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To cite this version:

Tristan Barbeyron, François Thomas, Valérie Barbe, Hanno Teeling, Chantal Schenowitz, et al.. Habitat and taxon as driving forces of carbohydrate catabolism in marine heterotrophic bacteria: example of the model algae-associated bacterium Zobellia galactanivorans Dsij T. Environmental Microbiology, 2016, Ecology and Physiology of Marine Microbes, 18 (12), pp.4610-4627. 10.1111/1462-2920.13584 . hal-02137896

HAL Id: hal-02137896

https://hal.science/hal-02137896v1

Submitted on 23 May 2019

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Environmental Microbiology - Research Article

Habitat and taxon as driving forces of carbohydrate catabolism in marine heterotrophic bacteria: example of the model algae-associated bacterium *Zobellia galactanivorans* Dsij^T

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Running title: Complete genome of *Zobellia galactanivorans*

Conflict of Interest

The authors declare no conflict of interest.

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Keywords: marine heterotrophic bacteria, bacteria - algae interactions, carbon cycle, sulfated polysaccharides, comparative genomics

Originality – significance statement

Marine heterotrophic bacteria (MHB) are crucial players in the recycling of organic carbon in the sea and especially of algal polysaccharides. However the precise roles of MHB in the carbon cycle are difficult to define, mainly due to our limited knowledge on the degradation pathways for algal polysaccharides in comparison to those for land plant polysaccharides. Here we contribute to close this gap by describing an in-depth physiological and genomic analysis of the marine flavobacterium *Zobellia galactanivorans* DsiJ^{T}, a model organism for studying the bioconversion of algal polysaccharides. Bioinformatic and experimental approaches confirm the exceptional capacity of this flavobacterium for using carbohydrates among MHB. *Z. galactactanivorans* DsiJ also displays multiple adaptive traits to a macroalgae-associated lifestyle, supporting that this bacterium is a suitable model for studying seaweed-bacteria interactions. On a more holistic level, a comparative analysis of 126 MHB genomes, including *Z. galactactanivorans*, demonstrate that the carbohydrate catabolism of MHB is influenced by phylogeny as well as habitat. As a consequence, clustering analysis of carbohydrate-active enzymes can be used as a predictive tool to assess a bacterial ecological niche within the carbon cycle.

Summary

The marine flavobacterium *Zobellia galactanivorans* Dsij^T was isolated from a red alga and by now constitutes a model for studying algal polysaccharide bioconversions. We present an in-depth analysis of its complete genome and link it to physiological traits. *Z. galactanivorans* exhibited the highest gene numbers for glycoside hydrolases, polysaccharide lyases and carbohydrate esterases and the second highest sulfatase gene number in a comparison to 125 other marine heterotrophic bacteria (MHB) genomes. Its genome contains 50 Polysaccharide Utilization Loci, 22 of which contain sulfatase genes. Catabolic profiling confirmed a pronounced capacity for using algal polysaccharides and degradation of most polysaccharides could be linked to dedicated genes. Physiological and biochemical tests revealed that *Z. galactanivorans* stores and recycles glycogen, despite loss of several classic glycogen-related genes. Similar gene losses were observed in most *Flavobacteriia*, suggesting presence of an atypical glycogen metabolism in this class. *Z. galactanivorans* features numerous adaptive traits for algae-associated life, such as consumption of seaweed exudates, iodine metabolism, and methylotrophy, indicating that this bacterium is well equipped to form profitable, stable interactions with macroalgae. Finally, using statistical and clustering analyses of the MHB genomes we show that their carbohydrate catabolism correlates with both taxonomy and habitat.

Introduction

Half of Earth's primary production occurs in marine environments (Field et al., 1998), mostly in the open ocean by phytoplankton. By contrast, macrophytes (macroalgae and seagrasses) only contribute significantly to primary production in coastal ecosystems (Smith, 1981; Gattuso et al., 1998). Polysaccharides constitute a large fraction of algal biomass. Cell wall polysaccharides constitute ~50% of dry weight in macroalgae (Kloareg and Quatrano, 1988) and up to 70% in microalgae that produce exopolysaccharides (EPS) (Malis Arad, 1988; Hoagland et al., 1993). Marine phototrophs store photoassimilates as intracellular polysaccharides (glycogen, starch or laminarin) (Michel et al., 2010). Moreover, polysaccharide-rich algal debris released in surface waters can form particles that can sink to the sediment. All these sources of polysaccharides represent potential substrate niches for marine heterotrophic bacteria (MHB). Diversity and (meta)genomic studies have unveiled the major role that some MHB clades play as carbon recyclers, mainly *Alpha*- and *Gammaproteobacteria*, *Bacteroidetes*, *Planctomycetes* and *Verrucomicrobia* (Kirchman, 2002; Glöckner et al., 2003; Giovannoni et al., 2005; Azam and Malfatti, 2007; Ivars-Martinez et al., 2008; Teeling et al., 2012; Buchan et al., 2014; Teeling et al., 2016). Yet, our understanding of the marine carbon cycle suffers from a lack of detailed biochemical knowledge, which is why genomic data from marine degraders are essentially annotated using knowledge from decades of research on the decomposition of terrestrial plants by bacteria and fungi (Gilbert et al., 2008). However, structures of phytoplankton EPS are mostly uncharacterized, and while polysaccharides from macroalgae are better understood, they exhibit an enormous chemical diversity and differ greatly from their terrestrial counterparts (Popper et al., 2011). Notably all marine algae produce sulfated polysaccharides that are absent from land plants (Ficko-Blean et al., 2015b). However, our understanding of the enzymatic decomposition of these marine polysaccharides is still poor. The discovery of glycoside hydrolases and polysaccharide lyases specifically targeting algal polysaccharides has accelerated during the last 15 years (Michel and Czjzek, 2013; Hehemann et al., 2014; Martin et al., 2014). Particularly *Zobellia galactanivorans* Dsij^T (Barbeyron et al., 2001) has so

1 far played a pivotal role for many of these advances. This marine flavobacterium was originally
2 isolated from a healthy red macroalga (*Delesseria sanguinea*) in Roscoff (Brittany, France) due to its
3 carrageenolytic activity (Potin et al., 1991) and has since been the source for numerous novel algae-
4 specific polysaccharidases (Martin et al., 2014). Notably, studies of this microorganism resulted in the
5 discovery of β-porphyranases and α-1,3-(3,6-anhydro)-L-galactosidases, enzymes that catalyze two
6 key steps in agar recycling. Some of the respective genes were laterally transferred from marine
7 bacteria to gut bacteria of the Japanese population, likely due to their traditional consumption of
8 edible seaweeds (Hehemann et al., 2010; Rebuffet et al., 2011). The first operons of marine bacteria
9 for alginate and mannitol consumption were also characterized in *Z. galactanivorans* (Thomas et al.,
10 2012; Groisillier et al., 2015). Other *Zobellia* species and uncultured strains were found in coastal
11 regions around the world (Nedashkovskaya et al., 2004; Schulze et al., 2006; Alonso et al., 2007).
12 Most strains were isolated from algae where interactions might range from symbiosis with green
13 seaweeds (Matsuo et al., 2005) to algicidal behavior towards dinoflagellates (Skerratt et al., 2002).
14 Recent studies indicate that *Zobellia* strains are an integral part of the core microbiota of healthy
15 seaweeds (Hahnke and Harder, 2013; Hollants et al., 2013; Martin et al., 2015; Marzinelli et al.,
16 2015).

17 We here report the analysis of the complete genome of *Z. galactanivorans* Dsij^T in the context of
18 comprehensive physiological studies. Moreover, the carbohydrate catabolism of *Z. galactanivorans*
19 was compared to that of 125 other MHB highlighting the multiple niches occupied by heterotrophic
20 bacteria in the marine carbon cycle.

21 **Results and discussion**

22 ***Z. galactanivorans has a pronounced capacity for algal polysaccharide degradation***

23 The *Z. galactanivorans* genome encodes 101 peptidase genes from 37 MEROPS families and genes
24 for 141 glycoside hydrolases (GH), 15 polysaccharide lyases (PL) and 18 carbohydrate esterases (CE)
25 (Table 1). Numerous GH and PL have modular architectures and 37 associated CBM were identified.

The carbohydrate active enzymes (CAZymes) cover a wide range of families (GH=44, PL=8, CE=9, CBM=14). Moreover, 71 sulfatases were identified (Fig. S1). They belong to 17 subfamilies of the S1 family (formyl-glycine dependent sulfatases) (see the SulfAtlas database, http://abims.sb-roscoff.fr/sulfatlas/) (Barbeyron et al., 2016). In comparison to 125 other sequenced MHB identified in the CAZY database (http://www.cazy.org/, Lombard et al., 2014), *Z. galactanivorans* had the highest number of GH, PL and CE, and the second highest number of sulfatases after *Rhodopirellula baltica* SH 1^T (Glöckner et al., 2003) (Table S1). Most of these CAZymes and sulfatases had signal peptides and were predicted to be either periplasmic or membrane-anchored (121/141 GH, 14/15 PL and 61/71 sulfatases), suggesting that they target external substrates. All essential genes of the Sec, TaT and Type-IX (unique to *Bacteroidetes*) secretion systems (McBride and Zhu, 2013) are present. Among the 28 proteins with C-terminal type IX secretion domains (TIGR04183 profile), 14 were CAZymes that mostly target algal polysaccharides (AgaA, AgaD, PorA, CgkA, LamC, LamD, LamE and AlyA7). These predictions agree with experiments showing that the beta-agarase AgaA and the kappa-carrageenase CgkA are extracellular enzymes (Barbeyron et al., 1998; Jam et al., 2005). The link between type IX secretion and algal polysaccharide degradation highlights the importance of this catabolism for *Z. galactanivorans*.

Moreover, a large capacity for substrate detection and uptake was identified. *Z. galactanivorans* possesses 119 TonB-dependent receptors (TBDR) genes, 71 of which are located adjacent to *susD*-like genes (Table S2). The latter code outer membrane lipoproteins that are unique to the *Bacteroidetes* phylum (Thomas et al., 2011b). They bind polysaccharides and thereby assist the TBDR in substrate uptake. Presence or absence of an associated *susD*-like gene thus indicates, whether a *Bacteroidetes* TBDR is specific for polymers (SusC-like) (Koropatkin et al., 2012) or for small molecules (FhuA-like) (Ferguson et al., 1998). *Z. galactanivorans* has the highest number of both SusC-like and FhuA-like TBDR among marine *Bacteroidetes* (Table S2), suggesting remarkable capacities for the uptake of both oligosaccharides and small molecules. *Z. galactanivorans* and *Zunongwangia profunda* have SusC-like/FhuA-like TBDR ratios of ~1.5 indicating a preference for polysaccharides. In contrast, other

MHB species have lower overall TBDR numbers and SusC-like/FhuA-like ratios around or below 1. Among the 31 predicted ATP-binding cassette (ABC) transporters in *Z. galactanivorans*, only 11 are importers, suggesting a minor role of ABC-transporters for the import of small carbohydrates from the periplasm to the cytoplasm. Only the mannitol ABC-transporter (ZGAL_4260-4662) is certain to be involved in carbohydrate uptake (Groisillier et al., 2015). Moreover, *Z. galactanivorans* lacks the phosphoenolpyruvate-carbohydrate phosphotransferase system (PTS). Therefore, sugar uptake likely relies on Major Facility Superfamily (MFS) transporters (26 genes) and sodium/solute symporters (SSS, 15 genes). This is in stark contrast with coastal *Alpha*- and *Gammaproteobacteria* which essentially use ABC-transporters and PTS for sugar uptake (Poretsky et al., 2010). Eighteen of the *Z. galactanivorans* TBDR are transducers that sense external compounds and transmit signals to the cytoplasm via interactions with anti-sigma factors (17 genes) and extracytoplasmic function sigma factors (34 genes) (Koebnik, 2005). The *Z. galactanivorans* genome also encodes 65 one-/two-component regulatory systems and 122 transcriptional regulators.

CAZymes, sulfatases, transporters and associated regulators in the *Z. galactanivorans* genome are mainly clustered into Polysaccharide Utilization Loci (PUL, Bjursell et al., 2006). Fifty PUL were identified (with 3 to 29 genes) and their putative substrates were predicted when possible (Fig. 1, Table S3). These PUL are likely operons, as was demonstrated for the alginolytic PUL29 (Thomas et al., 2012). Twenty-two PUL contained sulfatases suggesting sulfated polysaccharides as targets. The only PUL with a TBDR without associated *susD*-like gene (PUL25, Fig. 1) is predicted to import a small carbohydrate (mannosylglycerate) and not an oligosaccharide. These PUL comprise six MFS-transporter and eight SSS but no ABC-transporter genes, further supporting a rather minor role of ABC-transporters for sugar uptake.

In catabolic profiling with a large collection of carbohydrates *Z. galactanivorans* grew on 9 of 11 tested monosaccharides, 2 of 3 polyols, 5 of 5 disaccharides and 15 of 37 polysaccharides (Table 2). Polysaccharides comprised those typical for seaweeds (agar, porphyran, kappa-, iota-, and lambda-carrageenans, alginate, laminarin, some fucoidans) and polysaccharides that occur in algae as well as

in plants (starch, β-1,3-1,4-glucan, xylan, galactan). No polysaccharides of animal origin were used, confirming that *Z. galactanivorans* is highly specialized on macrophyte degradation.

Presence of genes corresponding to the utilization of each of the substrates was checked (Table S4). In most cases the observed catabolic capacities could be explained on the genomic level, but some interesting discrepancies were found. For example, *Z. galactanivorans* uses L-fucose, L-rhamnose, lambda-carrageenan, some fucoidans, and arabic gum, but lacks homologs to genes that are known to mediate degradation of these substrates in other organisms. This implies that *Z. galactanivorans* likely has evolved new, yet uncharacterized degradation pathways for these substrates. Conversely, utilization was predicted for D-ribose, D-galacturonate, D-sorbitol, glycogen, cellulose, pectin, pachyman, paramylon, xyloglucan, mannan, galactomannan, arabinogalactan, gluconolactone, and chitin, yet this could not be confirmed experimentally. Depending on substrate types, different explanations can be proposed. For glycogen, pachyman and paramylon their three-dimensional structure is a likely factor, since chemically similar, but structurally different polysaccharides are used (starch, laminarin and curdlan, respectively). *Z. galactanivorans* probably does not detect and/or uptake these polysaccharides. Consistently, the recognition properties of the SusD-like proteins are dominated by the three-dimensional conformation of their ligands (Koropatkin et al., 2008). For other carbohydrates (e.g. cellulose, xyloglucan), the substrate specificity of some CAZymes was likely incorrectly predicted despite our careful annotation strategy. This is not unexpected in some highly polyspecific families such as GH5 (Aspeborg et al., 2012). It is more surprising for GH28 and PL1 which are considered specific to pectins (http://www.cazy.org/). Thus these enzymes might display new, uncharacterized specificities. Another alternative scenario is that the necessary genes may not be expressed under the tested conditions.

***Analyses of carbohydrate biosynthetic pathways unravel an unusual glycogen metabolism in* Flavobacteriia**

Z. galactanivorans possesses 56 glycosyltransferases (GT) belonging to 11 GT families (Table 1), indicating a significant capacity for carbohydrate biosynthesis. Most of these genes (31/56) are localized within 14 clusters including additional carbohydrate-related genes (Table S5). Six clusters include GT2 and GT4 and are probably responsible for the production of lipopolysaccharides (LPS) or extracellular polysaccharides (EPS). *Z. galactanivorans* may synthesize two carbohydrate osmolytes: trehalose, using a bimodular trehalose-6-phosphate synthase/phosphatase (GT20, ZGAL_101), and sorbitol by a glucose-fructose oxidoreductase (ZGAL_4647).

Two glycogen biosynthetic genes are present, namely for glycogen synthase (ZGAL_2539, *glgA*, GT5) and the 1,4-alpha-glucan branching enzyme (ZGAL_518, *glgB*, GH13_9). Surprisingly *glgC*, encoding the ADP-glucose pyrophosphorylase responsible for precursor synthesis, and the three genes encoding essential enzymes to recycle stored glycogen (glycogen phosphorylase, GT35; alpha-1,4-glucanotransferase, GH77; debranching enzyme, *glgX*, GH13_11) are missing (Ball and Morell, 2003). These genes are also absent in most other *Flavobacteriia*. Genbank BLASTp searches identified 40 *Flavobacteriia* genera with GT5. Among these, 10 lack *glgC* and only 3, 2 and 6 have genes for glycogen phosphorylase, alpha-1,4-glucanotransferase and a debranching enzyme, respectively (Table S6). Moreover none of the 40 genera has a complete set of glycogen-related genes.

To test glycogen synthesis/recycling in *Z. galactanivorans*, cells were grown with nitrogen limitation and excess of glucose to stimulate carbon storage. After three days, ethanol-insoluble material was recovered with a yield of ~100 $mg.L^{-1}$ culture. ^{1}H-NMR analysis of the extract revealed a typical glycogen spectrum (Fig. S2a), with signals from H-1(1→4) [5.38 ppm], H-1(1→6) [4.98 ppm] and H4 [3.44 ppm] as determined in pure glycogen (Zang et al., 1991). Transmission electron microscopy revealed intracellular glycogen granules (Fig.2) and biochemical quantification showed that glycogen accumulates up to 1,662 nmol eq.glucose.mg^{-1} protein in the cytoplasm. This stored glycogen was consumed when cells were transferred to medium without any carbon source (Fig. S2b), and granules were invisible after six days (Fig. 2). Since *Z. galactanivorans* synthesizes and recycles its own glycogen, despite the loss of classic glycogen-related genes, it must have evolved

new, uncharacterized enzymes for its glycogen metabolism. To our knowledge, this is the first case reporting an alternative glycogen metabolism in bacteria. Since classic glycogen-related genes are lacking in many other *Flavobacteriia* as well, we speculate that these alternative genes have emerged early in *Flavobacteriia* evolution and are active in many other *Flavobacteriia* as well.

Adaptive traits to a macroalgae-associated lifestyle

To live on seaweeds implicates extensive biological and physicochemical constraints for bacteria (Egan et al., 2013). Genome analysis unraveled numerous traits indicating that *Z. galactanivorans* is well adapted to interact with macroalgae (Fig. 3). First, the genome contains two polyketide synthases (ZGAL_384, ZGAL_4387) that are candidates for the synthesis of polycyclic compounds such as thallusin. This phytohormone produced by *Z. uliginosa,* the closest known relative to *Z. galactanivorans* (Barbeyron et al., 2001; Nedashkovskaya et al., 2004), induces the normal morphogenesis of green seaweeds (Matsuo et al., 2003; Matsuo et al., 2005). If thallusin production is confirmed, presence of *Z. galactanivoran*s could be beneficial for some algal hosts. Second, *Z. galactanivoran*s can feed on seaweed exudates such as mannitol (Table 2), which brown algae produce in large quantities (Rousvoal et al., 2011; Groisillier et al., 2014). This capacity is due to a mannitol-inducible operon (Fig. 1) (Groisillier et al., 2015). In the presence of mannitol *Z. galactanivorans* forms thicker biofilms, which suggests that this exudate favors the colonization of algal surfaces (Salaun et al., 2012). *Z. galactanivorans* also likely uses the red macroalgae exudate digeneaside (mannosylglycerate) (Kremer, 1980), since PUL25 contains a GH63 mannosylglycerate hydrolase (ZGAL_2226) and a glycerate kinase (ZGAL_2229), which can convert digeneaside into D-mannose 6-phosphate and 3-phospho-D-glycerate (Fig. 1).

In response to stress macroalgae can rapidly produce reactive oxygen species (ROS) and reactive nitrogen species (RNS) as defense mechanism (Potin et al., 2002; Cosse et al., 2007; Kumar et al., 2015). *Z. galactanivorans* is well equipped to cope with such oxidative bursts (Table S7). Superoxide (O_2^-) ions can be quenched by two distinct superoxide dismutases (ZGAL_114 and ZGAL_1583). The

detoxification of hydrogen peroxide (H_2O_2) is conducted by eight peroxidases of four families. *Z. galactanivorans* features an extensive thiol-based defense, with one glutathione reductase, three thioredoxin reductases, nine thioredoxins and ten peroxiredoxins. ROS-damaged proteins are repaired by five methionine sulfoxide reductases. A copper-containing azurin (ZGAL_3047) also participates in ROS-defense (Seib et al., 2006). *Z. galactanivorans* possesses a nitric oxide (NO) reductase (NorBC, ZGAL_4311) and a nitrous oxide (N_2O) reductase (NosZ, ZGAL_2236), which convert NO to dinitrogen (N_2) (Table S7). We did not find any NO synthase (Crane et al., 2010), but a nitrate assimilation pathway (Luque-Almagro et al., 2011), including a nitrate reductase (ZGAL_3212) and a bimodular nitrate reductase/nitrite reductase (ZGAL_1625), was identified. Consistently, *Z. galactanivorans* uses nitrate as a sole nitrogen source (data not shown). However, the nitrite reductase module of ZGAL_1625 is homologous to NasB from *Bacillus subtilis* JH642*,* which does not release NO but ammonia (Ogawa et al., 1995). Therefore, there appears to be no endogenous source of NO in *Z. galactanivorans* and NorBC/NosZ likely function as a detoxification system of algal NO.

Seaweeds can concentrate halides from seawater and release volatile halogenated organic compounds (VHOCs) in response to biotic and environmental stress (Potin et al., 2002; Cosse et al., 2007). Moreover, cells of brown algal kelps accumulate huge amounts of iodine in their apoplast, forming an iodine reservoir implicated in chemical defense and antioxidative activities (Verhaeghe et al., 2008). *Z. galactanivorans* has three vanadium-dependent haloperoxidases (vHPOs) belonging to the very same family of enzymes involved in iodine uptake and VHOCs emission in macroalgae (Küpper et al., 1998; Colin et al., 2005). We recently demonstrated that ZGAL_1262 and ZGAL_2088 are genuine iodoperoxidases (Fournier et al., 2014). The additional presence of an iodotyrosine dehalogenase (ZGAL_4305) suggests that *Z. galactanivorans* has its own iodine metabolism. To test this hypothesis, we compared the intracellular iodine content of *Z. galactanivorans* and a control marine bacterium without any vHPO gene (*Pseudoalteromonas haloplanktis* TAC125, Medigue et al., 2005). After 4 days of growth in ZoBell medium, the iodine content of *P. haloplanktis* cells was below the detection limit (<2 $\mu g.g^{-1}$ dry weight), whereas pellets of *Z. galactanivorans* contained 6.1 ±

1.2 µg.g^{-1} dry weight (Table S8). Assuming that bacterial cell density is ranging between 0.392 g.cm^{-3} and 1 g.cm^{-3} (Loferer-Krossbacher et al., 1998), the corresponding intracellular iodine concentration ranges between 18 and 48 µM. These values are two orders of magnitude above the oceanic iodine concentration ranging from 0.44 to 0.49 µM (Küpper et al., 1998), suggesting that *Z. galactanivorans* cells can accumulate iodine. This iodine accumulation, together with the activity of the characterized iodoperoxidases (Fournier et al., 2014) and of the putative iodotyrosine dehalogenase, possibly helps *Z. galactanivorans* to cope with algal VHOCs and the high concentration of iodine in kelp cell walls.

Z. galactanivorans has several genes that could help in competition with other microorganisms, which might be beneficial for the colonization of algal surfaces (Egan et al., 2013). *Z. galactanivorans* possesses the essential genes for gliding motility (McBride and Zhu, 2013), and therefore can rapidly spread on surfaces (Barbeyron et al., 2001). A complete ribulose monophosphate (RuMP) pathway (Hanson and Hanson, 1996) was also predicted. On land plants, methylotrophs use the RuMP pathway to detoxify and assimilate the methanol indirectly released by the action of pectin methylesterases (Vorholt, 2012). Like pectins, agars and carrageenans are methylated (Lahaye and Rochas, 1991; Van de Velde, 2008) and removal of methyl groups during degradation would produce methanol. The RuMP pathway could thus be advantageous when degrading red algal cell walls to cope with the release of this toxic compound. Consistently, *Z. galactanivorans* possesses numerous CE (Table 1) and uses methylated-iota-carrageenan (Table 2). Two key enzymes of the RuMP pathway (3-hexulose-6-phosphate synthase, ZGAL_3942; 3-hexulose-6-phosphate isomerase, ZGAL_3941) are only present in few species from eight flavobacterial genera (including some found on algae: *Cellulophaga*, *Maribacter*), suggesting that this trait is rather uncommon in *Flavobacteriia*. Due to a homoserine lactone lyase (ZGAL_4600), *Z. galactanivorans* may degrade communication molecules produced by competing bacteria, interfering with their quorum sensing and their ability to form biofilms (Huang et al., 2006). Finally, *Z. galactanivorans* has a PL9_4 (ZGAL_3183) homologous to DssA from *Paenibacillus koleovorans* TB^{T}, which degrades the sheath polysaccharide from

Sphaerotilus natans (Takeda et al., 2000). Thus *Z. galactanivorans* may attack the EPS of other bacteria as a counter-defense mechanism.

Carbohydrate catabolism in marine heterotrophic bacteria depends on habitat and taxon

The growing number of MHB genomes from diverse taxa offers a great opportunity to assess their potential roles as carbohydrate recyclers in various environments, although this has rarely been studied systematically. We compared the number of GH, PL and sulfatases in MHB genomes according to either taxonomy or habitat (Fig. 4). Marine *Bacteroidetes* have been ascribed a pivotal role in polymer degradation (Kirchman, 2002; Bauer et al., 2006; Thomas et al., 2011b). Accordingly, their GH, PL and sulfatase genomic content was on average significantly higher than in *Alpha*- and *Gammaproteobacteria* (Fig. 4 A-C). However, these averages mask a substantial inter-species variability, with several *Bacteroidetes* representatives being very poor in CAZymes (e.g. *Owenweeksia hongkongensis* UST20020801^{T}, *Croceibacter atlanticus* HTCC2559^{T}) and two extreme outliers with high values (*Z. galactanivorans*, *Zunongwangia profunda*) (Table S1). Therefore, the role of marine *Bacteroidetes* as polysaccharide degrading specialists cannot be generalized at the phylum level. In comparison, the CAZyme genomic content of *Gammaprotobacteria* appears relatively homogenous (Fig. 4 A-C). The gammaproteobacterium with the highest CAZyme frequencies, *Pseudoalteromonas atlantica* T6c (GH=66, PL=5, sulfatases=18, Table S1), has a lower degradation potential than the corresponding upper echelon of the *Bacteroidetes*. *Alphaproteobacteria* constitute a very homogenous group, and their average CAZyme repertoire is limited (GH=19, PL=1, sulfatases=3). Interestingly *Pelagibacter ubique* HTCC1062 and *Pelagibacter* sp. IMCC9063, which belong to the most abundant group of MHB (SAR11 clade, Giovannoni et al., 2005), exhibit the poorest polysaccharide degradation capacity (GH=4, PL=0, sulfatase=0 and GH=5, PL=0, sulfatase=0, respectively). Most of the GH of these bacteria are involved in the modification of their own peptidoglycans (GH23, GH73 and GH103, Table S1). Thus the SAR11 *Alphaproteobacteria* appear to play no significant role in the degradation of marine polysaccharides.

We tested the effect of habitat on the CAZyme gene repertoire of MHB (Fig. 4 D-F). Bacteria from macrophytes had the highest mean values (GH=53, PL=5, sulfatase=17), although the large variability among distinct species precludes statistical significance. It is worth noting that *Z. galactanivorans* appears as an outlier even in this population. By contrast, phytoplankton-associated bacteria had similar profiles, but with lower median and mean values and a lower variance. This richness in CAZyme genes is likely related to the diversity of neutral, uronic and sulfated polysaccharides that marine photosynthetic organisms produce. The differences between the habitats "Macrophytes" and "Phytoplankton" likely reflects differences in the diversity and complexity of polysaccharides between more complex multicellular macrophytes with tissue differentiation (Popper et al., 2011) and more uniform unicellular phytoplankton species. Animal-associated MHB on the other hand constitute a more even group with lower overall degradation capacities. Surprisingly, members of this group possess very few sulfatases (Fig. 4F), although animal extracellular matrices include sulfated glycosaminoglycans (GAG) (Jackson et al., 1991). Nonetheless, animal pathogens do not necessarily degrade GAG, as shown by the freshwater fish pathogen *Flavobacterium psychrophilum* JIP02/86, which has very few GH and no PL or sulfatase genes (Duchaud et al., 2007). Bacteria isolated from surface seawater and sediments displayed rather uniform and moderate CAZyme repertoires. A commonality of both habitats is that they receive algal and animal debris. A large repertoire of carbohydrate binding modules, endo-polysaccharidases, of exo-glycosidases and of modifying enzymes such as carbohydrate esterases and sulfatases is needed to degrade the mostly insoluble complex polysaccharides present in cell walls. This is well known for plants (Gilbert et al., 2008) and has recently also been shown for algal cell walls (Hehemann et al., 2012; Thomas et al., 2012; Thomas et al., 2013; Labourel et al., 2014; Labourel et al., 2015; Jam et al., 2016). By this initial attack the matrix is loosened and a wide array of less complex soluble oligosaccharides is released into the seawater. The bacteria utilizing these oligosaccharides and the predigested algal cell walls thus require fewer types of enzymes and therefore feature a smaller and less diverse repertoire of CAZymes and sulfatases. Nonetheless the habitat "Surface seawater"

includes extreme outliers: e.g. *Simiduia agarivorans* SA1^T (PL=14, Fig. 4E), *Coraliomargarita akajimensis* 04OKA010-24^T (sulfatases=49), *R. baltica* (sulfatases=112, Fig. 4F). A possible explanation is that "Surface seawater" is not only a habitat by itself but also a transitory environment for some MHB. Indeed, *Planctomycetes* are abundant on kelps (Bengtsson et al., 2012) and *S. agarivorans*, as an agarolytic bacterium (Lin et al., 2012), probably also lives on red macroalgae. PL and sulfatase repertoires of MHB isolated from deep marine sediments are poor (Fig. 4E, F), but more variable regarding GH (Fig. 4D). Even potent degraders from this habitat have very few PL and sulfatases (*Verrucosispora maris* AB-18-032^T: GH=99, PL=4, sulfatase=0; *Zunongwangia profunda*: GH=125, PL=0, sulfatase=6, Table S1). This preference for neutral polysaccharides is likely related to the enzymatic processes affecting organic particles throughout their sinking from surface to ocean interior (Azam and Malfatti, 2007). Considering the richness in PL and sulfatases of algae-associated bacteria, uronic and sulfated polysaccharides should be mainly decomposed directly on algal hosts and in surface seawater, while deep sediments likely receive organic particles enriched in the most recalcitrant polysaccharides (e.g. cellulose, chitin). MHB from deep seawater constitute a very homogenous group in terms of CAZyme genes with repertoires resembling those of bacteria isolated from deep marine sediments, which indicates that both may receive similar polysaccharide substrates. Finally, hydrothermal vent bacteria constitute the group with the least CAZyme genes and the lowest CAZyme variance. This suggests that polysaccharide utilization is less crucial for MHB at hydrothermal vents where chemolithoautotrophic primary production predominates. In Table S9 we evaluate the combined influence of taxonomy and habitat on the mean carbohydrate-associated genetic repertoire of the 126 MHB. The resulting tendencies that we observed appear to be additive, that is they were either amplified (e.g. "rich bacterial group" and "rich habitat") or attenuated (e.g. "rich bacterial group" and "poor habitat"). For instance, *Bacteroidetes* species ("rich group") living on macrophytes ("rich habitat") display indeed the highest gene numbers, while *Bacteroidetes* from marine sediments or deep seawaters ("poorer habitats") exhibited lower capacities. Moreover, bacteria from the same habitat but from different taxa did not display similar potentials and thus

likely occupy distinct ecological niches. On macrophytes and phytoplankton, *Bacteroidetes* have a richer repertoire than *Alpha-* and *Gammaproteobacteria*. Thus, these *Proteobacteria* could be scavengers, partially feeding on oligosaccharides released by *Bacteroidetes*. Accordingly, *Bacteroidetes* are the first group of bacterioplankton that profit from diatom blooms, followed later by *Proteobacteria*, suggesting a sequential degradation of algal-derived compounds (Teeling et al., 2012; Teeling et al., 2016).

To refine the prediction of the ecological roles of MHB, we performed a double-clustering analysis of the 126 MHB and their enzymatic repertoires (Fig. S3). Such an approach was already successfully used for terrestrial fungal degraders (Couturier et al., 2012). Strikingly, most protein clades (carbohydrate-related group, CRG) encompass (sub)families predicted to act in similar pathways, based on current literature, allowing substrate prediction for 17 CRG. For instance, CRG7 (GH13_9_GlgB, GT5, GH13_11_GlgX, CBM48, GH77, GT35) and CRG10 (GH28, GH105, PL1, CE8, CE12) are predicted to be specific for the metabolism of endogenous glycogen and for pectin catabolism, respectively. Thus co-occurrence of some (sub)families in the 126 MHB carries sufficient information to group biologically-related families. The relevance of using subfamilies for polyspecific families is also illustrated. GH16 enzymes are scattered in different CRGs: laminarinases in CRG11 and kappa-carrageenases, beta-agarases and beta-porphyranases in CRG15. This clustering approach can also be a powerful tool to identify missing enzymes in incomplete pathways. CRG15 is certainly specific for the degradation of red algal sulfated galactans (agars and carrageenans), since it includes the above-mentioned GH16 kappa-carrageenases, beta-agarases and beta-porphyranases, the GH117 enzymes to complete agar degradation (Rebuffet et al., 2011), and five subfamilies of sulfatases (S1_15, S1_19, S1_25, S1_28, S1_29). These latter subfamilies are currently uncharacterized and thus constitute promising candidates for identifying agar- and carrageenan-specific sulfatases.

This clustering determined 15 bacterial groups (BG), most likely corresponding to specific ecological functions. For instance, BG3 corresponds to a “super-degrader” group, notably including *Z. galactanivorans*, *F. agarilytica*, *Zunongwangia profunda, P. atlantica, R. baltica* and *C. akajimensis*.

This clade encompasses bacteria of distinct phyla, but no *Alphaproteobacteria*, as expected from the boxplot analysis. BG3 habitats mainly correspond to “Macrophyte” and “Phytoplankton”. Consistently, BG3 bacteria mostly shared richness in sulfatases and in CRG specific for “soft” algal polysaccharides (e.g. laminarin, alginate, sulfated galactans). BG1 and BG15 comprise bacteria specialized for neutral, recalcitrant polysaccharides (mainly cellulose and chitin, respectively). BG1 comprises *Actinomycetes* and *Proteobacteria* from sediments, while BG15 only includes *Gammaproteobacteria* from seawater, sediments, or animals. BG7 includes most of the “poorest degraders”: numerous *Alpha*- (including SAR11), *Delta*- and *Epsilonproteobacteria*, but also one *Bacteroidetes* (*Owenweeksia hongkongensi*). Their habitats are essentially seawater, deep marine sediments and hydrothermal vents. Finally, this unsupervised clustering approach emphasizes that species within one genus may have different polysaccharide degradation potentials and occupy distinct glycan niches, as shown for the genera *Alteromonas* (Ivars-Martinez et al., 2008) and *Polaribacter* (Xing et al., 2015). For instance, the "super-degrader" *Pseudoalteromonas atlantica* clusters with BG3, while *Pseudoaltermonas haloplanktis* and *Pseudoalteromonas* sp. SM9913 belong to BG11 and BG15, two groups with limited CAZyme repertoires.

Concluding remarks

In-depth genomic analysis and physiological experiments indicate that *Z. galactanivorans* has exceptional capacities to utilize algal polysaccharides. This marine *Bacteroidetes* also displays numerous adaptive traits that enable formation of a profitable, stable association with macroalgae. These results fortify the status of *Z. galactanivorans* as a multifaceted model organism for the study of bacteria-algae interactions. On a more holistic level, our comparative genomic analyses demonstrate that the carbohydrate catabolism of MHB is influenced by phylogeny as well as habitat. As a consequence, clustering analysis of carbohydrate-active enzymes can be used as a predictive tool to assess a bacterial ecological niche within the carbon cycle.

Experimental procedures

Genome sequencing and annotation

Sequencing of *Z. galactanivorans* genome was undertaken using the same random shotgun approach as described for *Salinibacter ruber* M8 (Peña et al., 2010). Briefly, three clone libraries (A=3 kbp, B=10 kbp, C=25 kbp) were constructed, clones were picked at random, and insert-containing vectors were purified and subsequently end-sequenced (66539 (A), 24704 (B), 10387 (C)) using dye-terminator chemistry on ABI3730 Sanger sequencers (Applied Biosystems, Carlsbad, USA). Assembly was performed as described previously (Vallenet et al., 2008). Additional 410 sequences were needed for finishing, resulting in a fully closed circular chromosome of 5,521,712 bp (42.8% GC). Gene finding and automatic annotation were performed with the GenDB system (Meyer et al., 2003) resulting in the detection of 4,738 protein coding sequences (CDS), 40 tRNA genes and two rRNA operons. GenDB was also used for the manual refinement of the functional annotations of all encoded proteins. Protein localizations were predicted using SignalP 3.0 (Bendtsen et al., 2004), LipoP 1.0 (Juncker et al., 2003) and TMHHM 2.0 (Krogh et al., 2001) with default parameters. Modularity was checked using Pfam (Finn et al., 2016) and taken into account in the annotation. Protein families were also determined using the specialized databases MEROPS (Rawlings et al., 2016) and CAZY (Lombard et al., 2014). Functions were attributed based on the closest characterized homologues. For all polyspecific enzyme families the function was attributed based on phylogenetic trees including the query sequence and characterized homologues, as previously shown (Stam et al., 2006). Proteins were aligned using MAFFT with iterative refinement (FFT-NS-i) using the BLOSUM62 scoring matrix (Katoh and Standley, 2013). Maximum-likelihood trees were reconstructed in MEGA 6.0.6 from manually refined alignments, using 100 bootstraps. The parameters were prior determined using the “Find best protein model (ML)” function (Tamura et al., 2013). The sequence of the *Z. galactanivorans* genome was deposited at EMBL (accession: FP476056).

Carbohydrate catabolic profiling

Growth of *Z. galactanivorans* was tested in triplicate in the marine mineral medium published by (Thomas et al., 2011a) complemented with a large collection of carbohydrates as sole carbon sources (2 $g.L^{-1}$, Table S10).

Extraction and NMR characterization of intracellular polysaccharide

Z. galactanivorans was grown at 20°C in 300 mL marine mineral medium (MMM) with 10 $g.L^{-1}$ glucose and 0.2 $g.L^{-1}$ NH_4Cl. After 3 days, cells were centrifuged for 20 min at 27,000***g***, 4°C, rinsed twice in filtered seawater (FSW) and stored at -20°C until analysis. Ethanol-insoluble polysaccharides were extracted from cell pellets using a modified procedure from Ernst et al (Ernst et al., 1984). Briefly, cells were boiled for 3 hours in 50% KOH and centrifuged for 10 min at 27,000***g***. The supernatant was filtered at 0.2 µm and mixed with 3 volumes of ethanol. Insoluble material was recovered by centrifugation, rinsed with 80% ethanol and resuspended in 1 mL ultrapure water. Water was exchanged with D_2O by two freeze-drying cycles. The extract was analyzed by ^1H-NMR at 70°C and the spectrum was compared with that of pure glycogen from rabbit liver (Zang et al., 1991).

Quantification of intracellular glycogen content

Z. galactanivorans was grown at 20°C in 30 mL MMM containing 10 $g.L^{-1}$ glucose and 0.2 $g.L^{-1}$ NH_4Cl, in five independent replicate cultures. When stationary phase was reached, cells were centrifuged for 30 min at 4,000***g***, rinsed in 10 ml FSW, resuspended in 30 mL Marine Mineral Medium (MMM, Thomas et al., 2011a) without carbon source and incubated at 20°C. Aliquots for glycogen quantification (1 mL) were sampled daily for 5 days and after 8 days. Intracellular glycogen content was quantified using an adapted procedure from Parrou and François (Parrou and Francois, 1997). Cells were pelleted (5 min at 13,000 ***g***), resuspended in 125 µL 0.25 M Na_2CO_3 and incubated at 98°C for 4 hours. The pH was neutralized by addition of 75 µL 1 M acetic acid and 300 µl 0.2 M sodium acetate pH 5.2. Glycogen was digested by addition of 1.5 µL of amyloglucosidase (final concentration 1.8 $mU.mL^{-1}$) and incubation overnight at 57°C with agitation. Samples were

centrifuged for 5 min at 1,000 ***g***. The amount of reducing ends released (RE_{AMG+}) was quantified on 80 µL of supernatant using the ferricyanide assay (Kidby and Davidson, 1973), with duplicate readings of the absorbance at 420 nm, using a standard curve of 0.25-1.5 mM glucose. In parallel, the amount of reducing ends was measured in controls without amyloglucosidase treatment (RE_{AMG-}). Protein content (PC) was measured on 50 µl of extract using the Bradford method and a standard curve of 0.05-1 $mg.mL^{-1}$ bovine serum albumin. For each extract, glycogen content was expressed in nmol eq. glucose per mg of protein according to the following equation:

$$glycogen\ content = \frac{RE_{AMG+} - RE_{AMG-}}{PC}$$

Visualization of intracellular glycogen by transmission electron microscopy

Z. galactanivorans was grown in excess glucose/limited nitrogen and subsequently transferred to minimum medium without carbon source as described above. Aliquots (1 mL) were sampled before transfer and 6 days after the transfer. Samples were centrifuged 5 min at 14,000***g***, rinsed in 1 mL FSW and fixed with 4% glutaraldehyde, 0.25 M sucrose in 0.2 M sodium cacodylate buffer (SCB) for 3 hours, followed by successive washing steps in SCB containing: 0.25 M sucrose and 0.22 M NaCl (one week); 0.15 M sucrose and 0.27 M NaCl (10 min); 0.05 M sucrose and 0.33 M NaCl (10 min); 0.35 M NaCl (10 min). Samples were post-fixed with 1.5% osmium tetroxide, 0.35 M NaCl in SCB for 1 h at 4°C and rinsed three times in SCB, 0.35 M NaCl before dehydration in graded ethanol series (30, 50, 70, 90, 100% (v/v), twice for 15 min each). Samples were transferred to propylene oxide and progressively embedded in Epon (25% (v/v) for 1 h; 75% overnight; 100% twice for 24 h), followed by a change of fresh Epon and polymerization 48 h at 60°C. Thin sections were stained with uranyl acetate and lead citrate and viewed on a JEOL JEM-1400 transmission electron microscope (JEOL, Tokyo, Japan) equipped with a Gatan Orius camera (Gatan France, Evry, France).

Measurement of iodine intracellular concentration

Z. galactanivorans and *P. haloplanktis* were grown in triplicate in ZoBell medium during 4 days at 20°C. After centrifugation, each cell pellet was freeze-dried, together with 20 mL of the

corresponding supernatant and sterile ZoBell medium as controls. Powder samples were used to determine total iodine contents at the Département d'Analyse Elémentaire, Service Central d'Analyses, Centre National de la Recherche Scientifique (Vernaison, France). After complete combustion in a Schöninger flask, the sample ashes were analyzed using anion-exchange chromatography, with UV detection for iodide. The detection limit was 2 µg of iodine per g of dried weight sample.

Comparative genomics

We compared the predicted capacities for carbohydrate degradation of *Z. galactanivorans* to that of other MHB. To have reliable and comparable annotations, we only took marine bacteria present in the CAZY database (http://www.cazy.org/b.html, Lombard et al., 2014) as of July 2015. In total 125 MHB were identified, and their isolation sources were determined by literature searches (Table S11). The genome sequences of these MHB are mainly high-quality draft and a few are completely closed (Lombard et al., 2010). Sulfatases were found in 63 MHB and the subfamilies of the 594 identified sulfatases were determined by phylogenetic analysis (Fig. S1) and in accordance with the recently published sulfatase classification database SulfAtlas (http://abims.sb-roscoff.fr/sulfatlas) (Barbeyron et al., 2016). For each genome (Table S1), we reported the number of genes per (sub)family of glycoside hydrolases (GH), glycosyltransferases (GT), polysaccharide lyases (PL), carbohydrate esterases (CE), carbohydrate binding modules (CBM) (based on the assignment of the CAZY database) and of sulfatases (based on our phylogenetic analysis, FigS1, and on the SulfAtlas database). The effect of habitat or taxonomy on the number of GH, PL and sulfatases per genome was tested with generalized linear mixed model in R 3.3.2 using a negative binomial distribution, followed by Tukey's pairwise comparisons with Benjamini-Hochberg correction for multiple testing. This analysis was represented as boxplots (Fig. 4). A cluster analysis was computed from a matrix with numbers of genes per family of GH, PL, CE and CBM, in the families GT5 and GT35 (endogenous glycogen metabolism) and per (sub)families of sulfatases (Table S12), in which highly polyspecific families (GH5, GH13, GH16, GH30) were replaced by their CAZY subfamilies for better resolution. Since the

GH16 family has not been officially subdivided yet, we defined substrate subfamilies based on phylogeny (Fig. S4) as previously shown (Barbeyron et al., 1998). This matrix was clustered per genome and per (sub)family with Cluster 3.0 (de Hoon et al., 2004). The (sub)family data were centered on the mean. The clustering method was "average linkage" using uncentered correlation for genome and (sub)family data.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

TB, FT, MC and GM are grateful for support by the French Government through the National Research Agency with regard to the "Blue Enzymes" ANR project (reference ANR-14-CE19-0020-01). HT, FOG and RA acknowledge support by the Max Planck Society. This work was supported in part by funding from the Consortium National de Recherche en Génomique (CNRG) to Genoscope and from the EU FP6 Network of Excellence (NoE) "Marine Genomics Europe". We thank Sophie Le Panse (Platform MERIMAG, SBR, FR2424, Roscoff, France) and Nelly Kervarec (NMR core service, UBO, Brest, France) for their help with electron microscopy and NMR analyses, respectively. We are grateful to Corinne Michel for her artistic drawing of Fig. 3.

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Figure Legends

Fig. 1. Examples of Polysaccharide Utilization Loci (PUL) illustrating the PUL diversity in *Z. galactanivorans*. Genetic organization of three demonstrated carbohydrate-related operons: (A) the alginolytic operons (Thomas et al., 2012) and (B) the mannitol utilization system (Groisillier et al., 2015). (C) The putative laminarin utilization system is divided into three PUL. (D) A PUL including glycoside hydrolase and sulfatase genes and thus predicted to be specific for an uncharacterized sulfated polysaccharide. (E) The putative mannosylglycerate utilization system is the only PUL of *Z. galactanivorans* including a TonB-dependent receptor without an associated *susD*-like gene. Gene function is color-coded: red, glycoside hydrolases (GH) or polysaccharide lyases (PL); orange, other carbohydrate-related genes (e.g. sugar kinase, sugar dehydrogenase); purple, putative regulation factor or signal transduction protein; blue, membrane proteins involved in binding/transport; green, sulfatase; grey, unknown function. The crystal structures of several characterized enzymes from these PUL are also represented: (A) the PL7 alginate lyase AlyA5 (PDB code 4BE3, Thomas et al., 2013); (C) the laminarinases LamA (PDB code 4BOW, Labourel et al., 2014) and LamC (PDB code 4CTE, Labourel et al., 2015). Both enzymes include a GH16 catalytic module, but LamC also features an appended laminarin-specific CBM6 (PDB code 5FUI, Jam et al., 2016). (D) the GH117 ZGAL_3597 (PDB code 4U6B, Ficko-Blean et al., 2015a). Abbreviations: SDR, short chain dehydrogenase; MFS, Major Facility Superfamily transporter; TBDR, TonB-dependent transporter; ABCT, ATP-binding cassette transporter; M2DH, mannitol-2-dehydrogenase; SSS, Solute sodium symporter.

Fig. 2. Visualization of glycogen in *Z. galactanivorans* cells by transmission electron microscopy. *Z. galactanivorans* was pre-cultured with nitrogen limitation and excess of glucose to stimulate carbon storage and subsequently transferred to minimum medium without carbon source. Representative images are shown for cells observed before (A, B, C) or 6 days after the transfer (D, E, F). Red arrows show examples of glycogen granules. Scale bars = 0.2 µm.

Fig. 3. Scheme of *Z. galactanivorans* interacting with red and brown macroalgae. A cell of *Z. galactanivorans* (zoomed view) interacting with a red macroalga and a kelp (brown algae). The proteins from *Z. galactanivorans* are displayed in yellow. The compounds specific to red macroalgae, specific to brown macroalgae, and common to both types of macroalgae are displayed in red, orange and green, respectively. The compounds from competing bacteria are shown in purple. The compounds resulting from *Z. galactanivorans* enzymatic processes are displayed in white. Abbreviations: EPS, exopolysaccharide; DssA, sheath polysaccharide lyase (PL9_4); AHL, acetylated homoserine lactone; F6P, fructose-6-phosphate; M2DH, mannitol-2-dehydrogenase; fk, frucktokinase; NorBC/NosZ: Nitric oxide reductase / Nitrous oxide reductase; vIPO, vanadium-dependent iodoperoxidase; GH63: GH63 family mannosylglycerate hydrolase; glck, glycerate kinase; M6P: mannose-6-phosphate; 3PG, 3-phospho-glycerate; RuMP cycle, ribulose monophosphate cycle; SOD: superoxide dismutase; AgaA-D: family GH16 β-agarases (from AgaA to AgaD); PorA-E: family GH16 β-porphyranases (from PorA to PorE); AhgA-B: family GH117 α-1,3-(3,6-anhydro)-L-galactosidase AhgA and AhgB; CgkA: family GH16 κ-carrageenase CgkA; CgiA: family GH82 ι-carrageenase (from CgiA1 to CgiA3); LamA-E, family GH16 laminarinases (from LamA to LamD) and family GH64 laminarinase LamE; AlyA1-A7; alginate lyases (PL6: AlyA4 and AlyA6; PL7: AlyA1, AlyA2 and AlyA5; PL14: ALyA7; PL17, AlyA3).

Fig. 4. Boxplots of the total number of glycoside hydrolase, polysaccharide lyase and sulfatase-encoding-genes in the genomes of 126 marine heterotrophic bacteria available in the CAZY database (http://www.cazy.org/), grouped according to taxonomy (A, B, C) or habitat (D, E, F). For statistical robustness, this analysis was restricted to the phylum *Bacteroidetes* and the classes *Alpha-* and *Gammaproteobacteria* as most represented taxa. Black dots and diamonds represent outliers and mean values, respectively. Letters denote significantly different groups (FDR <0.05). The number of bacterial genomes included in the analysis was as follows: Macrophyte (macroalgae and seagrasses), 8; Phytoplankton, 7; Animal, 6; Surface seawater, 42; Deep seawater, 12; Sediment, 20;

Deep sediment, 18; Hydrothermal vent, 13; *Alphaproteobacteria*, 18, *Gammaproteobacteria*, 53; *Bacteroidetes*, 21.

Fig. S1. Phylogenetic tree of the formylglycine-dependent sulfatases identified in the 126 marine heterotrophic bacteria. The phylogenetic tree was constructed using the maximum likelihood (ML) approach with the program MEGA 6.0.6 (Tamura et al., 2013). Numbers indicate the bootstrap values in the ML analysis (100 bootstraps have been performed). The gene systematic ID and the name of the bacterium is indicated in each sequence label. The sequences marked by red dots correspond to biochemically characterized sulfatases. Each clade has been attributed a subfamily number within the family S1 of sulfatases (formylglycine-dependent sulfatases) in coherence with the SulfAtlas database (http://abims.sb-roscoff.fr/sulfatlas) (Barbeyron et al., 2016). The subfamilies containing characterized sulfatases have been attributed the same substrate specificity.

Fig. S2. A. Proton NMR spectrum of *Z. galactanivorans* intracellular ethanol-insoluble material (50 $mg.ml^{-1}$, at 70°C). The spectrum of pure type III glycogen from rabbit liver (50 $mg.ml^{-1}$, at 55°C) is shown for comparison (modified from Zang et al., 1991, with the permission of Carbohydrate Research and Elsevier). **B.** Intracellular glycogen content in *Z. galactanivorans*, from 1 to 8 days after transfer to minimum medium without carbon source. Glycogen was quantified by enzymatic hydrolysis using an amyloglucosidase into glucose monomers detected by a reducing sugars assay, and normalized by protein quantity in each extract. Values are mean ± s.e.m. (n=5). Asterisks denote significant difference to Day 1 (paired t-test; *, $P<0.05$; **, $P<0.01$).

Fig. S3. Comparison of the repertoires of carbohydrate-related proteins of 126 marine heterotrophic bacteria available in the CAZY database using double hierarchical clustering. The matrix used for this clustering is available as Table S12. Left tree: Bacterial genomes (listed in Table S11). The taxonomy (in magenta) and habitat (in green) of each bacterium is indicated on the right. Taxonomy abbreviations: Ac, Actinobacteria; aP, Alphaproteobacteria, Aq, Aquificae; Ba, Bacteroidetes; Cl, Chlorobi; De, Deinococcus-Thermus, dP, Deltaproteobacteria; eP,

Epsilonproteobacteria; Fi, Firmicutes; gP: Gammaproteobacteria; P, Planctomycetes, Td, Thermodesulfobacteria; Tt, Thermotogae; uP, unclassified Proteobacteria; V, Verrucomicrobia. Habitat abbreviations: A, macroalgae-associated; DMS, deep marine sediments; DS: deep seawater; DV, deep hydrothermal vent; MA, marine animal-associated, MS, marine sediments; P, phytoplankton-associated; SS, surface seawater. Based on the clades, 15 bacterial groups (B. group) were defined. Top tree: carbohydrate-related (sub)families (GH, Glycoside hydrolases; PL, Polysaccharide lyases; CE, Carbohydrate esterases; CBM, Carbohydrate binding module; GT5 and GT35, glycosyltransferases from families 5 and 35; S1-n, sulfatase from family S1 (formylglycine-dependent) subfamily n; S3, sulfatase from family 3 (zinc beta-lactamase superfamily). The number of proteins per (sub)families was determined according to the CAZY database (http://www.cazy.org/) for the CAZYmes and according to Fig. S1 for the sulfatases (in concordance with the SulfAtlas database, http://abims.sb-roscoff.fr/sulfatlas). A substrate-specificity was attributed to 17 protein clades, referred to as carbohydrate-related groups (CRG), based on current literature. The values of each column have been centered and are represented according to a blue (negative) – yellow (positive) scale. The clustering was calculated using Cluster 3.0 (de Hoon et al., 2004) and represented by TreeView (Page, 1996).

Fig. S4. Phylogenetic tree of the glycoside hydrolases from family 16 (GH16) identified in the 126 marine heterotrophic bacteria. The phylogenetic tree was constructed using the maximum likelihood (ML) approach with the program MEGA 6.0.6 (Tamura et al., 2013). Numbers indicate the bootstrap values in the ML analysis (100 bootstraps have been performed). The sequences marked by black triangles and black dots correspond to biochemically characterized enzymes and to Z. galactanivorans enzymes, respectively. The sequence labels correspond to the gene systematic ID. For characterized sequences, the label also contains the EC number and the PDB code, when available. Each clade has been attributed a substrate specificity and a EC number based on the presence of characterized enzymes, as previously shown (Barbeyron et al., 1998).

1 **Table S1. Comparison of the repertoires of CAZYmes and of sulfatases of the 126 marine**
2 **heterotrophic bacteria. Worksheet "CAZY repertoire"**. Six protein categories were considered:
3 glycoside hydrolases (GH), glycosyltransferases (GT), polysaccharide lyases (PL), carbohydrate
4 esterases (CE), carbohydrate binding modules (CBM) and sulfatases (S1_n and S3). Per bacterial
5 genome is reported (in each protein category) the number of proteins in each protein (sub)family,
6 the total number of proteins, and the total number of proteins (sub)families. Each cell is colored
7 according to a conditional formatting of its column (three-color scale with blue for 0%, yellow for
8 50%, red for 100%, with the 100% corresponding to the highest number in each column). **Worksheet**
9 **"CAZY summary"**. Various indicators are calculated based on the data of the "CAZY repertoire"
10 worksheet. The bacterial genomes are sorted in decreasing order of total number of GH.
11 **Table S2: Comparison of TonB-dependent receptors (TBDR) gene occurrences in marine**
12 ***Bacteroidetes*.** For each genome, the total number of TBDR genes is indicated and subdivided into
13 TBDR gene next to a *susD*-like gene (SusC-like TBDR) or standalone TBDR gene (FhuA-like TBDR). The
14 ratio of these two categories of TBDR genes (SusC-like/FhuA-like) is given. Each cell is colored
15 according to a conditional formatting of its column (three-color scale with blue for 0%, yellow for
16 50%, red for 100%, with the 100% corresponding to the highest number in each column).
17 **Table S3. List and gene composition of the 50 Polysaccharide Utilization Loci (PUL) of *Zobellia***
18 ***galactanivorans*.** A putative substrate was proposed for each PUL, when possible. The PUL
19 comprising sulfatase genes are colored in green. The TonB-dependent transducers and their
20 associated RNA polymerase ECF-type sigma factor and anti-sigma factor are colored in orange.
21 **Table S4. List of the genes of *Zobellia galactanivorans* involved in carbohydrate degradation.** For
22 each carbohydrate tested as a carbon source (Cf. Table 2) the presence of the genes responsible for
23 its degradation was checked in *Z. galactanivorans* genome. The systematic ID of each homologous
24 gene is indicated.
25 **Table S5. List and gene composition of the 14 gene clusters encompassing glycosyltransferases.**

Table S6. List of the *Flavobacteriia* genera featuring glycogen synthase (GT5), Glucose-1-phosphate adenylyltransferase (GlgC), Glycogen phosphorylase (GT35), debranching enzyme GlgX (GH13_11), alpha-1,4-glucanotransferase (MalQ).

Table S7. List of the genes involved in the defense against the reactive species of nitrogen and oxygen and in nitrate assimilation.

Table S8. Total iodine contents of *Pseudoalteromonas haloplanktis* and *Zobellia galactinovorans* cultures. The measure were done using biological triplicates.

Table S9. Average number of glycoside hydrolases (GH), polysaccharide lyases (PL) and sulfatases (S) of the 126 marine heterotrophic marine bacteria according to their taxonomy and their habitat. The number of genome in each taxon and in each habitat is indicated between brackets.

Table S10. List of the carbohydrate source used for the catabolic profiling of *Zobellia galactanivorans*. Commercial provider and reference are indicated for each compound.

Table S11. List of 126 marine heterotrophic bacteria identified in the CAZY database (http://www.cazy.org/). For each bacterium is indicated the taxonomic affiliation, the type of marine habitat, the isolation location, the geographic coordinates and the depth (when available), the species name of the associated host (when applicable), the bibliographic reference. It is noteworthy that only bacteria isolated from genuine marine habitats have been selected. For instance, the potent degrader *Saccharophagus degradans* 2-40 has not been considered in this study. Although this bacterium has sometimes been described as a marine bacterium (Weiner et al., 2008), it was in fact isolated from a halophilic land plant (*Spartina alterniflora*) in a salt marsh (Andrykovitch and Marx, 1988). Consistently, S. *degradans* has a repertoire of CAZYmes reminiscent of that of the terrestrial degrader *Cellvibrio japonicus* (Weiner et al., 2008). Its alginate-related genes were also acquired by horizontal gene transfer (Thomas et al., 2012). This bacterium possesses several β-agarases and is able to degrade neutral agarose. However, it features no β-porphyranase and even more significantly no sulfatase, indicating a limited capacity to degrade natural agars which are sulfated.

1 **Table S12. Matrix used for the double hierarchical clustering of the 126 marine heterotrophic**

2 **bacteria and of their carbohydrate-related proteins.**

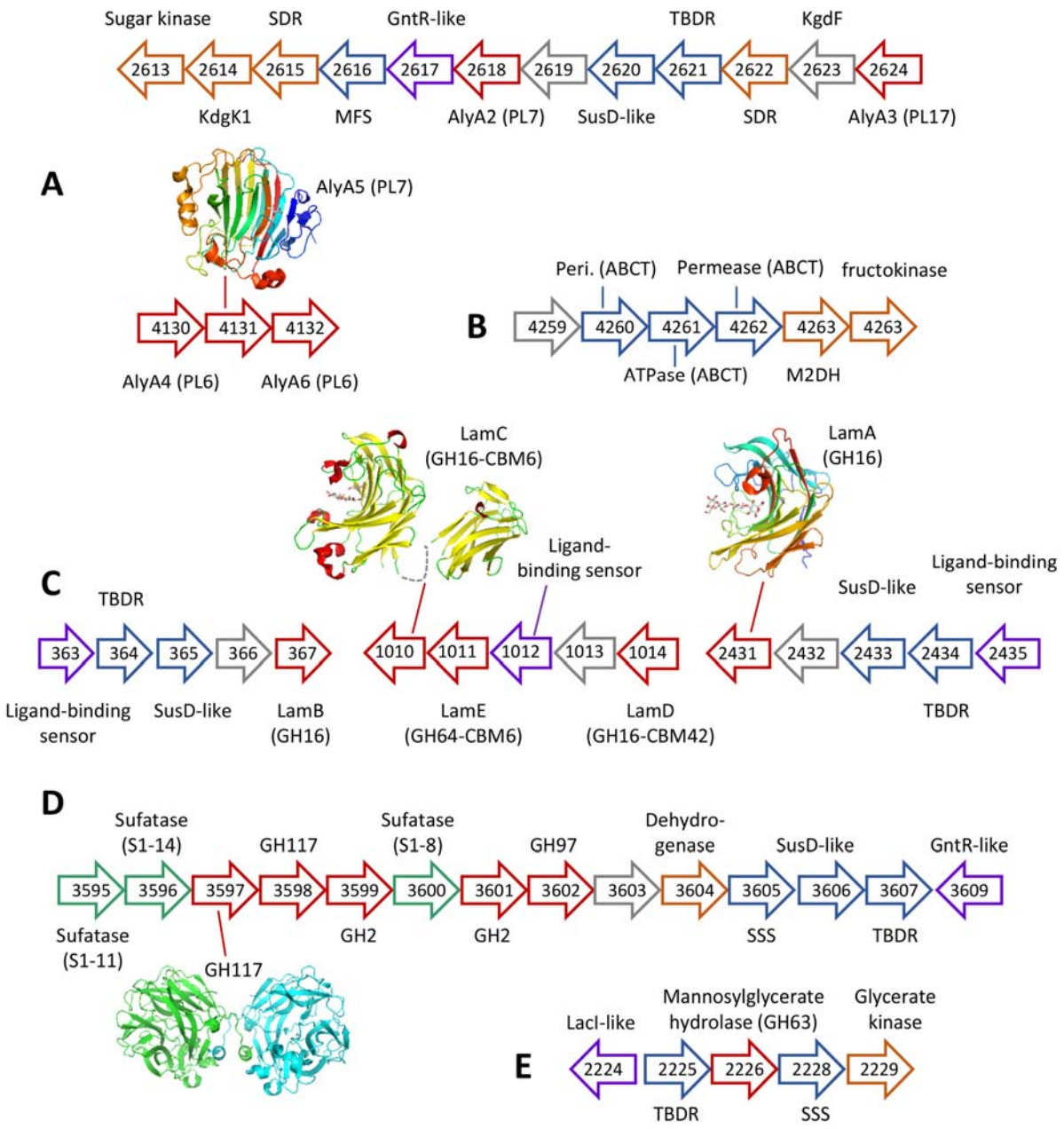
Table 1. *Zobellia galactanivorans* repertoires of carbohydrate active enzymes, sulfatases and peptidases. Seven protein categories were considered: glycoside hydrolases (GH), glycosyltransferases (GT), polysaccharide lyases (PL), carbohydrate esterases (CE), carbohydrate binding modules (CBM), formylglycine-dependent sulfatases (Sulf. S1-) and peptidases (Pept.). The number of proteins (#) is indicated per CAZY family, sulfatase subfamily and MEROPS family (highlighted in grey). The total of proteins (T) and of protein families (FT) per protein category is also indicated.

																T	FT
GH	**1**	**2**	**3**	**5**	**9**	**10**	**13**	**15**	**16**	**18**	**20**	**23**	**27**	**28**	**29**	**141**	**44**
#	1	9	5	3	1	3	6	2	16	2	4	2	1	2	18		
GH	**30**	**31**	**33**	**36**	**43**	**63**	**64**	**65**	**73**	**78**	**82**	**88**	**89**	**92**	**95**		
#	1	2	1	2	2	1	1	1	1	2	3	2	1	1	7		
GH	**97**	**105**	**109**	**110**	**113**	**114**	**115**	**116**	**117**	**120**	**127**	**129**	**130**	**NC**			
#	6	3	1	2	1	1	1	2	6	1	4	1	1	8			
GT	**1**	**2**	**4**	**5**	**9**	**19**	**20**	**28**	**30**	**51**	**83**					**56**	**11**
#	1	21	18	2	1	1	1	1	1	6	3						
PL	**1**	**6**	**7**	**9**	**12**	**14**	**17**	**NC**								**15**	**8**
#	2	2	3	2	1	1	1	3									
CE	**1**	**4**	**6**	**7**	**9**	**11**	**14**	**15**	**NC**							**18**	**9**
#	5	3	3	1	1	1	2	1	1								
CBM	**4**	**6**	**16**	**22**	**32**	**35**	**42**	**47**	**48**	**50**	**51**	**57**				**37**	**14**
#	1	5	2	4	1	2	1	2	1	8	2	6					
Sulf S1-	**7**	**8**	**11**	**14**	**15**	**16**	**17**	**19**	**20**	**22**	**23**	**24**	**25**	**27**	**28**	**71**	**17**
#	4	8	6	2	6	8	8	3	8	1	4	2	3	2	4		
Sulf S1-	**29**	**34**															
#	1	1															
Pept.	**A2**	**A8**	**C40**	**C45**	**C56**	**M1**	**M3**	**M4**	**M12**	**M14**	**M15**	**M16**	**M19**	**M20**	**M22**	**101**	**37**
#	1	1	4	1	4	8	1	2	1	4	2	2	1	6	2		
Pept.	**M23**	**M24**	**M28**	**M29**	**M42**	**M48**	**M50**	**M61**	**S1**	**S8**	**S9**	**S12**	**S13**	**S14**	**S16**		
#	6	4	5	1	1	3	1	2	1	2	6	9	1	2	1		
Pept.	**S26**	**S41**	**S49**	**S54**	**S66**	**T3**	**U48**										
#	1	6	1	4	1	1	2										

Table 2: Carbohydrate utilization by *Zobellia galactanivorans*. Growth was tested with a collection of carbohydrates as sole carbon source (using biological triplicates). The "genes" column indicates the presence (+) or absence (-) of the genes responsible for the utilization of the corresponding substrate based on pathways characterized in other organisms. Detailed information of these genes is reported in Supplementary Table S6. The theoretical pathway is incomplete for D-galactose (4/5 enzymes) and sucrose (3/4 enzymes), although these substrates are assimilated. The substrates highlighted in blue are assimilated, but the corresponding genes in other organisms are not conserved in *Zbl.* galactanivorans. The substrates highlighted in green are not assimilated, despite the presence of conserved genes potentially involved in their utilization pathway

Carbon source	Growth	Genes	Carbon source	Growth	Genes	Carbon source	Growth	Genes
D-glucose	+	+	Lactose	+	+	MLG	+	+
D-galactose	+	+ **(4/5)**	Starch	+	+	Fucoidan (*P. can*)	-	-
L-arabinose	+	+	Glycogen	-	+	Fucoidan (*A. nod.*)	+	-
D-mannose	+	+	Dextran	-	-	Fucoidan (*L. hyp.*)	+	-
L-fucose	+	-	Dextran sulfate	-	-	Xanthan	-	-
L-rhamnose	+	-	Cellulose (Avicel)	-	+	Xylan	+	+
D-xylose	+	+	Agar	+	+	Xyloglucan	-	+
D-fructose	+	+	Porphyran	+	+	Mannan	-	+
D-ribose	-	+	κ-carrageenan	+	+	Galactomannan	-	+
N-acetylglucosamine	+	+	ι-Carrageenan	+	+	galactan (arabic gum)	+	-
Sialic acid	**n.t.**	+	Methylated ι-carrageenan	+	+	Arabinogalactan	-	+
D-Galacturonic acid	-	+	λ-Carrageenan	+	-	Gluconolactone	-	+
D-mannitol	+	+	Alginate	+	+	Chitin	-	+
glycerol	+	+	Ulvan	-	-	Chitosan	-	-
D-Sorbitol	-	+	Pectin	-	+	Chondroitin sulfate A	-	-
Sucrose	+	+ **(3/4)**	Laminarin	+	+	Chondroitin sulfate B	-	-
Maltose	+	+	Pachyman	-	+	Chondroitin sulfate C	-	-
D-Melibiose	+	+	Paramylon	-	+	Hyaluronic acid	-	-
D-Raffinose	+	+	Curdlan	+	+	Heparin	-	-

1



2

3 **Figure 1**

4

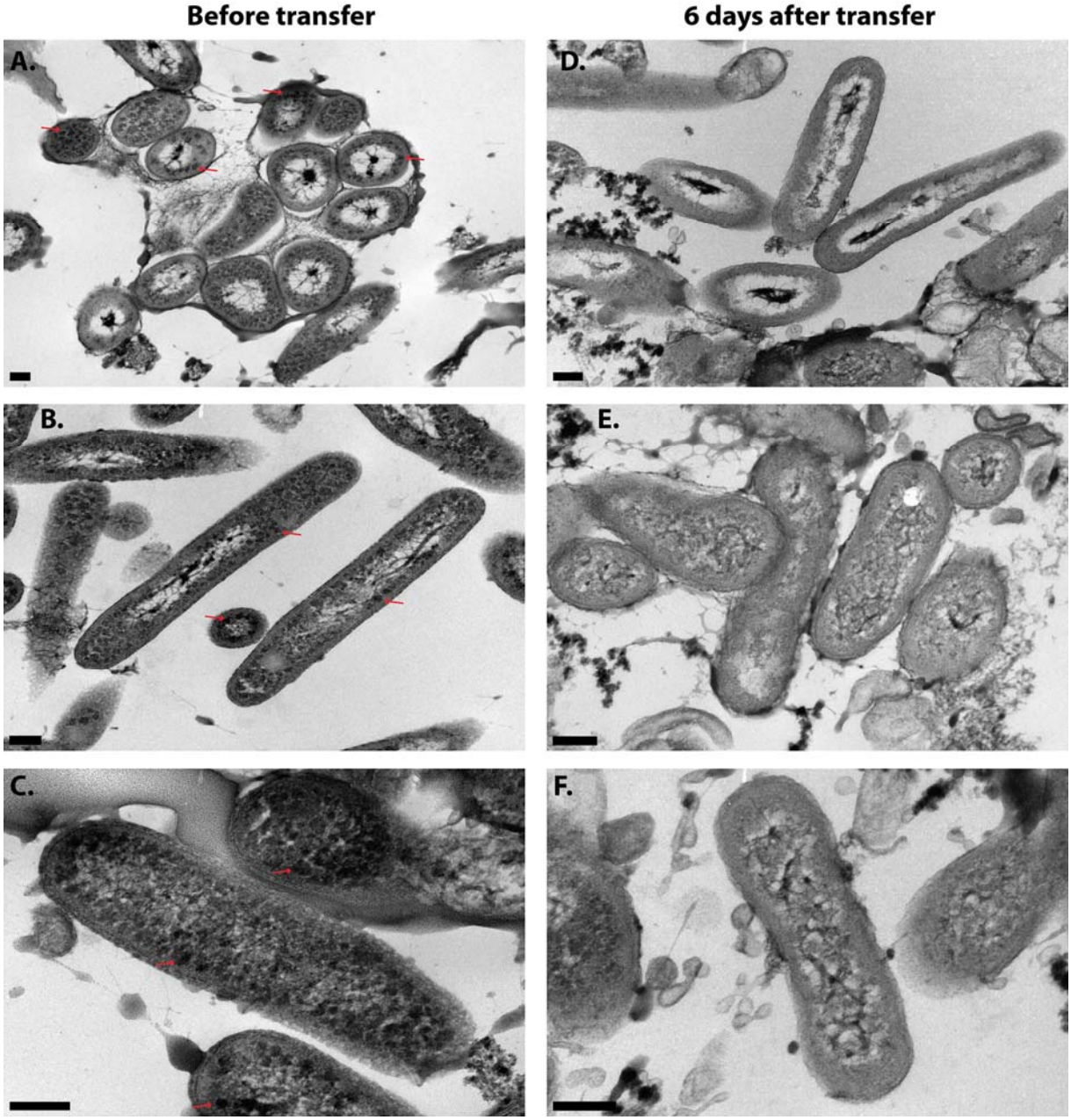


Figure 2

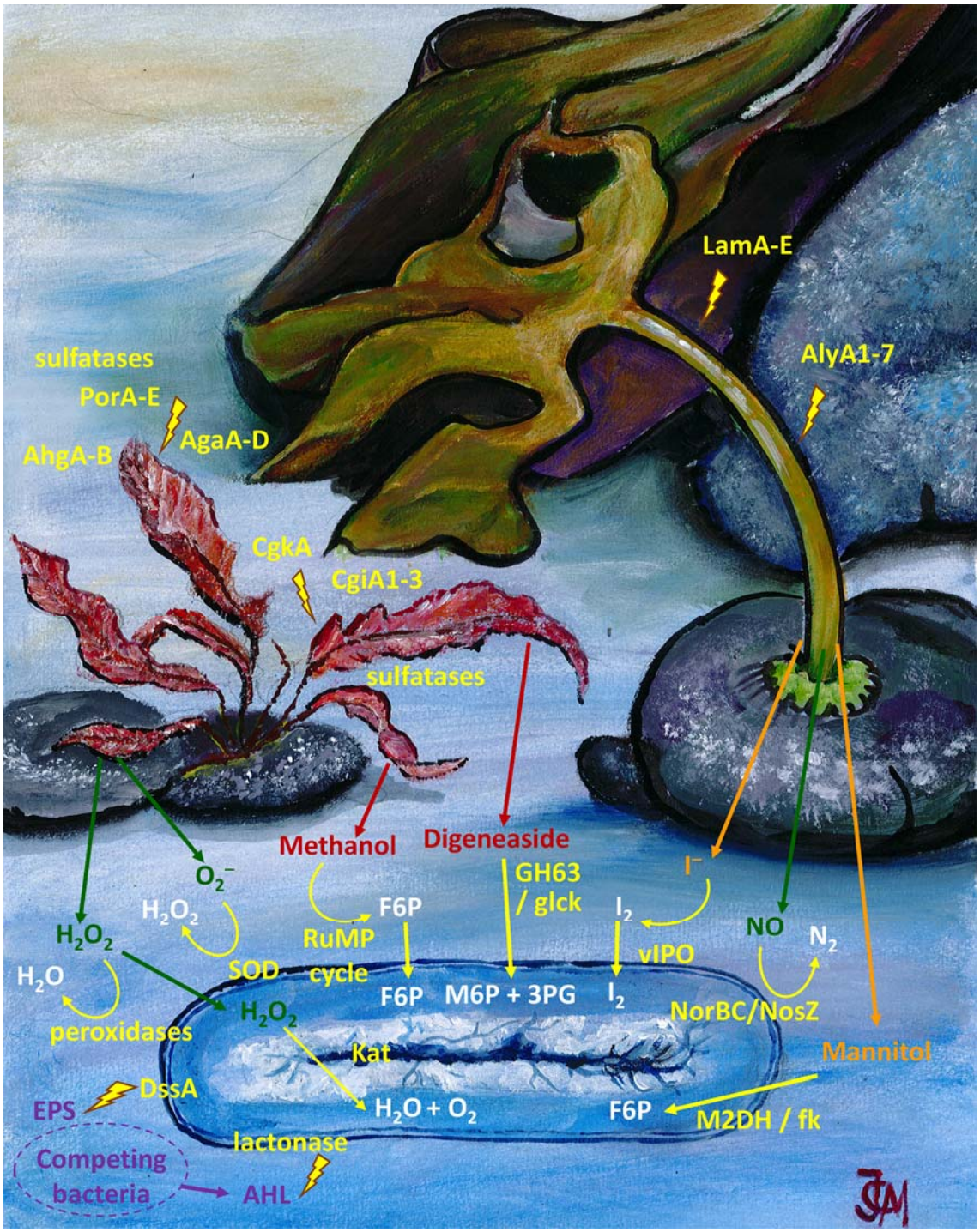

LamA-E
AlyA1-7
sulfatases
PorA-E
AgaA-D
AhgA-B
CgkA
CgiA1-3
sulfatases
Methanol
Digeneaside
GH63
/ glck
O_2^-
H_2O_2
F6P
RuMP
cycle
I_2
I^-
NO
N_2
H_2O_2
SOD
vIPO
H_2O
F6P
M6P + 3PG
I_2
NorBC/NosZ
peroxidases
H_2O_2
Kat
Mannitol
DssA
EPS
$H_2O + O_2$
F6P
M2DH / fk
lactonase
Competing
bacteria
AHL

Figure 3

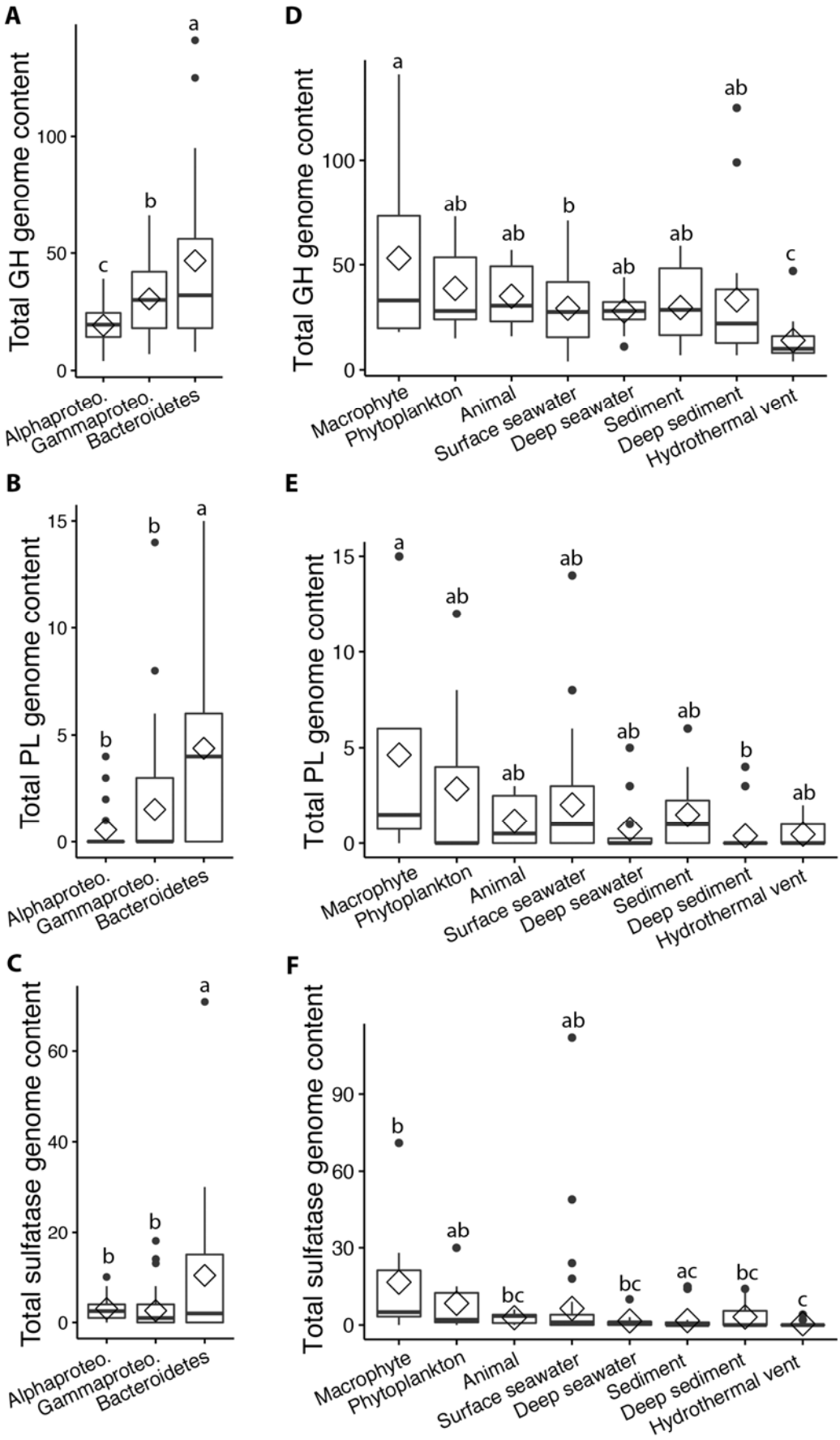

A
Total GH genome content
Alphaproteo.
Gammaproteo.
Bacteroidetes
B
Total PL genome content
C
Total sulfatase genome content
D
E
F
Macrophyte
Phytoplankton
Animal
Surface seawater
Deep seawater
Sediment
Deep sediment
Hydrothermal vent

Figure 4