



Marine Polysaccharide Sulfatases

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Polysaccharides are the most abundant and the most complex organic molecules in the ocean. In contrast to land polysaccharides, many marine polysaccharides are highly sulfated; in particular, the cell walls of macroalgae harbor a high diversity of sulfated polysaccharides (carrageenans, agarans, fucoidans, ulvans, etc.). These sulfated polysaccharides, biosynthesized by macroalgal primary producers, represent an important food source for heterotrophic organisms. Their biodegradation requires a set of enzymes that can cleave the glycosidic linkages of the carbohydrate backbone (called glycoside hydrolases) and the sulfate ester groups (called polysaccharide sulfatases). This review first provides an overview of the current state of knowledge on the classification and mechanisms of sulfatases in general. Then, based on an exploration of marine genomic and metagenomics data that reveals the diversity of carbohydrate sulfatases, it focuses on strategies to predict these sulfatases. In particular, the modularity of sulfatases and their location in marine polysaccharide utilization loci (PUL) provide clues as to their potential substrates and can drive future functional assays. Finally, the review underscores the low number of currently biochemically characterized marine carbohydrate sulfatases (e.g., agarases, carrageenases, and fucose sulfatases). Bottlenecks encountered in studies on sulfatases likely lie in the difficulties in purifying them and producing them in heterologous systems.

Keywords: sulfatases, polysaccharides, polysaccharide utilization loci, module, carrageenan, agars, genomic

INTRODUCTION

After carbon, oxygen, and nitrogen, sulfur is the most abundant element in living organisms. Sulfur participates in the composition of cysteine and methionine, which are key amino acids for protein folding (e.g., disulfide bonds) and essential for the reactivity of many enzymes as catalytic amino acids or complexing cofactors (e.g., iron-sulfur cofactors). Sulfur is also found in vitamins, steroids, and many carbohydrates including polysaccharides, proteoglycans, and glycolipids. Sulfur is assimilated by living organisms in the form of inorganic sulfate ions, which are the second most abundant anions in seawater. The concentration of sulfate ions in the oceans ranges from 25 to 28 mM; these values are very high compared with those found in freshwater and soil (10–50 μ M) (Wright and Colling, 1995; Friedlander, 2001; Bochenek et al., 2013).

The abundance of sulfate ions and its ubiquity has resulted in a wide distribution of sulfated molecules and more especially, sulfated polysaccharides in marine organisms. In contrast to land polysaccharides, a huge number of marine polysaccharides are decorated with sulfate ester groups. Except for sulfated glycosaminoglycans such as chondroitin sulfate or heparan sulfate found in the matrix of animal cells, anionic polysaccharides found in terrestrial organisms carry uronic groups (i.e., pectins, hyaluronic acid, polysialic acids). The occurrence of sulfated polysaccharides in macroalgae, marine angiosperms, and seagrasses has been proposed to be an adaptation to marine environments (Aquino et al., 2005; Olsen et al., 2016), suggesting that the sulfation

of polysaccharides is a marker of marine origins. Nevertheless, in all cases, the biosynthesis of sulfated polysaccharides requires the activation of sulfate in PAPS (3'-phosphoadenosine-5'-phosphosulfate), catalyzed by PAPS synthase, and then the grafting of a sulfate ester group onto the carbohydrate via a specific sulfotransferase (Klaassen and Boles, 1997; Zhang et al., 1998).

The most studied sulfated marine polysaccharides are those extracted from the cell walls of macroalgae due to the industrial interest in their hydrocolloid properties. Agarans and carrageenans are the most exploited polysaccharides from red algae and the wide diversity of red algae polysaccharides has been reviewed recently (Usov, 2011). Similarly, the sulfated polysaccharides extracted from brown and green algae also show high diversity. Several reviews have highlighted their structural complexity and their potential applications (see, for example, Kloareg and Quatrano, 1988; Berteau and Mulloy, 2003; Lahaye and Robic, 2007; Pomin and Mourão, 2008). The diversity of sulfated polysaccharides biosynthesized by marine microorganisms, such as bacteria and microalgae, has been less explored so far. However, sulfate groups were found to decorate the carbohydrate backbone of several secreted polysaccharides and matrix lipo-polysaccharides biosynthesized by marine bacteria (Mancuso Nichols et al., 2005; Nazarenko et al., 2011). Composition analyses attested also the structural diversity of sulfated polysaccharides biosynthesized in microalgae but only a few polysaccharide structures have been resolved (Hoagland et al., 1993; Gügi et al., 2015). For example, studies and structural analyses of the main cell-wall polysaccharide in diatoms—a group of ecologically important marine organisms—suggest that it is a sulfated glucuronmannan (Percival and McDowell, 1967; Willis et al., 2013).

Marine polysaccharides make up a large part of algal biomass and are a food source for heterotrophic organisms. This reservoir of sulfated polysaccharides are produced by concomitant fixation of sulfur and carbon by photosynthetic organisms. Enzymatic degradation of complex sulfated polysaccharides requires a set of enzymes that can cleave the glycosidic bond and remove any decorations, such as sulfate groups, from the carbohydrate backbone. Specific glycoside hydrolases or polysaccharide lyases depolymerize marine polysaccharides break the glycosidic bonds via hydrolysis and β -elimination mechanisms, respectively. Concerted action with sulfatases can result in the production of neutral mono- and oligosaccharides then used for energy consumption.

The avalanche of gene sequences produced during the numerous marine genomics and metagenomics programs have revealed the important role of sulfate in marine biology (Glöckner et al., 2003; Teeling et al., 2012). Genomes of polysaccharide-degrading bacteria reveal the co-occurrence of glycoside hydrolases and polysaccharide lyases with sulfatases. More interestingly, in the bacterial genus *Bacteroidetes* known to include many polysaccharide-degrading strains, all the genes coding for polysaccharide-degrading enzymes leading to the saccharification of sulfated polysaccharides are clustered in the genome in so-called “polysaccharide utilization loci” (PUL) (Sonnenburg et al., 2006; Flint et al., 2008; White et al., 2014). More recently, the discovery of marine polysaccharide-degrading

enzymes in *Bacteroidetes* strains found in the microbiota of the human gut—likely acquired by gene transfer from marine organisms associated with seafood (Hehemann et al., 2010)—suggest that the investigation of the enzymatic degradation of marine polysaccharides goes beyond the ecological understanding of the ocean cycle, and also involves human health. Likewise, genomic analyses of human microbiota have revealed numerous PULs with sulfatase genes involved in the degradation of many sulfated polysaccharides, probably of marine origin.

Therefore, the purpose of this review is to summarize the work dealing with marine polysaccharide sulfatases starting with a short introduction to sulfatase classification, followed by a highlight on the diversity of marine polysaccharides sulfatases revealed by genomic data mining and then a description of the marine polysaccharide sulfatases that have been biochemically characterized to date. Glycosaminoglycan sulfatases will not be considered here, although several chondroitin or heparin sulfatases have been identified in marine prokaryotes (Han et al., 2014; Wang et al., 2015a).

MECHANISM AND STRUCTURE OF SULFATASES

Sulfatases are grouped into four classes based on sequence homology, crystallographic structure and mechanisms. Type I sulfatase—or formylglycine-dependent sulfatase—encompasses the vast majority of the known sulfatases and to date, all the biochemically characterized carbohydrate sulfatases (Hanson et al., 2004). These sulfatases require post-translational conversion of a cysteine or a serine into a formylglycine amino acid residue essential for catalysis. The oxidation of amino acids is catalyzed by a formylglycine-generating enzyme (FGE), also named sulfatase-modifying factor 1 (SUMF1), which recognizes the consensus amino acid sequence C/S-X-P-X-R (Cosma et al., 2003; Dierks et al., 2003; Sardiello et al., 2005; Bojarová and Williams, 2008).

In anaerobic bacteria, cysteine and serine residues can be modified by another family of enzymes called anaerobic sulfatase-maturing enzymes (anSMEs) (Berteau et al., 2006). Crystallographic structure of FGE (Bond et al., 1997; von Bülow et al., 2001) and anSME (Goldman et al., 2013) demonstrate that their mechanisms differ in the use or non-use of oxygen molecules, respectively. A recent review covers the state of knowledge on these enzymes as well as their potential biotechnological applications (Appel and Bertozzi, 2015).

Based on inhibition experiments and the analysis of the crystal structure of sulfatase complexes, two mechanisms of desulfation have been proposed, differing essentially in the hydration state of the formylglycine residue at the beginning of the reaction (Bond et al., 1997; Lukatela et al., 1998). However, both mechanisms generally agree that the last steps of the reaction involve a covalent bond between the sulfate anion and the formylglycine amino acid, and the regeneration of formylglycine by hydrolysis (**Figure 1**). The reaction results in the retention of the carbon configuration, the chirality of the carbon center being unaffected by the desulfation catalysis (Williams et al., 2014; **Figure 2**).

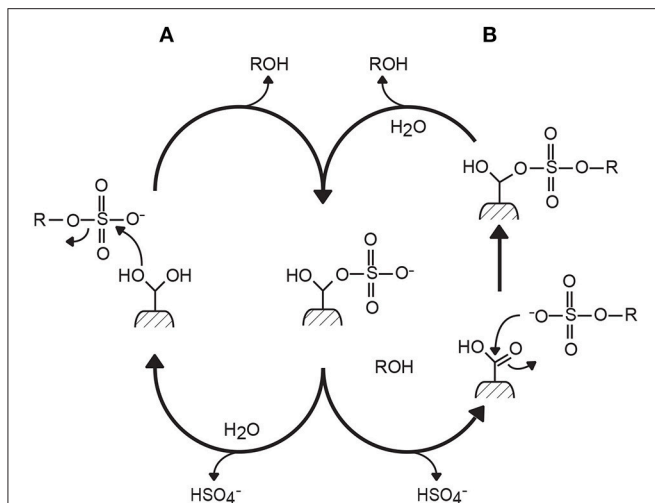


FIGURE 1 | Two mechanisms of action proposed for formylglycine-dependent sulfatases (the type I sulfatase family).

Mechanisms (A,B) differ by the hydration state at the beginning of the reaction. However, they both lead to the formation of a covalent intermediate between the sulfate ester group and formylglycine amino acid (Bond et al., 1997; Lukatela et al., 1998)

Type II sulfatase encompasses Fe(II) α -ketoglutarate-dependent alkylsulfatases, which belong to the dioxygenase superfamily (Müller et al., 2004). The catalysis requires molecular oxygen (O_2) and α -keto-glutarate as a co-substrate and leads to the oxidation of the primary sulfate ester group in the aldehyde and concomitantly to the oxidative decarboxylation of the α -keto-glutarate into succinate. Type III sulfatases are represented by enzymes related to Zn^{2+} - or Mn^{2+} -dependent metallo- β -lactamases. The cleavage of the C-OS bond occurs via a nucleophilic attack of the carbon by a molecule of water activated by the binuclear metal ion complex located in the active site (Hagelueken et al., 2006). The loss of the sulfate group results in the inversion of the configuration of the carbon (Figure 2). No carbohydrate sulfatases fall in the type II or type III sulfatase categories.

Two other sets of enzymes represent another potential sulfatase families. One sulfatase belonging to the superfamily of amidohydrolases has been purified from the marine bacteria *Pseudoalteromonas carrageenovora*; the recombinantly expressed enzyme is active on a synthetic substrate (i.e., methylemberliferyl sulfate), but showed less activity than the native purified enzyme (Genicot et al., 2014). The galactose-6-sulfurylase (discussed below in Section Predicted marine polysaccharide sulfatases) catalyzes the formation of anhydrogalactose concomitantly to the release of the sulfate group (Rees, 1961a,b).

PREDICTED MARINE POLYSACCHARIDE SULFATASES

The massive compilation of gene sequences through the numerous genome and metagenome sequencing programs has led to an exponential increase in the number of putative proteins

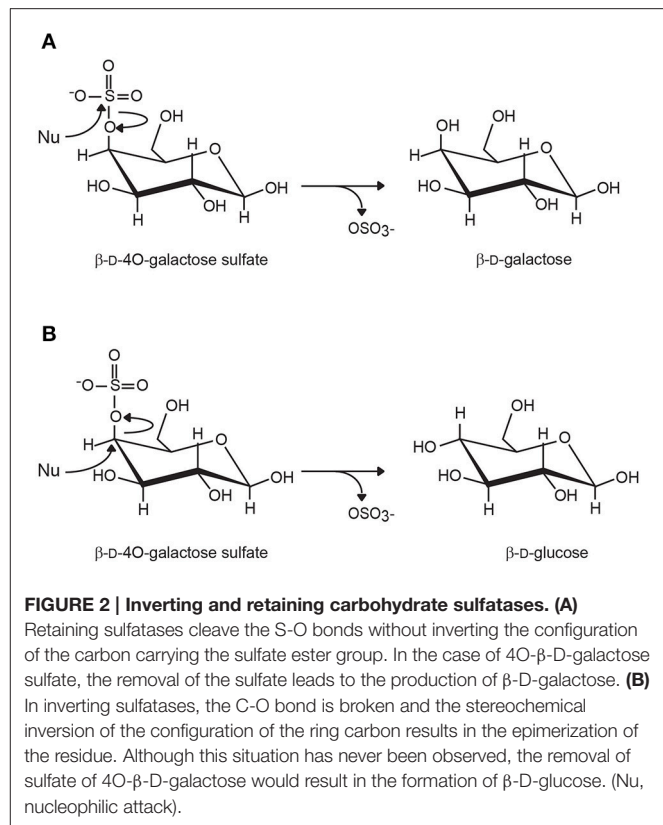


FIGURE 2 | Inverting and retaining carbohydrate sulfatases. (A)

Retaining sulfatases cleave the S-O bonds without inverting the configuration of the carbon carrying the sulfate ester group. In the case of 4O- β -D-galactose sulfate, the removal of the sulfate leads to the production of β -D-galactose. (B) In inverting sulfatases, the C-O bond is broken and the stereochemical inversion of the configuration of the ring carbon results in the epimerization of the residue. Although this situation has never been observed, the removal of sulfate of 4O- β -D-galactose would result in the formation of β -D-glucose. (Nu, nucleophilic attack).

whose functions have, for the most part, not yet been determined (Hanson et al., 2010). Despite the development of bioinformatics tools, prediction of protein function requires experimental validation, including a comprehensive overview of metabolic pathways and functional characterization of the proteins. Like other classes of proteins, sulfatases have seen the number of predicted genes increase exponentially in databases, particularly owing to the massive sequencing of marine organisms. The precise function of the sulfatases, such as the nature of their sulfated substrates (carbohydrate, lipids, metabolites, etc.), cannot be predicted based on sequence comparison with biochemically characterized sulfatases due to the lack of data. Based on the surge in sulfatase gene sequences and their genomic environment, probable function and preferred substrates of carbohydrate sulfatases can be suspected.

Modular Sulfatases

Most sulfatases are made of one catalytic module; however, in rare cases, two sulfatase modules are found in the same protein, both having the predicted catalytic amino acid residues. More interestingly, some proteins are multi-modular, containing one sulfatase catalytic module and one glycoside hydrolase catalytic module. For example, the protein found in the bacterium *Nonlabens ulvanivorans*, combines two catalytic modules, one attributed to a sulfatase, and the other to a glycoside hydrolase belonging to family GH78 (rhamnosidases). This organization reflects a genomic rearrangement adapted to the degradation of ulvan, the sulfated cell-wall matrix polysaccharide found

in green algae of the genera *Ulva* and *Enteromorpha* (Kopel et al., 2014). Ulvan, composed of 3O-sulfate-rhamnose, is likely more efficiently degraded by a bi-modular protein. Similarly, the bi-modular protein found in *Flammeovirga* sp. SJP92 genome (GenBank accession no. PRJNA306821), composed of a xylosidase (GH10) linked to a sulfatase, suggests evolution for optimized degradation of sulfated xylan, found especially in the cell walls of red algae.

A rapid survey of the Uniprot (The UniProt Consortium, 2015) and CAZy databases (Terrapon et al., 2015) revealed several modular sulfatases (Figure 3), suggesting that a thorough analysis of database should multiply examples of modular sulfatases. Observations of these modular proteins indicate that the preferred substrate of these sulfatases is a sulfated carbohydrate and provide strong clues as to the sugar residues involved.

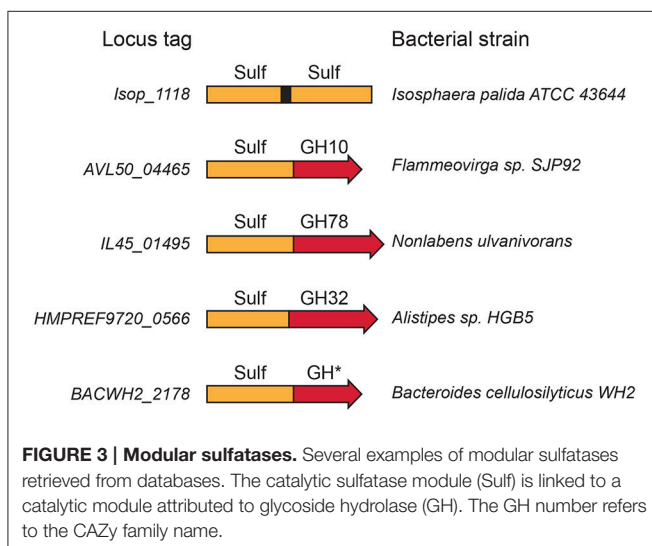
Sulfated Polysaccharide Utilization Loci

PULs are clusters of genes co-localized in the genome that encode proteins involved in the same polysaccharide-degradation pathway, starting from the detection of the polysaccharide to its depolymerization and uptake of the degradation products (Flint et al., 2008; White et al., 2014; Terrapon et al., 2015). PULs are found in *Bacteroidetes*, which are recognized as important polysaccharide degraders given the number polysaccharide-degrading enzymes found in their genomes. The starch utilization loci of the human gut symbiont *Bacteroidetes thetaiotaomicron* was the first PUL to be described (Anderson and Salyers, 1989). Starch is first bound by the outer membrane SusD protein, and then undergoes partial hydrolysis by an amylase, SusG. The malto-oligosaccharides are then imported into the cell by SusC, which is a TonB-dependent transporter. The complete degradation of malto-oligosaccharides is ensured by SusA and SusB, located in the periplasm. This gene organization is used as paradigm for the prediction of other PULs and always include proteins that are homologous

to SusC and SusD and a series of polysaccharide-degrading enzymes.

Because protein expression of PUL is co-regulated, transcriptomic methods can monitor the expression level of proteins of the targeted PUL when the bacteria are grown in presence of selected polysaccharides, thereby identifying the function of the identified PUL (Martens et al., 2008, 2011; McNulty et al., 2013; Despres et al., 2016). Co-expression of proteins located in the same PUL by carbohydrate inducers validate the PUL organization and give some indication as to the putative polysaccharides metabolized *in vivo*. PULs found in *Bacteroidetes* of the human microbiota have been recorded in the CAZy database (<http://www.cazy.org/PULDB/>) and experimentally analyzed PULs are also included (Terrapon et al., 2015). Many PULs, including the experimentally analyzed ones, contain sulfatases and glycoside hydrolases (or polysaccharide lyases), suggesting that these sulfatases are active on carbohydrates.

The first documented marine PUL includes proteins involved in the degradation of porphyran (the biosynthetic precursor of agarose; Figure 4). It contains several glycoside hydrolases, sulfatases, and a set of proteins of unknown function. This PUL has been transferred laterally from marine *Bacteroidetes* to human microbiota *Bacteroidetes* (Hehemann et al., 2010). Many other PULs dedicated to the degradation of sulfated polysaccharides have been observed in several bacteria genomes or metagenomes (Gómez-Pereira et al., 2012; Mann et al., 2013; Kabisch et al., 2014; Hahnke et al., 2015; Xing et al., 2015; Pansch et al., 2016; Sun et al., 2016). The functions of PULs are hypothesized based on sequence homology of glycoside hydrolases found in the PUL with characterized enzymes. Then, the presumed enzyme activities encountered in the PUL are compared with the literature available on the composition or structure of marine polysaccharides to attribute the putative metabolized polysaccharide to the PUL. Additional information, such as the location where the *Bacteroidetes* strains have been isolated or sequenced (e.g., surface of an algae, microalgae bloom) may also help refine the inference.



BIOCHEMICALLY CHARACTERIZED MARINE POLYSACCHARIDE SULFATASES

Agaran and Carrageenan Sulfatases

Agarans and carrageenans are found in the cell wall of many red algae (Rhodophyta). They are sulfated galactans made of D-galactose alternately linked by α - and β - glycosidic bonds. In agarans, the α -linked galactose belongs to the L series. These polysaccharides are classified according the position and number of sulfate ester groups and the occurrence of α -anhydrogalactose. The best-known agaran is agarose, appreciated for its gelling properties. Other agarans, such as the less-studied porphyran or funoran, are found in many species of red algae. The diversity of carrageenans is very high, based on the high number of different repetition moieties that have been reported; however, κ - (kappa-), ι - (iota-), hybrid κ -/ ι - (also called κ 2-carrageenan), and λ - (lambda-) carrageenans are the most frequent marine

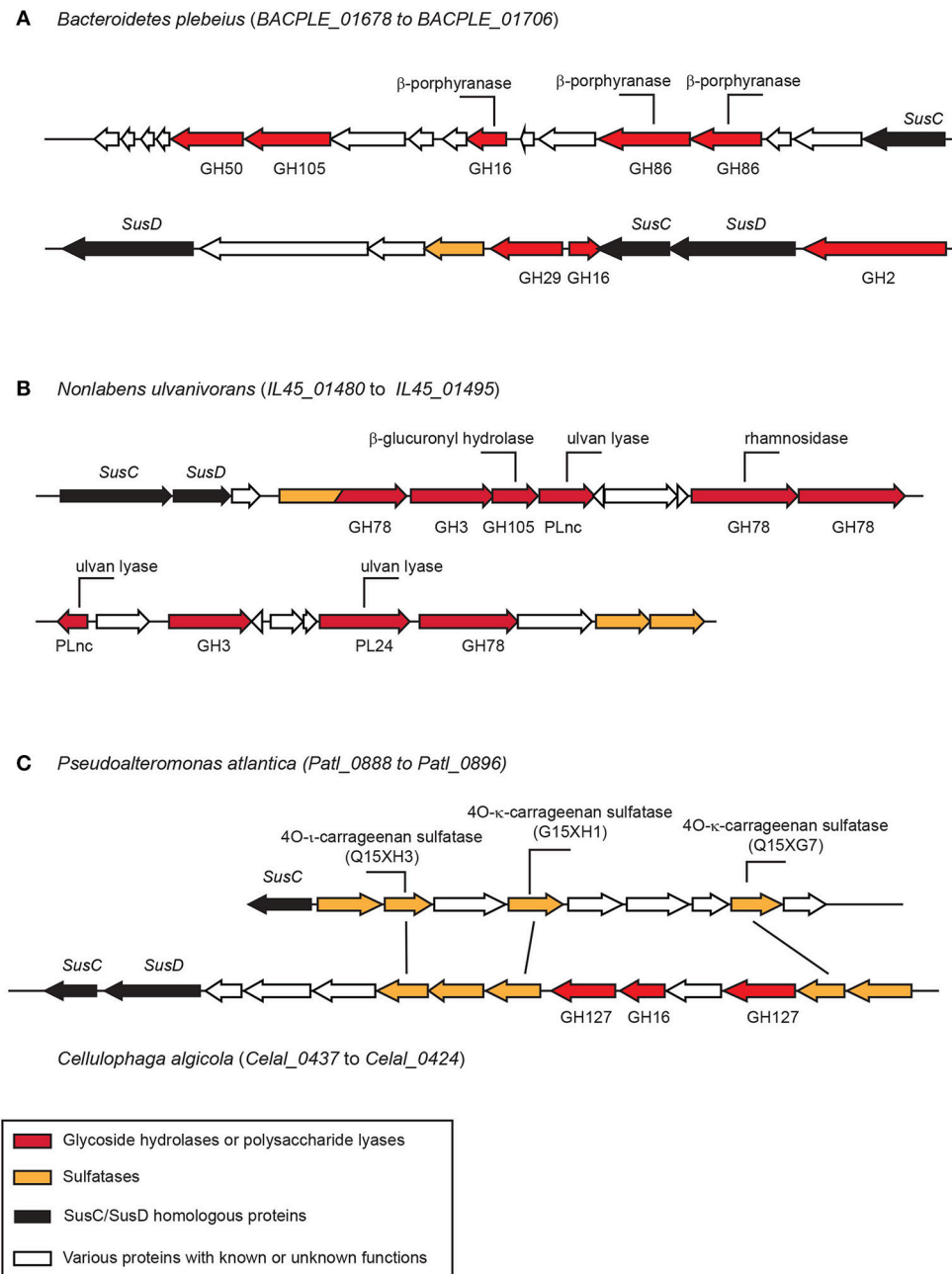


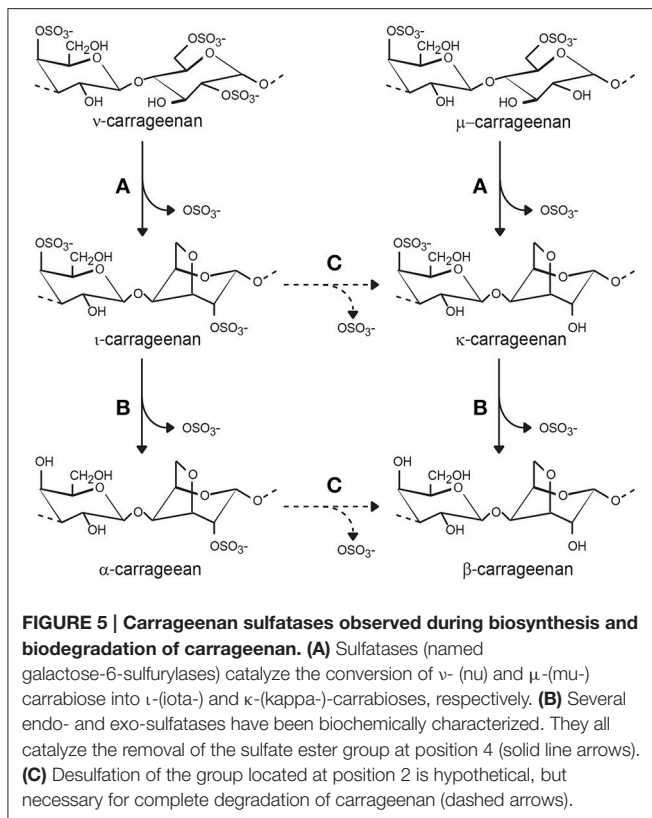
FIGURE 4 | Examples of polysaccharide utilization loci (PUL) containing sulfatases. (A) The porphyranase PUL in *Bacteroidetes plebeius* found in the human microbiota (Hehemann et al., 2012). **(B)** Ulva degradation PUL observed in *Nonlabens ulvanivorans* isolated from the feces of *Aplysia* sp. (Kopel et al., 2014). **(C)** Carrageenan PUL in *Cellulophaga algicola*. The sulfatases found in this PUL have strong homologies with the biochemically characterized sulfatase of *Pseudolateromonas atlantica* T6c (Préchoux et al., 2013, 2016). The function of the biochemically characterized enzymes are indicated on their corresponding genes.

polysaccharides in industry, employed as gelling, or thickening ingredients (Usov, 2011).

Enzymatic degradation of red algal polysaccharides by glycoside hydrolases which cleave the glycosidic bonds have been observed in a wide diversity of marine bacteria (Michel et al., 2006). Porphyranases have also been found in the genome in human gut microbiota *Bacteroidetes* strains. Surprisingly,

agaran- and carrageenan-degrading enzymes (i.e., agarases, porphyranases, or carrageenases) are not predicted from the genome sequence analysis of agarophyte and carrageenophyte algae (Bhattacharya et al., 2013; Collén et al., 2013).

Despite the high number of described agaran- and carrageenan-depolymerizing enzymes, only a few sulfatases have been biochemically characterized. They are all observed



in marine bacteria and as for agarases and carrageenases, no sulfatases have been predicted from the analysis of red algae genomes (Ho, 2015).

The pioneering work of Weigl and Yaphe (1966) demonstrated the first carrageenan sulfatase activity in protein extracts prepared from the marine bacterium *P. carrageenovora*. Ten years later, this enzyme—a 4O- κ -carrabiose sulfatase of about 55 kDa—was purified and NMR revealed an exo-type mode of action. The sulfate ester group located at the non-reducing end of oligo- κ -carrageenans produced by *P. carrageenovora* κ -carrageenase are specifically eliminated, suggesting that the sulfatase intervenes after enzymatic depolymerization of κ -carrageenan (McLean and Williamson, 1979, 1981).

More recently, a similar approach was undertaken with the carrageenolytic strain *Pseudoalteromonas atlantica* T6c whose genome has been sequenced (Copeland et al., 2006). An endo- ι -carrageenan-sulfatase active on ι -carrageenan was isolated and biochemically characterized. The enzymes catalyzes the specific removal of the sulfate at position 4 of the β -linked galactose resulting in the conversion of ι -carrabiose into α - (alpha-) carrabiose repetition units (Figure 5) (Préchoux et al., 2013; Préchoux and Helbert, 2014). The gene coding for the sulfatase has been cloned and expressed recombinantly in *Escherichia coli*. These experiments demonstrate that the endo-4O- ι -carrageenan-sulfatase is a type I sulfatase, a formylglycine-dependent sulfatase.

Another carrageenan-sulfatase was isolated from *P. atlantica* T6c, but it catalyzes the desulfation of κ -carrabiose, resulting in a neutral β -carrabiose repetition moiety (Figure 5).

Overexpression of this endo-4O- κ -carrageenan sulfatase in *E. coli* also validated its grouping in the type I sulfatase family (Préchoux et al., 2016). Interestingly, in contrast to the first carrageenan sulfatase investigations, these studies revealed endo-acting carrageenan sulfatases that are efficient on polymers instead of oligosaccharides, suggesting that degradation of carrageenan may follow different pathways. One pathway involves the depolymerization of carrageenan prior to desulfation of oligo-carrageenans and another pathway supposes the desulfation of the polysaccharides before the depolymerization of α - or β -carrageenans by yet-to-be-discovered α - and β -carrageenases.

Desulfation of agars has also been reported in several marine bacteria strains: *P. carrageenovora* (Kim et al., 2005), *Pyrococcus furiosus* (Jung et al., 2012), *Thermotoga marina* (Lee et al., 2013), *Marinomonas sp. FW-1* (Wang et al., 2015b), and *Flammeovirga pacifica* (Gao et al., 2015). In all cases, the modalities of desulfation were not demonstrated, notably, the position of the eliminated sulfate ester group. Heterologous expression of *T. marina* sulfatase (Lee et al., 2013) demonstrates that it belongs to the type I family. Interestingly, *P. carrageenovora* sulfatase has homology with type III β -lactamase-like sulfatase. The gene was originally identified in neighboring genes involved in κ -carrageenan degradation, suggesting potential involvement in sulfated galactan metabolism (Barbeyron et al., 1995).

The galactose-6-sulfurylases, which intervene at the last step in the biosynthesis of agarans and carrageenans, catalyze the removal of sulfate ester groups at position 6 in galactose. The hydroxyl group in position 3 of the galactose residue attacks the carbon, resulting in the loss of the sulfate group. This nucleophilic substitution leads to the inversion of the carbon configuration, the formation of 3,6 anhydrogalactose and inversion of the 4C_1 chair conformation to 1C_4 (Figure 5). In this reaction, the molecule of water required for hydrolysis is replaced by an endogenous hydroxyl group; therefore, the term galactose-6-sulfurylase seems inappropriate (Usov, 2011).

The first evidence for the formation of an anhydro-ring was obtained by Rees (1961a,b) using protein extracts from *Porphyra* sp. incubated on porphyran purified from the same algae. Similar approaches conducted with carrageenophyte algae also lead to the formation of an anhydro-ring in κ - and ι -carrageenan (Wong and Craigie, 1978; Zinoun et al., 1997; Genicot-Joncour et al., 2009). The pure enzyme was sequenced and expression of the corresponding gene was attempted in *E. coli* without success. The function of the gene could not be validated experimentally, but its occurrence in recently sequenced red algal genomes confirm its algal origin and may explain the difficulty of expressing a eukaryotic protein in a prokaryotic cell (Collén et al., 2013).

Fucoidan Sulfatases and Others

Fucoidans represent a class of sulfated polysaccharides composed mainly of α -L-fucose found in the cell walls of brown algae and in marine invertebrates (i.e., worms, sea urchins). The structure of fucoidans depends on their biological origin and differs among algal species. Fucoidans are co-products of the alginate industry, which make them attractive as bioactive compounds and several potential applications have been examined (Berteau and Mulloy, 2003; Pomin and Mourão, 2008). Several marine strains have been shown to produce the enzymatic arsenal to degrade brown

algal fucoidans (Michel and Czjzek, 2013). Fucanolytic enzymes include glycoside hydrolases and sulfatases. Gene sequences of fucanases have been validated by overexpression of active enzymes (Colin et al., 2006). However, although fucoidan sulfatase activity has been demonstrated in several bacterial strains, its sequence is still unknown and as for agarases and carrageenases in red algae, no sulfatase genes are predicted in brown algal genome (Cock et al., 2010). Sulfatases active on polymeric substrates or specific to oligosaccharide chain ends have been reported, hinting at the unexplored diversity of fucoidan sulfatases (Daniel et al., 2001).

Desulfation of marine sulfated glycosylated metabolites is grossly unexplored: one report demonstrates a 4O-xylose sulfatase in a protein extract of the liver of the marine mollusk *Littorina kurila* (Kusaykin et al., 2006). The enzyme is active on the sulfated xylose residue of holostan triterpene glycoside (Kusaykin et al., 2006).

OUTLOOK

Sequencing of marine life has shed light on the diversity of carbohydrate sulfatases. The modularity of sulfatases and the genome organization of *Bacteroidetes* (i.e., PUL organization) have the potential to drive future functional characterization of the large amount of sulfatase sequences. Ultimately, the comprehensive classification of sulfatases based on sequence homology, allowing prediction of function is a challenging task for future work, calling for bioinformatics analyses (Wegner et al., 2013; Barbeyron et al., 2016) and crystallographic description of catalytic sites to identify the key amino acids involved in substrate recognition.

Functional characterization can benefit from genetic tools, such as gene mutation, to inactivate encoded proteins, and from more classic approaches, such as biochemical analyses of the enzymes purified from the organism in which they have been detected or heterologous expression in *E. coli*. However,

only very few carbohydrate sulfatases have been biochemically characterized, underlining the difficulties in preparing them to purity. Recombinant marine bacterial sulfatases have been obtained, but always with a low level of activity. This low activity can be explained by the low rate of post-translational maturation of sulfatases in classic proteins expression systems that are naturally unable to convert cysteine and serine into formylglycine amino acid. Co-expression of sulfatases with known maturing enzymes (i.e., FGE, AnSME) can result in the production of a low amount of active enzymes, but better knowledge of the sulfatase maturing system—which probably involves several yet-to-be-discovered proteins—is a bottleneck to the production of active recombinant sulfatases.

The diversity of sulfatases lies in the diversity of sulfated polysaccharides present in the oceans. The composition and structure are simply unknown for most of them. Therefore, a comprehensive analysis of sulfatase function requires, in parallel, novel data on marine polysaccharides. Macro- and micro-algae, the primary producers of the oceans, harbor an unexplored and massive source of sulfated polysaccharides that contain both carbon and inorganic sulfate ions. Deciphering the turnover of marine polysaccharides involving sulfatases and other enzyme partners, will enhance our molecular understanding of the biogeochemistry of the ocean.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

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