadomonas* (Lee et al. 2018; Jung et al. 2017a, b), *Microbulbifer* (Su et al. 2017), *Micrococcaceae* (Xu et al. 2018), *Pseudoalteromonas* (Chi et al. 2015a, b; Oh et al. 2010a, b), *Pseudomonas* (Hsu et al. 2015), *Saccharophagus* (Kim et al. 2010, 2017, 2018; Lee et al. 2013), *Simiduia* (Tawara et al. 2015), *Thalassomonas* (Liang et al. 2014), and *Vibrio* (Liao et al. 2011) whereas few were from soil bacteria *Streptomyces* (Temuujin et al. 2011, 2012), *Cohnella* (Li et al. 2015) and one exclusive source was the activated sludges of sewage plant from which *Cellvibrio* (Osamu et al. 2012) had been identified with agarolytic property.

To get the desired enzyme in bulk quantities with more heterogeneity and enhanced properties, the technique of recombination was adopted, where the gene possessing the agarolytic activity was cloned and expressed in suitable host systems. Hence, the genes which encoded β-agarase were isolated from different microbial sources and were expressed in a host system which is most commonly the *Escherichia coli* (*E. coli*). The other system that was used as the host for cloning and expression of β-agarase was *Streptomyces lividans TK24* (Temuujin et al. 2011). A fungal host system of *Pichia pastoris GS115* has also been recently used for the expression of β-agarase gene (Xu et al. 2018). These recombinant β-agarases have a major share in the multibillion dollar enzyme market. Hence, in this review, we hashed out in detail about the different features of recombinant β-agarases reported after 2010 that were classified under different families, their sources, molecular weights, and the host in which they were expressed. The effect of different metal ions, their stability at different pH and temperatures, and the kinetic properties of those recombinant β-agarases along with their degrees of degradation were also conversed.

Molecular characteristics of the recombinant β-agarases

Glycoside hydrolases have been categorized into more than 150 families based on the amino acid sequence similarities. Majority of glycoside hydrolases are predicted to have conserved catalytic machinery and molecular mechanism (Gebler et al. 1992). The relation between the structural fold and the function of the enzymes helps in the evolution of the sequence-based glycoside hydrolase classification (Allouch et al. 2003). The substantial similarity in molecular mechanism, catalytic residues, and tertiary structure assists in grouping the glycoside hydrolases with common evolutionary ancestry into larger group termed as clans (Henrissat and Bairoch 1996). All the glycoside hydrolases have their corresponding catalytic domain and a few of the GH families may possess a non-catalytic carbohydrate-binding module (CBM) along with their catalytic domain. These CBMs are classified into 84 families, out of which CBM6 and CBM13 are only found in agarases (Alkotaini et al. 2016). The selected GH families of β-agarases, their clan, mechanism, 3D structure, and their substrate specificity are tabulated in Table 1. The

Table 1 Mechanism, structure, substrate specificity, and clan grouping of selected GH families of β-agarases

Family	Clan	Mechanism	Structure	Substrate specificity
GH16	B	Retaining	β-Jelly roll	β-1,4 or β-1,3 glycosidic bonds of glucans and galactans
GH39	A	Retaining	$(\beta/\alpha)_8$	Arabinoxylans
GH50	A	Probably Retaining	$(\beta/\alpha)_8$ barrel fold	Agarose
GH86	A	Probably Retaining	$(\beta/\alpha)_8$	Agarose and porphyran
GH118	–	Inverting	–	Agarose

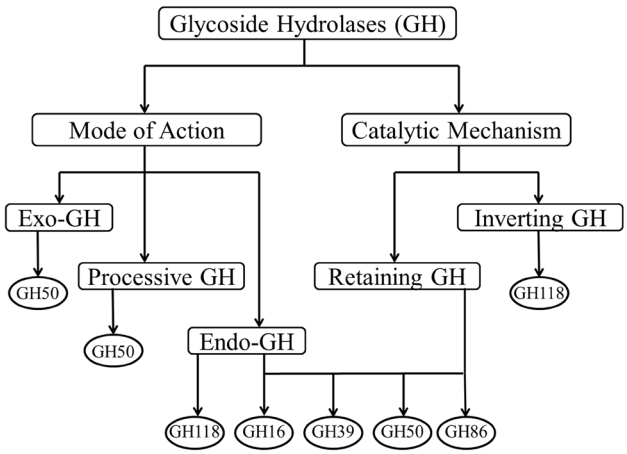


Fig. 1 Classification of glycoside hydrolases and the respective GH families of recombinant β-agarases. Based on mode of action and catalytic mechanism, the recombinant β-agarases are grouped into different families of glycoside hydrolases

classification of the glycoside hydrolase families based on their mode of action and catalytic mechanism is depicted in Fig. 1.

The GH family members act on their substrates either by one-step inversion or by two-step retention hydrolysis mechanism (Ha et al. 2011). The families GH16, GH39, GH50, and GH86 perform their hydrolysis by retention mechanism and the GH118 family members perform their hydrolysis by inversion mechanism. The only difference between the inverting and retaining enzymes is the distances between the two catalytic residues that are positioned on either side of the substrate-binding pocket of the enzymes, where the average distance is higher in inverting enzymes than the retaining enzymes. Excluding this residual distance, the active sites of both the retaining and inverting enzymes appear alike (Giles 2014). The inverting glycoside hydrolases execute the process of hydrolysis by direct displacement method, whereas the retaining glycoside hydrolases perform by double displacement method (Hehemann 2009). By the superimposition of the catalytic domains of one GH family member on the other distantly related GH family member helps in the location of the extremely conserved regions easily (Allouch et al. 2003). The catalytic domain of the glycoside hydrolases has glutamate or/and aspartate as their key residues (Xie et al. 2013). Pertaining to the catalytic residues of glycoside hydrolases, the substrate-binding cleft has a positive subsite that interacts with the non-reducing end of the substrate and a negative subsite that interacts with the sugar ring. Between these positive and negative subsites, the glycosidic bond cleavage point is located (Hehemann 2009). The exo-glycoside hydrolases have smaller active sites in the form of tunnels or pockets where the end-terminal chain of the substrate interacts and gets cleaved. On the other hand, grooves or clefts are found in the endo-glycoside hydrolases that are largely open and they cleave at the central portion of the substrate. In the endo-acting glycoside hydrolases, the catalytic channel is formed by the concave sheet that is crossed by a cleft which makes the molecule to appear slightly elongated (Allouch et al. 2004). The β-agarases of all the families possess endolytic action on their substrates. A few glycoside hydrolases possess both endolytic and exolytic activities that fall under processive glycoside hydrolases in which the action of the enzyme does not allow the cleaved substrate to get detached off from its long-chain polysaccharide (Giles 2014).

The recombinant β-agarases belong to the glycoside hydrolase families GH16, GH39, GH50, GH86, and GH118. The existing protein structures are about 42 in GH16, 8 in GH39, 2 in GH50, 2 in GH86, and 1 in GH118 family, respectively. Out of which only 6 β-agarases structures from GH16 and 4 β-agarases structures from GH50 are reported so far. Of all the families, GH16 is found to be the largest family with 6844 members with heterogeneous function in which 205 members are characterized. The members of GH16 family include enzymes like xyloglucan:xyloglucosyltransferase, keratan-sulfate endo-1,4-β-galactosidase, endo-1,3-β-glucanase, endo-1,3(4)-β-glucanase, licheninase, κ-carrageenase, xyloglucanase, endo-β-1,3-galactanase, β-porphyranase, hyaluronidase, endo-β-1,4-galactosidase, chitin β-1,6-glucanosyltransferase, and endo-β-1,4-galactosidase along with β-agarase, and hence, this GH16 family of glycoside hydrolases can be termed as polyspecific family (http://www.cazy.org/). All the GH16 agarases were found to

act on its substrate endolytically. The active site of the GH16 family proteins is cleft-shaped which is bounded by the loops extending from the β-sheets that are aligned in a curved sandwich manner. In GH16 agarases, it was observed that the GH16 catalytic domain is located directly adjacent to the signal peptide at the N-terminal and at the C-terminal which the CBM6 domain was witnessed (Ekborg et al. 2006). The carbohydrate-binding module of the glycoside hydrolases has glycine, alanine, and/or arginine as their key residues (Xie et al. 2013). It is anticipated that the CBM increases the concentration of enzyme on the surface of the polysaccharide, thereby improving the activity of the enzyme on the substrates that are insoluble in nature (Allouch et al. 2004). Hsu et al. 2015 observed three carbohydrate-binding modules (CBM6) at the C-terminal end. At the conserved regions of the GH16 catalytic domain, the catalytic residue glutamate will be present at the 232 and 237 position, whereas aspartate will be present at the 234 position. The amino acids at the motif, active site, ion-binding site, and sugar-binding site are highly conserved in the GH16 agarases (Fu and Kim 2010). Any difference at the substrate-binding site makes the enzyme to get differed in their substrate specificities (Allouch et al. 2003).

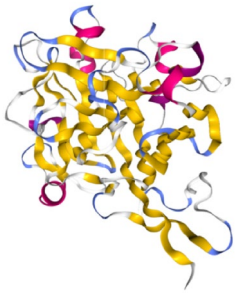
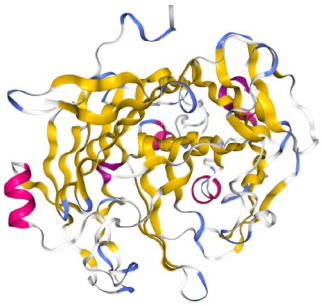
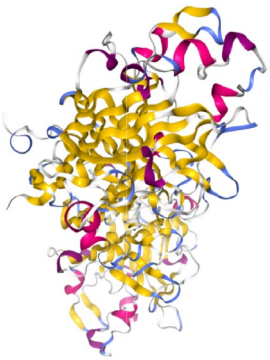
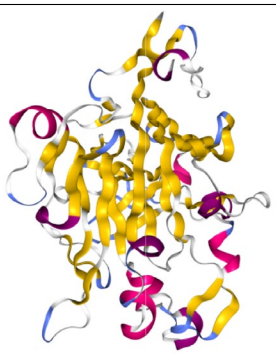
The GH39 family has 19 characterized proteins of 1963 members which include α-L-iduronidase and β-xylosidase (http://www.cazy.org/). The only β-agarase of GH39 family was reported by Jung et al. 2017 which acted endolytically on its substrate. At the N-terminal, the GH39 domain was observed in this agarase, and hence, it fell under GH39 family. Among 599 GH50 members, there are about 23 characterized agarases. By the analogy acquired from the superimposition of GH50 enzymes with the clan GH-A enzymes, the catalytic residues of the GH50 enzymes have been construed. It was witnessed that the GH50 β-agarases have the ability to act as endo, exo, and processive glycoside hydrolases (Hehemann 2009). According to Pluvinage et al. (2013, these processive glycoside hydrolases are predicted to have two glutamate residues in their active site tunnel which is a TIM barrel fold, and at the opening position of the active site, an auxiliary β-sandwich domain which is hypothesized to be a CBM is fused with the main barrel that benefits the substrate binding. The GH86 family comprises of β-porphyranase along with β-agarase. We could find 8 characterized enzymes out of 126 members. The β-agarase members of the GH86 family act on their substrates endolytically. A few agarases of GH86 family had CBM6 domain along with the GH86 catalytic module (Hou et al. 2015). The smallest of glycoside hydrolase family is GH118 where three members are characterized out of eight members in total. All the β-agarases of this family were found to act endolytically on the substrate. As reported by Xie et al. (2013), the members of GH118 family have a larger substrate-binding cleft. To date, the families GH50 and GH118 solely have β-agarase activity in their family, and hence, they can be termed as monospecific families (http://www.cazy.org/).

The structural properties and the 3D structures of all the available β-agarases are tabulated in Table 2. All the tabulated agarases have single unique protein chain. The GH16 agarase MtAgaA was asymmetric with a GH16 catalytic domain and a CBM6 domain displaying β-jelly roll topology with two antiparallel β-sheets. The global stoichiometry of ZgAgaB was homo-2-mer-A2 where all the other agarases had monomer-A as their global stoichiometry. The global symmetry of the listed agarases in Table 2 is found to be asymmetric except the agarase ZgAgaB where it has cyclic global symmetry (Takagi et al. 2015).

The sources of different recombinant agarase genes, their molecular weights, and the hosts in which they were expressed are listed in Table 3. The molecular weight of the recombinant β-agarases from the marine sources varied through an extensive range from 32 to 132 kDa. The recombinant β-agarases with the highest molecular weight so far was AgaJ9 with a molecular weight of 132 kDa (the monomeric form of AgaJ9) which was expressed in *E. coli strain ER2566* and was sourced from a seawater bacterium *Gayadomonas joobiniege G7*. This recombinant enzyme degraded agarose rapidly and hence concluded to break down agarose endolytically. This was categorized under the GH39 family that encoded 1205 amino acids containing protein with 23 amino acid signal peptide (Jung et al. 2017). The lowest molecular weight of 32 kDa was witnessed from a couple of different β-agarases. One was from the soil bacterium *Streptomyces coelicolor A3* (*2*) which was expressed in *Streptomyces lividans TK24*. This was an endotype β-agarase gene (DagA) which encoded a protein with 309 amino acids and a signal peptide chain with 30 amino acids in its N-terminus. To produce the enzyme efficiently, Temuujin et al. (2011) used three kinds of strong promoters, ermEp, tipAp, and sprTp, of which ermEp produced DagA more efficiently that belonged to the GH16 family. The other 32 kDa β-agarase was observed as an intracellular fusion protein that also belonged to the GH16 family and was from *Pseudoalteromonas* sp. *AG52* which was isolated from red seaweed, *Gelidium amansii*, and was overexpressed in *E. coli*. This gene was named as AgaA and it encoded a 290 amino acids containing protein (Oh et al. 2010a, b).

In contrast to all other sources, the only bacterium that was isolated from a different source was *Cellvibrio* sp. which was from the activated sludges of municipal sewage plant. The AgaA gene of this *Cellvibrio* sp. encoded a primary translation product whose molecular weight was 79 kDa with 713 amino acids. The nucleotide sequence of AgaA belonged to the GH86 family, and it was cloned and expressed in *E. coli DH5α* (Osamu et al. 2012). For the production of reducing sugars from *Gracilaria verrucosa*, red algae, about three recombinant

Table 2 Structural properties and 3D structure of available β-agarases

PDB ID	Gene	Microorganism	3D Structure	Residue Count	Chains	Sequence Length	Ligands	Reference
GH16								
3WZ1	MtAgaA	*Microbulbifer thermotolerans* JAMB-A94		282	A	282	Sodium ion Glycerol	Takagi et al 2015
4ASM	AgaDcat	*Zobellia galactanivorans*		363	B	363	Calcium ion Imidazole Di-hydrogen ethyl ether	Hehemann et al 2012
1O4Z	ZgAgaB	*Zobellia galactanivorans*		1384	A B C D	346	Sodium ion Magnesium ion 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid	Takagi et al 2015
4ATF	AgaB-E189D	*Zobellia galactanivorans*		1232	A B C D	308	Sodium ion β-D-galactose 3,6-anhydro-L-galactose	Hehemann et al 2012

agarase genes corresponding Aga50A, Aga50D, and NABH, which belonged to endotype GH118 family were cloned into *E. coli* from *Saccharophagus degradans 2-40*. The molecular weights of these recombinant agarases Aga50A, Aga50D, and NABH were 87.8 kDa, 89.7 kDa, and 44.0 kDa respectively (Kim et al. 2018). From a different host system, other than

Table 2 (continued)

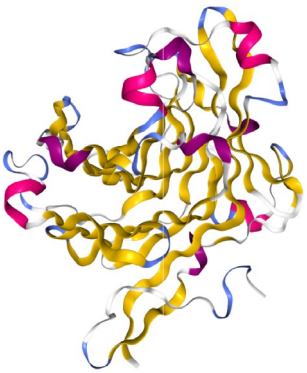
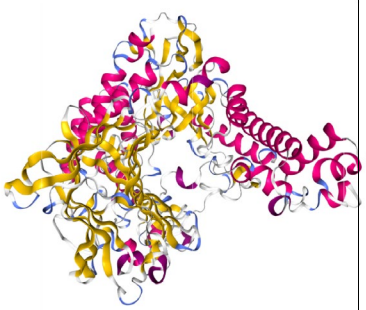
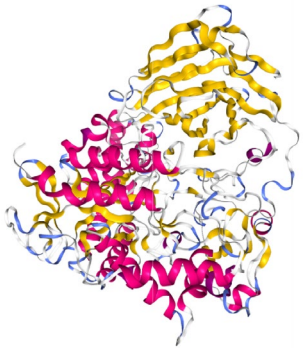
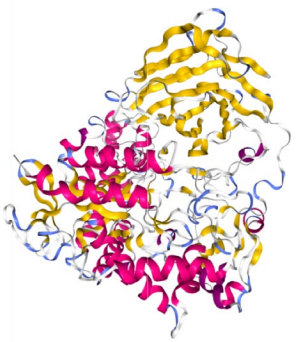
1O4Y	ZgAgaA	*Zobellia galactanivorans*		288	A	288	Sodium ion Sulfate ion Calcium ion	Takagi et al 2015
1URX	βAgaA	*Zobellia galactanivorans*		282	A	282	Calcium ion β-D-galactose α-D-galactose 3,6-anhydro-L-galactose	Allouch et al 2004
GH50								
4BQ2	Aga50D	*Saccharophagus degradans*		3000	A B C D	750	Calcium ion Glycerol	Pluvinage et al 2013
4BQ3	Aga50D	*Saccharophagus degradans*		3000	A B C D	750	Calcium ion Glycerol Neoagarobiose	Pluvinage et al 2013

Table 2 (continued)

4BQ4	Aga50D	*Saccharophagus degradans*		1500	A B	750	Calcium ion Glycerol β-D-galactose 3,6-anhydro-L-galactose	Pluvinage et al 2013
4BQ5	Aga50D	*Saccharophagus degradans*		1500	A B	750	Calcium ion Glycerol β-D-galactose 3,6-anhydro-L-galactose	Pluvinage et al 2013

E. coli, an endotype β-agarase of GH118 family was documented. A gene, BN3 from *Micrococcaceae* sp., was cloned and overexpressed in *Pichia pastoris GS115* through electro transformation. The molecular mass of this agarase was between 35 and 100 kDa (Xu et al. 2018). A pair of agarases with both exolytic and endolytic activity had been witnessed by Liang et al. 2014 and Temuujin et al. 2012. A 90 kDa GH50 agarase from a seawater bacterium, *Thalassomonas agarivorans BCRC 17492* constituted with 774 amino acids protein along with 26 amino acid signal peptide was observed in this gene and it was designated as AgaB1 (Liang et al. 2014). The other agarase was mined from *Streptomyces coelicolor A3* (*2*) and was expressed in *Streptomyces lividans TK24* which is a unique host when compared to all other hosts that were used in the expression of the β-agarase gene. This agarase Sco3487 displayed a molecular mass of 83.9 kDa which had 798 amino acid-containing protein with 45 amino acids' signal peptide chain (Temuujin et al. 2012).

Physiochemical properties of the recombinant β-agarases

Physiochemical properties of 37 recently studied recombinant agarases are summarized in Table 4. Majority of the agarases were from coastal sediment, sea water, and marine algae, which explicitly convey that most of the agarases were from marine sources. Based on the analysis of physiochemical properties of recombinant agarase, an optimum temperature of 60 °C was found to be the maximum optimum temperature so far and it was reported from the recombinant agarase YM01-3 from a marine bacterium *Catenovulum agarivorans YM01T*. The pH stability of this agarase was found to be between 4 and 9 and the optimum pH was 6 (Cui et al. 2014). A recombinant agarase, AgaA, from marine *Pseudomonas vesicularis MA103* indicated optimum activity at varied lower temperatures such as 16 °C, 20 °C, and 24 °C (Hsu et al. 2015). The AgaJ9 was a cold-adapted β-agarase that could retain 80% of its activity even at a temperature of 5 °C. The enzyme was stable at a temperature below 30 °C with optimum activity at 25 °C, and the pH stability was between 4 and 8 with pH 5 as optimum. Jung et al. (2017) observed AgaJ9 in both monomeric and dimeric forms. Without regard to both the isomeric forms, on all tested conditions, the enzymatic properties were in similar. From a soil bacterium *Streptomyces coelicolor A3* (*2*), two similar agarose degrading enzymes were isolated (DagA and Sco3487). DagA was found to be stable at a pH range of 5–8 (Temuujin et al. 2011), but Sco3487 was found stable only at pH 7, though it had similar optimum properties as DagA (Temuujin et al. 2012).

Table 3 Molecular characteristic of recombinant β-agarases from different sources

Gene	Bacterium	Source of the bacterium	Host	Amino acids	Signal peptide (amino acids)	Molecular weight (kDa)	References
GH 16 endotype agarases							
YM01-1	*Catenovulum agarivorans YM01*	Marine bacterium	*E. coli BL21 (DE3)*	331	23	37.7	An et al. (2018)
Aga2	*Cellulophaga omnivescoria W5C*	Marine bacterium	*E. coli BW25113 (DE3)*	–	–	58	Ramos et al. (2018)
agaB	*Flammeovirga* sp. *SJP92*	–	*Escherichia coli*	849	19	91	Dong et al. (2016)
AgaYT	*Flammeovirga yaeyamensis strain YT*	Surface of a red algae, *Gracilaria tenuistipitata*	*Escherichia coli*	503	–	56.5	Yang et al. (2011)
ID2563	*Microbulbifer* sp. *Q7*	Guts of sea cucumbers	*E. coli BL21 (DE3)*	–	19	65	Su et al. (2017)
AgJ11	*Gayadomonas joobiniege G7*	Coastal seawater	*E. coli ER2566*	317	26	35	Jung et al. (2017a)
Aga16B	*Saccharophagus degradans 2-40T*	Marine bacterium	*E. coli BL21 (DE3)*	–	–	63.7	Kim et al. (2017)
AgaH92	*Pseudoaltero-monas* sp. *H9 KCTC23887*	Marine bacterium	*E. coli DH5α*	445	19	51	Chi et al. (2015a)
AgaA	*Pseudomonas vesicularis MA103*	Seawater	*Escherichia coli*	985	29	103.4	Hsu et al. (2015)
AgaG1	*Alteromonas* sp. *GNUM-1*	Marine Algae	*Escherichia coli*	301	19	59	Chi et al. (2014)
YM01-3	*Catenovulum agarivorans YM01T*	Seawater	*E. coli BL21 (DE3)*	420	No signal peptide	46	Cui et al. (2014)
AgaH71	*Pseudoaltero-monas hodoen-sis H7*	Coastal seawater	*Escherichia coli*	290	21	59	Chi et al. (2015b)
AgaA	*Pseudoalte-romonas* sp. *AG52*	Red seaweed, *Gelidium amansii*	*Escherichia coli*	290	–	32	Oh et al. (2010a)
DagA	*Streptomyces coelicolor A3(2)*	Soil bacterium	*Streptomyces lividans TK24*	309	30	32	Temuujin et al. (2011)
GH 16 agarases							
agaG1	*Alteromonas* sp. *GNUM1*	Surface of *Sargassum serratifolium*	*E. coli BL21 (DE3)*	–	–	–	Seo et al. (2014)
aga672	*Aquimarina agarilytica ZC1*	Surface of marine red alga *Porphyra haitanensis*	*E. coli DH5α*	–	–	98	Lin et al. (2017)
Aga4436	*Flammeovirga* sp. *OC4*	Deep sea bacterium	*Escherichia coli*	456	23	51	Chen et al. (2016)
AgaTM2	*Simiduia* sp. *Strain TM-2*	Marine sediment	*E. coli BL21 (DE3)*	587	–	64	Tawara et al. (2015)
Agy1	*Saccharophagus* sp. *AG21*	Red seaweed *Gelidium amansii*	*Escherichia coli*	636	–	69	Lee et al. (2013)

Table 3 (continued)

Gene	Bacterium	Source of the bacterium	Host	Amino acids	Signal peptide (amino acids)	Molecular weight (kDa)	References
AgrP	*Pseudoaltero-monas* sp. *AG4*	Red algae, *Chondrus crispus*	*E. coli BL21* (*DE3*)	290	21	33	Oh et al. (2010b)
AgaM1	*Flammeovirga* sp. *MY04*	Mangrove sediment	*E. coli BL21* (*DE3*)	712	No signal peptide	80	Di et al. (2018)
GH39 endotype agarase							
AgaJ9 monomer	*Gayadomonas joobiniege G7*	Sea water	*E. coli ER2566*	1205	23	132	Jung et al. (2017b)
GH50 endotype agarase							
AgaW	*Cohnella* sp. *strain LGH*	Soil	*Escherichia coli*	891	26	97	Li et al. (2015)
HZ2	*Agarivorans* sp. *HZ105*	Marine sediment	*E. coli BL21* (*DE3*)	920	–	107	Lin et al. (2012)
AgaACN41	*Vibrio* sp. *strain CN41*	Coastal sediment	*E. coli BL21* (*DE3*)	990	42	110	Liao et al. (2011)
GH50 exotype agarase							
AgWH50C	*Agarivorans gilvus WH0801*	Fresh seaweed	*Escherichia coli*	740	–	82	Liu et al. (2014b)
Aga50D	*Saccharophagus degradans 2-40*	Marine bacterium	*Escherichia coli*	–	–	84	Kim et al. (2010)
GH50 exo and endo agarase							
AgaB1	*Thalassomonas agarivorans BCRC 17492*	Seawater	*Escherichia coli*	774	26	90	Liang et al. (2014)
Sco3487	*Streptomyces coelicolor A3(2)*	Soil bacterium	*Streptomyces lividans TK24*	798	45	83.9	Temuujin et al. (2012)
GH50 agarase							
AgWH50A	*Agarivorans gilvus WH0801*	Fresh seaweed	*E. coli BL21* (*DE3*)	942	21	105	Liu et al. (2014a)
GH86 agarase							
AgaA	*Cellvibrio* sp.	Activated sludges at municipal sewage plant	*E. coli DH5α*	713	No signal peptide	79	Osamu et al. (2012)
GH86 endotype agarase							
AgaP4383	*Flammeovirga pacifica WPAGA1*	Deep sea sediment	*Escherichia coli*	965	–	110	Hou et al. (2015)
AgaJ5	*Gayadomonas joobiniege G7*	Sea water	*E. coli ER2566*	805	30	89.278	Lee et al. (2018)
GH118 agarase							
AgaJA2	*Agarivorans* sp. *JA-1*	Seawater	*Escherichia coli*	478	38	48	Lee et al. (2012)
GH118 endotype agarase							
AgaXa	*Catenovulum* sp. *X3*	Seawater	*E. coli BL21* (*DE3*)	529	28	52	Xie et al. (2013)
Other unspecified agarases							
BN3	*Micrococcaceae* sp.	–	*Pichia pastoris GS115* (electro transformation)	–	–	Between 35 and 100	Xu et al. (2018)

Table 3 (continued)

Gene	Bacterium	Source of the bacterium	Host	Amino acids	Signal peptide (amino acids)	Molecular weight (kDa)	References
Aga50A, Aga50D and NABH	*Saccharophagus degradans*	*Gracilaria verrucosa*, a red algae	*E. coli BL21* (*DE3*)	–	–	87.8, 89.7 and 44.0	Kim et al. (2018)

An agarase from *Micrococcaceae* sp. was found to be stable at highly acidic pH of 3 and it was stable till 9 which could be considered as slightly basic, while 7 was its optimum pH. This agarase showed maximum activity at 45 °C and was stable below the same temperature (Xu et al. 2018). The agarases, AgaJ5 and AgJ11 from *G. joobiniege G7*, were the only agarases found to be optimum in acidic pH 4.5 with the stability between pH of 4–5.5 (Lee et al. 2018; Jung et al. 2017a, b). Furthermore, the agarase, AgaP4383 from *Flammeovirga pacifica WPAGA1*, showed optimum activity at alkaline pH 9 and found to stable between pH 5 and 10 (Hou et al. 2015). From mangrove sediments, an agarase AgaM1 was isolated from *Flammeovirga* sp. *MY04* and was overexpressed in *E. coli*. This agarase found to have a wide range of temperature stability from 30 to 60 °C with 50 °C as its optimum temperature. The pH stability was from 5 to 10 which can also be considered as broader range (Di et al. 2018). Interestingly one isolate, *Cellvibrio* sp., was from activated sludges and the agarase, AgaA, from this organism had an optimum pH of 6.5 and an optimum temperature of 42.5 °C. It was stable at temperatures lesser than 40 °C (Osamu et al. 2012).

Kinetic properties of the recombinant agarases and their degrees of degradation

The kinetic studies that were conducted using agarose as the only substrate are tabularized in Table 5. In addition, we have also discussed about a few reports where substrates like agar, seaweeds, and agar oligosaccharides like neoagarooctaoses (NA8), neoagarohexaoses (NA6), and neoagarotetraoses (NA4) that were used to discover the kinetics of the agarases. The specific activities of the reported β-agarases varied through a wide range from a minimum of 0.57 U/mg to a maximum value of 1418 U/mg. Enzyme activity (U) was defined as the amount of enzyme that liberated 1 µmol of reducing sugar per minute under the assay conditions. The high specific activity agarase, AgaH92, was from a marine bacterium *Pseudoalteromonas* sp. *H9 KCTC23887* that degraded agarose into NA4 and NA6 and utilized agar as the sole carbon source (Chi et al. 2015a, b).

As agar is the substrate for the agarase, the presence of agar in the cell wall of many macro algae allows it to act as a direct substrate other than agar. A few studies have been conducted using different macroalgae species as the substrate for agarase. The agarase AgaTM2 acted on seaweeds and degraded them into NA4 and NA6 (Tawara et al. 2015). In the study conducted by Hou et al. (2015), where *Gracilaria lemaneiformis* was used as the substrate for the recombinant agarase AgaP4383 exhibited a V_{max} of 21 U/mg and a K_M of 32.41 mg/ml where it produced NA4 and NA6 as the products. Many other different agarases such as AgaB, ID2563, Aga16B, Aga4436, AgaH92, AgaA, Aga2, YM01-3, Agy1, DagA, AgaM1, and AgrP also yielded NA4 and NA6 as the degradation product from the substrate agarose, and exceptionally, they were obtained as products by the action of AgrP on agar (Dong et al. 2016, Su et al. 2017; Kim et al. 2017; Chen et al. 2016; Chi et al. 2015a, b; Hsu et al. 2015; Ramos et al. 2018; Cui et al. 2014; Lee et al. 2013; Temuujin et al. 2011; Di et al. 2018; Oh et al. 2010). Of all the agarases, the K_M of this agarase, AgaP4383, was found to be higher which makes us understand that the binding affinity of this agarase with the substrate is lesser, as the substrate is not directly accessible to the enzyme to act on it. In the same study, agar was also used as the substrate and its kinetic properties were studied. The agar substrate had a K_M of 8.53 mg/ml which revealed that the binding affinity of the enzyme towards agar was greater than the seaweed substrate and its V_{max} was 102 U/mg where it clearly depicts that if the substrate is given directly the rate of the reaction would be higher when compared to the reaction rate of the seaweed degradation. The highest V_{max} of 1.14×10^4 U/mg was observed by the activity of YM01-3 whose K_M was 3.78 mg/ml (Cui et al. 2014).

In the study of degradation mechanism by AgaACN41 from a sediment bacterium *Vibrio* sp., *strain CN41* revealed that it can degrade agarose, NA8, and NA6 into neoagarotetraose (NA4) with a K_M of 3.54 mg/ml and a V_{max} of 3 U/mg (Liao et al. 2011). The other case is where agarose, NA6 and NA4 were provided as the substrate for Sco3487 where it yielded neoagarobiose (NA2) as product with a V_{max} of 10.75 U/mg and a K_M of 4.87 mg/ml (Temuujin et al. 2012). In a few cases, two different substrates were given and their kinetics was compared with each other. One among them was AgaJ9, where agarose and NA6 were given as substrates. The K_M values of agarose and NA6 were 1.43 mg/ml and 2.16 mg/ml, whereas V_{max} values of agarose and NA6 were 10.7 U/mg and 58.8 U/mg, respectively. AgaJ9 degraded agarose into NA2, NA4, and NA6, while NA6 was

Table 4 Physiochemical properties of recombinant β-agarases

Gene	Bacterium	Source of the bacterium	Optimum pH	Optimum temperature (°C)	pH stability	Temperature stability	References
YM01-1	*Catenovulum agarivorans YM01*	Marine bacterium	7	50	6–9	< 45 °C	An et al. (2018)
Aga2	*Cellulophaga omnivescoria W5C*	Marine bacterium	8	45	5–9	35–55 °C	Ramos et al. (2018)
BN3	*Micrococcaceae* sp.	–	7	45	3–9	< 45 °C	Xu et al. (2018)
Aga50A Aga 50D and NABH	*Saccharophagus degradans*	*Gracilaria verrucosa*, a red algae	7	37	6–9	< 40 °C	Kim et al. (2018)
agaB	*Flammeovirga* sp. *SJP92*	–	8	45	–	45–55 °C	Dong et al. (2016)
AgaP4383	*Flammeovirga pacifica WPAGA1*	Deep sea sediment	9	50	5–10	< 40 °C	Hou et al. (2015)
AgWH50A	*Agarivorans gilvus WH0801*	Fresh seaweed	6	30	6	20–40 °C	Liu et al. (2014a)
agaG1	*Alteromonas* sp. *GNUM1*	Surface of *Sargassum serratifolium*	7	40	5–8	37–45 °C	Seo et al. (2014)
AgaYT	*Flammeovirga yaeyamensis strain YT*	Surface of a red algae, *Gracilaria tenuistipitata*	8	40	–	–	Yang et al. (2011)
Aga672	*Aquimarina agarilytica ZC1*	Surface of marine red alga *Porphyra haitanensis*	7	25	7–11	< 40 °C	Lin et al. (2017)
AgaJ5	*Gayadomonas joobiniege G7*	Sea water	4.5	30	4.5–5.5	Retained 40% of enzymatic activity at 10 °C	Lee et al. (2018)
ID2563	*Microbulbifer* sp. *Q7*	Guts of sea cucumbers	6	40	6–9	30–60 °C	Su et al. (2017)
AgaJ9	*Gayadomonas joobiniege G7*	Sea water	5	25	4–8	< 30	Jung et al. (2017b)
AgJ11	*Gayadomonas joobiniege G7*	Coastal seawater	4.5	40	4–5	30–40 °C	Jung et al. (2017a)
Aga16B	*Saccharophagus degradans 2-40T*	Marine bacterium	7.5	55	5.5–8.5	45–60 °C	Kim et al. (2017)
Aga4436	*Flammeovirga* sp. *OC4*	Deep sea bacterium	6.5	50–55	5–10	30–80 °C	Chen et al. (2016)
AgaH92	*Pseudoalteromonas* sp. *H9 KCTC23887*	Marine bacterium	6	45	5–9	40–50 °C	Chi et al. (2015a)
AgaTM2	*Simiduia* sp. *Strain TM-2*	Marine sediment	8	35	–	–	Tawara et al. (2015)
AgaA	*Pseudomonas vesicularis MA103*	Seawater	–	16, 20, 24	–	–	Hsu et al. (2015)
AgaW	*Cohnella* sp. *strain LGH*	Soil	7	50	6–10	< 50 °C	Li et al. (2015)
AgWH50C	*Agarivorans gilvus WH0801*	Fresh seaweed	6	30	6–7	20–30 °C	Liu et al. (2014b)

Table 4 (continued)

Gene	Bacterium	Source of the bacterium	Optimum pH	Optimum temperature (°C)	pH stability	Temperature stability	References
AgaG1	*Alteromonas* sp. *GNUM-1*	Marine algae	7	40	7–8	25–45 °C	Chi et al. (2014)
YM01-3	*Catenovulum agarivorans YM01T*	Sea water	6	60	4–9	< 50 °C	Cui et al. (2014)
AgaH71	*Pseudoaltero-monas hodoen-sis H7*	Coastal sea water	6	45	5–9	45–50 °C	Chi et al. (2015b)
AgaB1	*Thalassomonas agarivorans BCRC 17,492*	Sea water	7.4	40	7–9	30–40 °C	Liang et al. (2014)
Agy1	*Saccharophagus* sp. *AG21*	Red seaweed *Gelidium amansii*	7.5	55	5–9	45–50 °C	Lee et al. (2013)
AgaXa	*Catenovulum* sp. *X3*	Sea water	7.4	52	5–9	< 42 °C	Xie et al. (2013)
AgaA	*Cellvibrio* sp.	Activated sludges at municipal sewage plant	6.5	42.5	–	< 40 °C	Osamu et al. (2012)
AgaJA2	*Agarivorans* sp. *JA-1*	Sea water	7	35	6–8	25–45 °C	Lee et al. (2012)
HZ2	*Agarivorans* sp. *HZ105*	Marine sediment	7	40	–	–	Lin et al. (2012)
Sco3487	*Streptomyces coe-licolor A3(2)*	Soil bacterium	7	40	Only at 7	20–40 °C	Temuujin et al. (2012)
AgaACN41	*Vibrio* sp. *strain CN41*	Coastal sediment	7.5	40	7–8.6	< 40 °C	Liao et al. (2011)
DagA	*Streptomyces coe-licolor A3(2)*	Soil bacterium	7	40	5–8	25–40 °C	Temuujin et al. (2011)
Aga50D	*Saccharophagus degradans 2-40*	Marine bacterium	7	30	6–9	< 40 °C	Kim et al. (2010)
AgrP	*Pseudoaltero-monas* sp. *AG4*	Red algae, *Chon-drus crispus*	5.5	55	4.5–8	< 55 °C	Oh et al. (2010b)
AgaA	*Pseudoaltero-monas* sp. *AG52*	Red seaweed, *Gelidium amansii*	5.5	55	4.5–9	40–60 °C	Oh et al. (2010a)
AgaM1	*Flammeovirga* sp. *MY04*	Mangrove sedi-ment	7	50	5–10	30–60 °C	Di et al. (2018)

degraded into NA4 and NA2 (Jung et al. 2017). The simple agar oligosaccharide neoagarobiose (NA2) was obtained as the only degradation product by the action of three enzymes: Aga50A, Aga50D, and NABH (neoagarobiose Hydrolase) in a systematic fashion and the specific activities of these enzymes were 11.96, 13.18, and 10.25 U/mg, respectively (Kim et al. 2018). Upon action of AgWH50C and Aga50D on agarose, they both yielded neoagarobiose (NA2) as the degradation product (Liu et al. 2014b; Kim et al. 2010). The action of AgrP on agarose also yielded NA2 (Oh et al. 2010). With a unique degradation property, AgaXa from marine bacterium *Catenovulum* sp. *X3* yielded various neoagaro oligosaccharides as products. Those included NA6, NA8, NA10, and NA12. This was the only recombinant agarase to produce a wide variety of degradation products among all the agarases (Xie et al. 2013).

Effect of metal ions on β-agarase activity

The effect of various metal ions on the activity of recombinant agarases is presented in Table 6. Some of the distinctive effects of those metal ions are explained in detail below. The Ca^{2+} ions found to act as a thermo stability enhancer for a few agarases such as AgaTM2 from *Simiduia* sp. *Strain TM-2*, Agy1 from *Saccharophagus* sp. *AG21*, and for AgaA

Table 5 Kinetic properties of agarose degrading recombinant β-agarases

Gene	Hydrolysis product	K_m (mg/ml)	V_{max} (U/mg)	References
Aga2	NA4 and NA6	2.59	275.48	Ramos et al. (2018)
Aga50A, Aga 50D and NABH	NA2 (by all 3 systematically)	–	–	Kim et al. (2018)
AgaB	NA4 and NA6	3.99	700	Dong et al. (2016)
AgWH50A	NA4	5.97	0.781	Liu et al. (2014a)
AgaG1	NA2 and NA4	–	–	Seo et al. (2014)
AgaYT	NA2 and NA4	–	–	Yang et al. (2011)
Aga672	NA4, NA6, and NA8	59.8	154.3	Lin et al. (2017)
AgaJ5	NA6 (NA2 &NA4 in minor amounts)	8.9	188.6	Lee et al. (2018)
ID2563	NA4 and NA6	–	–	Su et al. (2017)
AgaJ9	NA2, NA4, and NA6	0.68	17.2	Jung et al. (2017b)
AgaJ11	NA2, NA4, and NA6	21.42	25	Jung et al. (2017a)
Aga16B	NA4 and NA6	7.7	18.3	Kim et al. (2017)
Aga4436	NA4 and NA6	–	–	Chen et al. (2016)
AgaH92	NA4 and NA6	–	–	Chi et al. (2015a)
AgaA	NA4 and NA6	–	–	Hsu et al. (2015)
AgaW	NA2 and NA4	3.43	387.11	Li et al. (2015)
AgWH50C	NA2	12.55	1.17	Liu et al. (2014b)
AgaG1	NA2 and NA4	3.74	23.8	Chi et al. (2014)
YM01-3	NA4 and NA6	3.78	1.14×10^4	Cui et al. (2014)
AgaH71	NA2, NA4, and NA6	28.33	88.25	Chi et al. (2015b)
AgaB1	NA2 (NA4 and NA6 very few amount)	–	–	Liang et al. (2014)
Agy1	NA4 and NA6	–	–	Lee et al. (2013)
AgaXa	NA6, NA8, NA10, and NA12	10.5	588.2	Xie et al. (2013)
AgaA	NA2, NA4, and NA6 (small amount)	–	–	Osamu et al. (2012)
AgaJA2	NA8	–	–	Lee et al. (2012)
HZ2	NA4	5.9	235	Lin et al. (2012)
Sco3487	NA4 and NA6	2.18	39.06	Temuujin et al. (2011)
Aga50D	NA2	41.9	17.9	Kim et al. (2010)
AgaM1	NA4 and NA6	1.82	357.14	Di et al. (2018)

from *Pseudoalteromonas* sp. *AG52*. This may be anticipated on account of the binding of Ca^{2+} ions to the catalytic cleft that is suspected to be present opposite to the substrate-binding site (Tawara et al. 2015; Lee et al. 2013; Oh et al. 2010a, b). And in the case of Ca^{2+} ions, it is supposed that its binding is essential for the agarase of GH16 family for its active form formation. The effect if Guanidine-HCl on AgaM1 was studied and the results showed that it increased the activity of the enzyme (Di et al. 2018). The protein denaturant urea contradictorily did not affect the agarases ID2563, YM01-1, and YM01-3 (Rajagopalan et al. 1961). In point of fact, the agarase ID2563 was resistant to urea and the agarase activity of YM01-1 and YM01-3 was not affected by urea (Su et al. 2017; An et al. 2018; Cui et al. 2014). However, the presence of urea negatively influenced the agarase AgaM1 (Di et al. 2018). The monovalent cation Ni^{+} hardly had any effect on the agarases YM01-1 and AgaJ9 (An et al. 2018; Jung et al. 2017).

A handful of metal ions positively influenced the activity of a few β-agarases. The redox reagent dithiothreitol (DTT) had stimulatory effects on the agarases AgaB, AgaW, AgaXa, AgaM1, and HZ2 (Dong et al. 2016; Li et al. 2015; Xie et al. 2013; Di et al. 2018; Lin et al. 2012). At higher concentrations of 9 mM and 10 mM, DTT increased the activity of HZ2 and AgaXa respectively (Lin et al. 2012; Xie et al. 2013). Outstandingly, this DTT did not affect the activity of any agarase negatively. The reducing effect of the DTT might have contributed in the prevention of the thiol oxidation into a disulfide bond which is present at the catalytic site of the enzyme. In the presence of 200 mM of NaCl, AgaB1 exhibited its maximum activity (Liang et al. 2014). Furthermore, the Na^{+} and K^{+} being present in the reaction mixture slightly activated the agarase activity of AgaJ5 (Lee et al. 2018).

Over and above, some of the metal ions had inhibitory or negative effects on the enzyme activity of the agarases.

Table 6 Effect of different metal ions on recombinant β-agarases

Gene	Ca^{2+}	Mg^{2+}	K^{+}	Fe^{3+}	Fe^{2+}	Zn^{2+}	Na^{+}	Ni^{2+}	Cu^{2+}	Mn^{2+}	Co^{2+}	SDS	EDTA	β-Me	References
YM01-1	ST	ST	ST	IB	–	–	NE	–	IB	IB	IB	NE	–	–	An et al. (2018)
Aga2	–	–	–	–	IB	ST	–	–	IB	IB	–	–	–	–	Ramos et al. (2018)
agaB	–	–	IB	IB	–	IB	–	–	IB	IB	IB	–	10 mM IB, 1 mM ST	1 mM ST, 10 mM IC	Dong et al. (2016)
AgaP4383	–	–	–	–	–	–	–	–	PIB	ST	ST	–	1 mM PIB, 30 mM TIB	NE	Hou et al. (2015)
AgWH50A	–	–	–	IB	–	–	–	–	TIB	IB	–	–	IB	–	Liu et al. (2014a)
ID2563	–	–	–	TIB	ST	TIB	ST	–	TIB	–	–	NE	–	ST	Su et al. (2017)
AgaJ9	IB	IB	NE	–	PIB	IB	NE	PIB	TIB	PIB	IB	–	PIB	–	Jung et al. (2017b)
AgJ11	TIB	–	–	–	TIB	TIB	–	PIB	TIB	TIB	PIB	–	IB	–	Jung et al. (2017a)
Aga4436	IB	IB	IB	–	IB	–	–	IB	IB	ST	ST	–	TIB	–	Chen et al. (2016)
AgaH92	–	IB	–	–	ST	NE	–	IB	NE	NE	–	–	IB	–	Chi et al. (2015a)
AgaW	ST	ST	ST	IB	–	IB	ST	–	PIB	IB	–	PIB	IB	–	Li et al. (2015)
AgWH50C	–	ST	–	–	–	–	–	–	TIB	ST	–	–	IB	–	Liu et al. (2014b)
AgaG1	1 mM SIB, 5 mM TIB	PIB	NE	–	–	1 mM PIB, 5 mM SIB	NE	–	–	1 mM SIB, 5 mM TIB	ST	–	NE	–	Chi et al. (2014)
YM01-3	ST	NE	ST	IB	–	–	ST	IB	IB	IB	–	NE	NE	–	Cui et al. (2014)
AgaH71	PIB	PIB	–	–	PIB	TIB	–	PIB	TIB	TIB	TIB	1 mM NE, 10 mM SIB	IB	–	Chi et al. (2015b)
Agy1	Increased TS	–	ST	–	ST	IB	ST	–	NE	IB	–	–	–	–	Lee et al. (2013)
AgaXa	–	ST	–	TIB	–	–	–	–	TIB	TIB	–	TIB	–	ST	Xie et al. (2013)
HZ2	IB	IB	IB	–	–	–	IB	–	–	–	–	IB	–	ST	Lin et al. (2012)
Sco3487	–	–	–	–	–	–	ST	–	–	TIB	ST	–	IB	–	Temuujin et al. (2012)

Table 6 (continued)

Gene	Ca^{2+}	Mg^{2+}	K^{+}	Fe^{3+}	Fe^{2+}	Zn^{2+}	Na^{+}	Ni^{2+}	Cu^{2+}	Mn^{2+}	Co^{2+}	SDS	EDTA	β-Me	References
DagA	0.2 mM NE, 1–10 mM IB	0.2 mM NE, 1–10 mM IB	–	–	–	0.2 mM NE, 1–10 mM IB		0.2 mM NE, 1–10 mM IB	TIB	TIB	ST	–	0.2 mM NE, 1–10 mM IB (0.2 mM of Co^{2+} recovered activity)	–	Temuujin et al. (2011)
Aga50D	10 mM NE, 100 mM activated	NE	10 mM NE, 100 mM slight IB	–	IB	IB	NE	–	IB	ST	–	–	–	–	Kim et al. (2010)
AgrP	ST	ST	ST	–	ST	IB	ST	–	IB	IB	–	–	IB	–	Oh et al. (2010b)
AgaA	2 mM Enhanced TS	–	ST	–	ST	TIB	–	–	TIB	–	–	–	IB	–	Oh et al. (2010a)
AgaM1	–	–	–	–	–	–	–	–	IB	–	–	IB	–	IB	Di et al. (2018)

β-Me β-mercaptoethanol, *ST* stimulated, *IC* increased, *IB* inhibited, *PIB* partial inhibition, *TIB* total inhibition, *SIB* strong inhibition, *NE* no effect, *TS* thermo stability

The partial inhibition of agarase activity by Ba^{2+} ions was detected in AgaW (Li et al. 2015). The Sr^{+} and Ba^{2+} ions inhibited the activity of Aga4436 (Chen et al. 2016). The occurrence of $CuCl_2$ and $FeCl_2$ decreased the activity of agarase from the marine bacterium *Saccharophagus degradans 2-40T* (Kim et al. 2017). A strong inhibition of enzyme activity on AgaXa and AgaP4383 was noticed by the presence of Al^{3+} and Cd^{2+} ions, respectively (Xie et al. 2013; Hou et al. 2015).

The recombinant agarase DagA from the soil bacterium *Streptomyces coelicolor A3* (*2*) exhibited assorted activities while varying the concentrations of different metal ions. On top of that, this agarase was not affected by Ca^{2+}, Mg^{2+}, Ni^{2+}, Zn^{2+}, EDTA (ethylene diamine tetra acetic acid), and EGTA (ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetra acetic acid) at a concentration of 0.2 mM. Under other concentrations starting from 1 to 10 mM, all the ions inhibited the activity and remarkably the presence of 0.2 mM of Co^{2+} totally recuperated the inhibitory effects of chelators (Temuujin et al. 2011). The effect of calcium chelating agent EGTA was studied by the same author on a different agarase Sco3487 and found that it inhibited the activity of the enzyme. The presence of monovalent silver cation, Ag^{+}, strongly inhibited the activity of the agarase reported by Hou et al. (2015) and Di et al. (2018). And notably, the recombinant agarase Aga672 from *Aquimarina agarilytica ZC1* did not require any metal ions or reagents to increase its activity (Lin et al. 2017).

Conclusion

In this review, we have attempted to report and discuss about the various properties and characteristics of the recombinant β-agarases those were stated after 2010 to till date. Most of the recombinant β-agarases were from marine sources and belonged to GH16, GH39, GH50, GH86, and GH118 families. The β-agarase genes isolated were cloned and were almost over expressed in *E. coli* as the host system. The stability and optimum activity of the enzyme was found to be in a wider pH and temperatures. This large heterogeneity observed in molecular, physiochemical, and catalytic characteristics of these agarases make them suitable for variety of industrial applications and increase its share in the enzyme market.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest for this article.

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