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EXPERIMENTAL ARTICLES

Diversity of Glycosidase Activities in the Bacteria of the Phylum *Bacteroidetes* Isolated from Marine Algae

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Abstract—Glycosidase activities of 177 strains of the phylum *Bacteroidetes*, belonging to 18 genera and isolated from the algae *Chondrus* sp., *Polysiphonia* sp., *Neosiphonia japonica, Saccharina crassifolia*, *Saccharina japonica, Chorda filum, Acrosiphonia sonderi*, and *Ulva fenestrata* collected in the littoral zones of the Sea of Okhotsk and the Sea of Japan, Pacific Ocean, were studied. According to the data obtained, glycosidases catalyzing hydrolysis of the β-glycoside bond were present in over 70% epiphytes of marine algae. It should be noted that α-galactosidases and the extremely rare enzymes, α-*N*-acetylgalactosaminidases, were more frequent in the *Bacteroidetes* than in the proteobacteria analyzed previously. It was found that the overwhelming majority of the bacteria of the dominant genera *Zobellia* and *Maribacter* contained the complete set of the tested glycosidases involved in degradation of algal polysaccharides. Apparently, the presence of the wide range of glycosidases in bacterial strains of these genera makes it possible for them to occupy diverse ecological niches under extreme conditions of the tidal zone. However, such important enzymes of the microbial lytic complex as α-galactosidases, β-galactosidases, or β-xylosidases, were not detected in the numerically important genus *Winogradskyella.* The noted difference in the metabolic profiles of the strains of these genera suggests the assumption that *Winogradskyella* strains play an unique role in the microbial communities, unrelated to the hydrolysis of such polysaccharides as agar and carrageenan. Significant differences in production of glycosidases among the different taxonomic groups were revealed, which is of importance for directed search of promising enzymes for biotechnology.

Keywords: α-galactosidase, α-mannosidase, α-L-fucosidase, β-galactosidase, α-*N*-acetylgalactosaminidase, β-xylosidase, β-*N*-acetylglucosaminidase, marine bacteria of the phylum *Bacteroidetes: Arenibacter, Cellulophaga, Maribacter, Zobellia, Formosa, Winogradskyella*, marine algae

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The major structural components of cell walls of most marine macroalgae are cellulose, organized in crystal microfibrils (in brown algae), and hemicellulose, comprising 1,3-β-D-xylan or 1,4-β-D-mannan (in green and red algae, respectively) [1]. Matrices of the cell walls of marine algae contain polyanionic homo- and heteropolysaccharides [1–5]: various fucoidan (sulfated fucans) structures and alginic acids in brown algae, agars and carrageenans (sulfated galactans) in red ones, or ulvans (sulfated rhamnoglucans, glucogalactans, arabinogalactans, and rhamnoxylogalactans) in green ones. Quantitative composition and structure of these polysaccharides vary depending on the taxonomic affiliation of the algae and their habitat [2–4]. Moreover, the following compounds are contained in seaweeds as storage carbohydrates: laminaran, in brown [6], floridean-starch, in red [7], and starch and laminaran, in green algae [8].

Degradation of polysaccharides is performed by the enzymes secreted by microorganisms associated with algae. These enzymes include various depolymerases acting on the biologically important glycoside bond of the polysaccharides. There are reports on the enzymes of marine proteobacteria and *Bacteroidetes* degrading alginate [9, 10], laminaran [11], as well as agar, carrageenan, and hemicellulose (1,3-β-xylan) [12], and enzymes of bacteria of the genus *Pseudoalteromonas* and the family *Flavobacteriaceae* involved in fucoidan and porphyran degradation [13–17]. Glycoside hydrolases of marine origin are used in hydrolysis and synthesis of various biologically active oligosaccharides [12].

Marine gram-negative heterotrophic pigmented bacteria of the phylum *Bacteroidetes* dominate in the primary epiphyte community [18]. They are known for their high metabolic activity [19] resulting from the diversity of their enzymatic systems. Transfer of the glycoside hydrolase genes from marine *Bacteroidetes* to the members of this phylogenetic cluster, which are

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an important component of the human colonic microbiota, was reported [17]. However, glycosidases synthesized by marine bacteria of the phylum *Bacteroidetes* associated with seaweed remain practically uncharacterized.

Earlier, we demonstrated that marine *Bacteroidetes* have a high potential as sources of fucoidanases, alginate lyases, and laminaranases [20], as well as biotechnologically important α-*N*-acetylgalactosaminidases [21, 22].

The goal of the present work was comparative study of glycosidase diversity in the *Bacteroidetes* isolated from red, green, and brown algae growing in the littoral zones of Far Eastern seas, together with matching of various glycosidase activities with the taxonomic position and source of bacterial producers. The results of the work may be used for characterization of the metabolic potential and selection of the biotechnologically promising strains of marine bacteria of the phylum *Bacteroidetes*.

MATERIALS AND METHODS

Strains of marine bacteria used in the work were isolated from red algae *Chondrus* sp. (Iturup Island, Sea of Okhotsk, October 1997), *Polysiphonia* sp. (Paramushir Island, Sea of Okhotsk, August 1992), and *Neosiphonia japonica* (Cove of Trinity, Posyet Bay, Sea of Japan, June 2000), brown algae *Chorda filum* (Iturup Island, Sea of Okhotsk, October 1997 and Cove of Trinity Bay, Posyet Bay, Sea of Japan, June 2000), *Saccharina crassifolia* (Iturup Island, Okhotsk Sea, October 1997), and *Saccharina japonica* (Cove of Trinity, Posyet Bay, Sea of Japan, June 2000), and green algae *Acrosiphonia sonderi* (Cove of Trinity, Posyet Bay, Sea of Japan, June 2000) and *Ulva fenestrata* (Cove of Trinity, Posyet Bay, Sea of Japan, August 1999 and June 2000).

Algal samples were collected aseptically in γ-ray-sterilized plastic bags. Samples of algal tissues (1 g per 10 mL of sterile seawater) were treated in a glass homogenizer. Microorganisms were isolated by direct plating of the algal homogenate applied in 0.1-mL aliquots per petri dish with marine agar (Difco, United States). Heterotrophic bacteria isolated from single colonies after incubation for 7 days at 28°C were purified by sequential transfers. The obtained pure bacterial cultures were stored in plastic tubes containing artificial seawater and glycerol (30%) as a cryoprotector at –80°C in the Collection of Marine Microorganisms of the Pacific Institute of Bioorganic Chemistry, Far Eastern Branch of the Russian Academy of Sciences.

Identification of bacterial strains. Bacteria were identified on the basis of their standard phenotypic, chemotaxonomic, and genomic characteristics as described previously [23]. Affiliation to a genus or species was determined by the nucleotide sequence of the 16S rRNA gene using the GenBank BLAST data base (http://www.ncbi.nlm.nih.gov/BLAST), confirmed by the physiological and biochemical characteristics of the strains.

Bacteria were cultured for 96 h at 28°C on a solid medium containing the following (g/L): bacto peptone, 5.0; yeast extract, 2.0; agar, 18.0 (Difco, United States); glucose, 1.0; KH_2PO_4, 0.2; K_2HPO_4, 0.2; $MgSO_4 \cdot 7H_2O$, 0.05; seawater, 500 mL; and distilled water, 500 mL; pH 7.6–7.8.

Glycosidase activity in bacteria was tested in ELISA plates. Each cell contained 0.05 mL suspension with approximately 50 μg bacterial biomass transferred to the plates with a platinum loop, together with 0.05 mL 0.025% of the relevant synthetic chromogenic glycoside substrate (0.05 M sodium phosphate buffer, pH 7.3). The plates were incubated for 3 h at room temperature.

To detect the α- and β-D-galactosidase, α-D-mannosidase, β-D-glucosidase, α-L-fucosidase, and α-*N*-acetylgalactosaminidase activities, *p*-nitrophenyl (*p*NP) derivatives of α- and β-D-galacto-, α-D-manno-, β-D-gluco-, α-L-fucopyranoside, and *N*-acetyl-α-D-galactosaminide (Sigma, United States), respectively, were used. To determine the β-*N*-acetylglucosaminidase and β-xylosidase activites, *p*NP-*N*-acetyl-β-D-glucosaminide (Chemapol, Czech Republic) and 4-methylumbelliferyl-β-D-xylopyranoside (Ferak, Germany), respectively, were used.

Enzyme activities were evaluated visually 5, 10, 15, 30, 60, 120, and 180 min after addition of the substrate by the appearance of yellow coloration due to formation of *p*-nitrophenol in the course of enzymatic reaction. Solutions of the respective glycosides and biomass suspensions were used as the controls.

RESULTS

The presence of β-D-glucosidase, β-D-galactosidase, β-*N*-acetyl-D-glucosaminidase, β-D-xylosidase, α-D-galactosidase, α-D-mannosidase, α-*N*-acetyl-D-galactosaminidase, and α-L-fucosidase activities was determined in 177 strains of marine bacteria of the phylum *Bacteroidetes* isolated from algae of the Sea of Okhotsk and the Sea of Japan. The results are presented in Table 1. As follows from the data, among the 18 identified taxa of the phylum *Bacteroidetes*, *Maribacter* (23.2%), *Zobellia* (22.6%), *Cellulophaga* (14.7%), *Formosa* (9.6%), and *Winogradskyella* (7.9%) were the most frequent. The genera *Aquimarina, Gillisia, Kriegella, Olleya, Pseudozobellia*, and *Salegentibacter* were each represented by a single strain (0.6%).

The most abundant enzymes among all of the studied epiphytes were β-glucosidases (81.9%), β-galactosidases (79.1%), and β-*N*-acetylglucosaminidases (78.5%). Less frequent were α-galactosidases (72.3%), β-xylosidases (71.3%), and α-mannosidases (65.5%). α-*N*-Acetylgalactosaminidases (37.9%) and α-L-fucosidases (34.5%) were relatively rare among glycosidases.

Table 1. Occurrence of glycosidases among marine *Bacteroidetes* isolated from various marine algae

Bacterial genus	Number of studied strains	Number of active strains							
		β-glucosidase	β-galactosidase	β-*N*-acetylglucosaminidase	α-galactosidase	β-xylosidase	α-mannosidase	α-*N*-acetylgalactosaminidase	α-fucosidase
Algibacter	6 (3.4)	6 (3.4)	5 (2.8)	3 (1.7)	2 (1.1)	1 (0.6)	3 (1.7)	0 (0)	3 (1.7)
Algoriphagus	3 (1.7)	2 (1.1)	3 (1.7)	1 (0.6)	2 (1.1)	2 (1.1)	3 (1.7)	0 (0)	1 (0.6)
Aquimarina	1 (0.6)	1 (0.6)	0 (0)	1 (0.6)	1 (0.6)	1 (0.6)	0 (0)	0 (0)	0 (0)
Arenibacter	10 (5.6)	8 (4.5)	8 (4.5)	7 (3.9)	10 (5.6)	9 (5.1)	9 (5.1)	9 (5.1)	7 (3.9)
Cellulophaga	26 (14.7)	22 (12.4)	22 (12.4)	21 (11.9)	23 (13.0)	17 (9.6)	15 (8.5)	4 (2.3)	1 (0.6)
Gillisia	1 (0.6)	1 (0.6)	1 (0.6)	1 (0.6)	1 (0.6)	0 (0)	0 (0)	0 (0)	0 (0)
Formosa	17 (9.6)	13 (7.3)	12 (6.8)	15 (8.5)	7 (3.9)	15 (8.5)	3 (1.7)	3 (1.7)	9 (5.1)
Kriegella	1 (0.6)	1 (0.6)	1 (0.6)	1 (0.6)	1 (0.6)	1 (0.6)	1 (0.6)	1 (1)	1 (0.6)
Maribacter	41 (23.2)	38 (21.5)	41 (23.2)	35 (19.8)	38 (21.5)	38 (21.5)	34 (19.2)	26 (14.7)	14 (7.9)
Mesonia	5 (2.8)	3 (1.7)	1 (0.6)	5 (2.8)	2 (1.1)	2 (1.1)	1 (0.6)	1 (0.6)	1 (0.6)
Olleya	1 (0.6)	1 (0.6)	0 (0)	1 (0.6)	1 (0.6)	1 (0.6)	0 (0)	0 (0)	1 (0.6)
Pseudozobellia	1 (0.6)	1 (0.6)	1 (0.6)	1 (0.6)	1 (0.6)	1 (0.6)	1 (0.6)	1 (0.6)	0 (0)
Roseivirga	2 (1.13)	2 (1.1)	2 (1.1)	0 (0)	2 (1.1)	2 (1.1)	2 (1.1)	2 (1.1)	0 (0)
Salegentibacter	1 (0.6)	1 (0.6)	0 (0)	1 (0.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Tenacibaculum	2 (1.13)	0 (0)	0 (0)	2 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ulvibacter	5 (2.82)	3 (1.7)	3 (1.7)	5 (2.8)	3 (1.7)	2 (1.1)	3 (1.7)	0 (0)	0 (0)
Winogradskyella	14 (7.9)	10 (5.6)	0 (0)	12 (6.8)	1 (0.6)	0 (0)	4 (2.3)	0 (0)	6 (3.4)
Zobellia	40 (22.6)	32 (18.1)	40 (22.6)	27 (15.3)	33 (18.6)	35 (19.8)	37 (20.9)	20 (11.3)	17 (9.6)
Total	**177 (100)**	**145 (81.9)**	**140 (79.1)**	**139 (78.5)**	**128 (72.3)**	**127 (71.8)**	**116 (65.5)**	**67 (37.9)**	**61 (34.5)**

Note: * in parentheses, the frequency of occurrence of the strains of a given genus in percent to the total number of studied strains is presented; ** in parentheses, the frequency of occurrence of the active strains in percent to the total number of studied strains is presented.

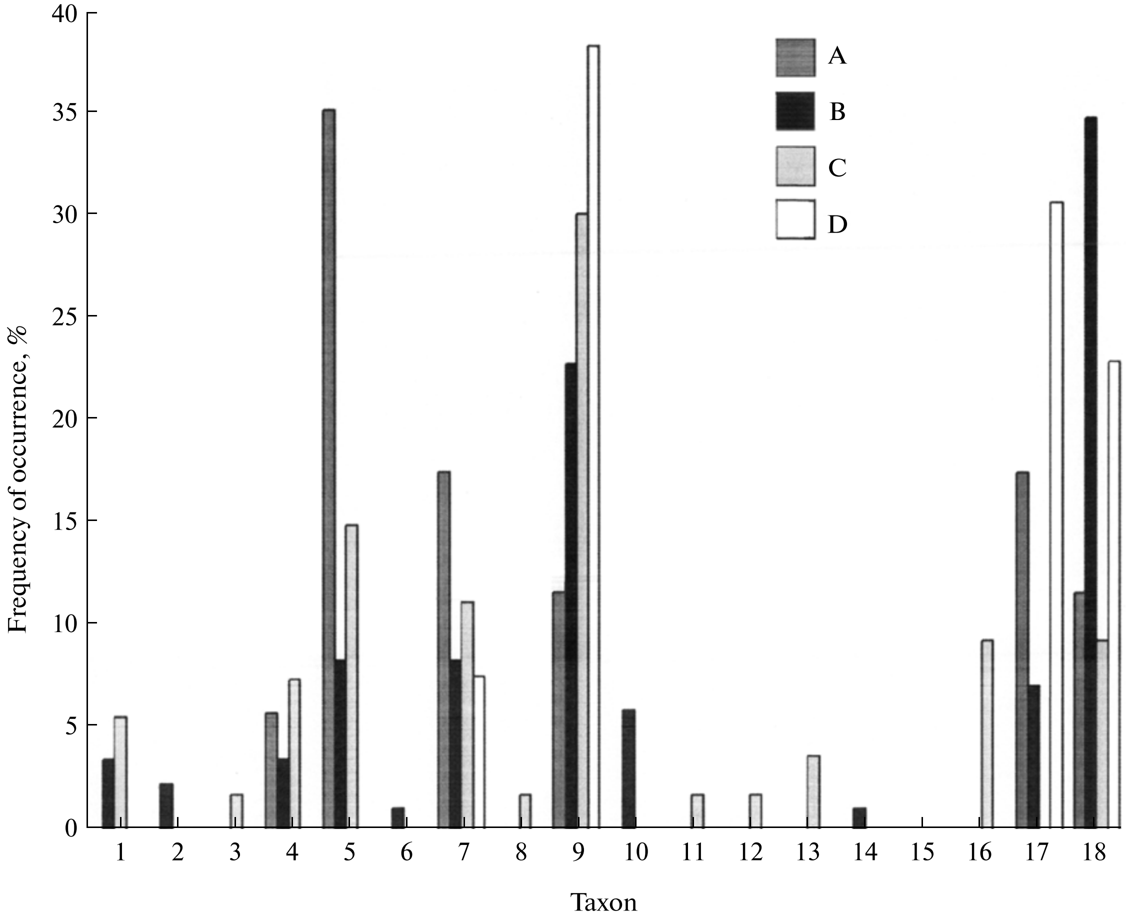

Fig. 1. Frequency of occurrence of bacteria of the following genera in microbial communities of sea algae *Saccharina japonica* (A), *Acrosiphonia sonderi* (B), *Ulva fenestrata* (C), and *Neosiphonia japonica* (D): *Algibacter* (1), *Algoriphagus* (2), *Aquimarina* (3), *Arenibacter* (4), *Cellulophaga* (5), *Gillisia* (6), *Formosa* (7), *Kriegella* (8), *Maribacter* (9), *Mesonia* (10), *Olleya* (11), *Pseudozobellia* (12), *Roseivirga* (13), *Salegentibacter* (14), *Tenacibaculum* (15), *Ulvibacter* (16), *Winogradskyella* (17), and *Zobellia* (18) (% of the total number of strains isolated from each alga).

The *Bacteroidetes* community of the green alga *Acrosiphonia sonderi* yielded the largest number of isolates (83). Bacterial genera *Zobellia* and *Maribacter* (Fig. 1 and Table 2) predominated in the community. The next largest community was the one of the green alga *Ulva fenestrata* (53 isolates), dominated by *Maribacter* and *Cellulophaga* (Fig. 1 and Table 2). Among the 17 isolates from the brown alga *Saccharina japonica* (Fig. 1 and Table 2), *Cellulophaga, Formosa*, and *Winogradskyella* prevailed considerably. Among the 13 isolates from the red alga *Neosiphonia japonica*, six *Maribacter* strains and four *Winogradskyella* strains were identified (Fig. 1 and Table 2). It should be noted that among the representatives of numerous microbial communities of the four above-mentioned algae of the Sea of Japan, no *Tenacibaculum* strains were found (Fig. 1), while this genus has been isolated from the algae of the Sea of Okhotsk.

From the microbial communities of *Chondrus* sp., *Polysiphonia* sp., *Chorda filum*, and *Saccharina crassfolia* bacteria of the phylum *Bacteroidetes* were isolated in minor amounts (Fig. 1).

Table 2 presents the results of the distribution of above-listed glycosidases among bacteria of the prevailing genera isolated from four algae, typical inhabitants of the littoral zones of the Cove of Trinity in the Posyet Bay of the Sea of Japan. *Zobellia* and *Maribacter*, isolated from *Acrosiphonia sonderi*, contained practically all of the tested glycosidases (Tables 1 and 2). Bacteria of the genus *Winogradskyella* isolated from the brown alga *Saccharina japonica* and the red alga *Neosiphonia japonica* contained the same, less representative set of glycosidases whose qualitative composition did not depend upon the strain isolation source. No α-galactosidases, β-xylosidases, α-*N*-acetylgalactosaminidases, or α-L-fucosidases were revealed in these strains (Table 2). The absence of α-L-fucosidase was noted in *Cellulophaga* isolates associated with the green alga *Ulva fenestrata*, while producers of this enzyme were revealed among associates of the brown alga *Saccharina japonica* (Table 2).

Table 2. Occurrence of glycosidases among the marine bacteria of the *Bacteroidetes* dominating genera of the associated with the marine algae

Taxon	Number of studied strains	Number of active strains							
		α-galactosidase	β-galactosidase	β-glucosidase	α-*N*-acetylglucosaminidase	β-*N*-acetylglucosaminidase	α-mannosidase	α-fucosidase	β-xylosidase
				Acrosiphonia sonderi					
Total number	83	58	68	64	32	59	57	28	64
Zobellia	29	24	29	22	15	17	28	10	26
Maribacter	19	19	19	18	10	14	15	7	18
				Ulva fenestrata					
Total number	53	34	39	38	17	42	29	16	36
Maribacter	17	15	17	15	14	17	16	7	15
Cellulophaga	7	6	6	5	0	6	2	0	5
				Saccharina japonica					
Total number	17	11	9	16	5	15	6	9	12
Cellulophaga	6	5	3	6	1	6	4	5	4
Formosa	3	3	1	2	1	1	0	2	3
Winogradskyella	3	0	0	3	0	3	1	3	0
				Neosiphonia japonica					
Total number	13	9	9	13	5	12	7	5	7
Maribacter	6	6	6	6	2	5	3	6	6
Winogradskyella	4	0	0	4	0	4	2	3	0

DISCUSSION

Application of traditional biochemical methods for screening of the metabolically active bacterial isolates under standard conditions using chromogenic glycosides made it possible to investigate the distribution of a number of glycosidases among the 177 strains of bacterial epiphytes of the algae of Far Eastern seas (Table 1) and indirectly evaluate the importance of bacterial epiphytes of the phylum *Bacteroidetes* in the degradation of alga.

Our results showed that communities of marine *Bacteroidetes* in each of the studied algae differed by species composition forming species-specific microbial associations (Fig. 1). High metabolic activity is apparently a prerequisite for the domination of certain bacterial genera over the others. The ability of bacteria of the genera *Zobellia* and *Maribacter* to produce the whole set of glycosidases tested indicates their physiological plasticity which determines their success in competition with other *Bacter\oidetes* (Table 2). At the same time, among the numerically important *Winogradskyella* isolates, independently of the host species, no producers of the important enzymes of such components of the lytic complex as α-galactosidases, β-glactosidases, or β-xylosidases were found. The difference in the metabolic profiles of the above-mentioned genera suggests that *Winogradskyella* occupies a specific ecological niche in the algal microbial communities unrelated to their involvement in hydrolysis of such polysaccharides as agar or carrageenan. The absence of producers of α-L-fucosidases among the *Cellulophaga* from the green alga *Ulva fenestrata*, in contrast to the representatives of this taxonomic group isolated from the brown alga *Saccharina japonica* (Table 2) may be explained by the different structures of the cell walls of these algae. For example, green algae have no fucose-containing oligo- or polysaccharides that could be degraded by this glycosidase.

Generally, the results presented in Table 1 and Figure 2 evidence that glycosidases catalyzing hydrolysis of the β-glycoside bond occurred in over 70% of the strains under study. α-Galactosidases and β-xylosidases were equally abundant. It should be noted that among the marine *Bacteroidetes*, epibionts of algae, α-galactosidases and α-*N*-acetylgalactosaminidases occurred more frequently than among the previously studied proteobacteria isolated form various sources [24–26].

β-Glucosidase (EC 3.2.1.21), which catalyzes hydrolysis of the β-glucoside bond in natural and synthetic β-glucosides and oligosaccharides, was the most abundant glycosidase among the studied epibionts (Table 1 and Fig. 2). Apparently, this is caused by its functions in the process of polysaccharide degradation by bacteria. Along with cellulase (endo-glucanase) and cellobiohydrolase, β-glucosidase is one of the three main types of enzymes involved in the degradation of cellulose [27], widely represented in marine algae. Moreover, together with endo-β-(1⟶3)-glucanase, β-glucosidase participates in utilization of other β-D-glucans widely occurring in marine environment, e.g. laminaran, by catalyzing hydrolysis of laminaribiose and gentiobiose to glucose [28].

The next abundant group included β-galactosidases (EC 3.2.1.23), β-xylosidases (EC 3.2.1.37), and α-galactosidases (EC 3.2.1.22), i.e., glycosidases cata-

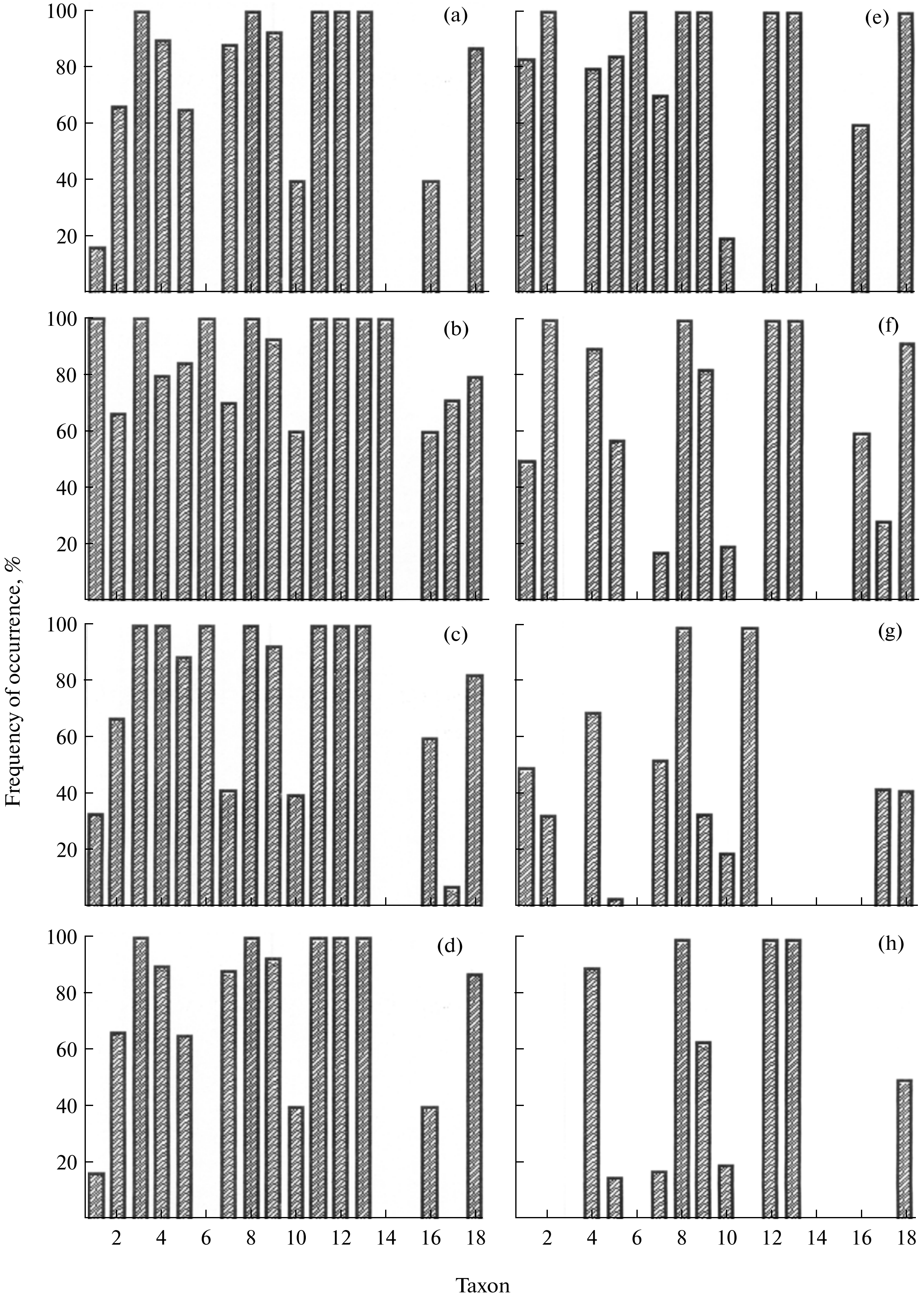

Fig. 2. Frequency of occurrence of producers of β-*N*-acetylglucosaminidases (a), β-glucosidases (b), α-galactosidases (c), β-xylosidases (d), β-galactosidases (e), α-mannosidases (f), α-fucosidases (g), and α-*N*-acetylgalactosaminidases (h) in bacteria of the following genera: *Algibacter* (1), *Algoriphagus* (2), *Aquimarina* (3), *Arenibacter* (4), *Cellulophaga* (5), *Gillisia* (6), *Formosa* (7), *Kriegella* (8), *Maribacter* (9), *Mesonia* (10), *Olleya* (11), *Pseudozobellia* (12), *Roseivirga* (13), *Salegentibacter* (14), *Tenacibaculum* (15), *Ulvibacter* (16), *Winogradskyella* (17), and *Zobellia* (18) (% to the total number of strains of the corresponding genus).

lyzing hydrolytic cleavage of the terminal residues from the non-reducing ends of various oligosaccharides, branched polysaccharides, as well as glycosides and carbohydrate components of various natural glycoconjugates (Table 1 and Fig. 2). These enzymes may act as components of microbial enzymatic systems responsible for biotransformation of hemicellulose (another widespread polysaccharide in marine algae), agar–agar and carrageenan. α-Galactosidases from the marine bacterium *Pseudoalteromonas* sp., isolated from the coelomic fluid of the shellfish *Crenomytilus grayanis* and the Sea of Okhotsk water, were reported [26, 29]. According to the CAZy structural classification, α-galactosidase of the strain *Pseudoalteromonas* sp. KMM 701, isolated from the water of the Sea of Okhotsk, was referred to family 36 of glycoside hydrolases [29]. A new 1,3-α-3,6-anhydro-l-galactosidase, performing the last step of agar degradation, was isolated from *Zobellia galactanivorans* [30]. No data was found in the literature on β-galactosidases and β-xylosidases of marine bacteria and their physiological function. However, β-xylosidases were used as marker enzymes in the studies of the structure and function of biofilm-forming river microbial communities [31].

There is no data on marine α-mannosidases (EC 3.2.1.24) or α-fucosidases (EC 3.2.1.51) of bacterial origin, the enzymes catalyzing the cleavage of mannose and fucose residues bound by α-glycoside bonds both at non-reducing ends of oligosaccharides and in branched polysaccharides. To summarize, all mentioned enzymes are components of the bacterial metabolic complex which carries out alga degradation and drives the Ocean carbon cycle in whole.

Among the studied epiphytes, a large number of producers of β-*N*-acetylglucosaminidases (EC 3.2.1.52) was noted. The glycosidase may possess both wide and narrow substrate specificities and catalyzes hydrolytic cleavage of either β-1,4-bound residues of either *N*-acetylglucosamine/*N*-acetylgalactosamine or *N*-acetylglucosamine alone from the non-reducing ends of glycoconjugates. Moreover, β-*N*-acetylglucosaminidase is identified as a chitobiase and is one of the components of the microbial chitinolytic complex, performing the degradation of the widely spread biopolymer, chitin, in marine environment. It may be stated that, along with chitinases, this enzyme is involved not only in carbon but also in nitrogen turnover in the environment surrounding the bacteria.

α-*N*-Acetylgalactosaminidase (EC 3.2.1.49) removes α-bound residues of *N*-acetamido-2-deoxy-α-D-galactopyranose from the non-reducing ends of glycosides and carbohydrate components of natural glycoconjugates. In our previous studies α-galactosidases, α-*N*-acetylgalactosaminidases, and β-*N*-acetylglucosaminidases were demonstrated to occur among the proteobacteria of the Sea of Okhotsk and Sea of Japan at the ratio of 28, 19, and 43%, respectively [25]. In the present study α-*N*-acetylgalactosaminidases were found to be rather rare enzymes among alga-associated marine *Bacteroidetes* as well, although they occurred more frequently in this group of bacteria, at the ratio of 38% (Table 1 and Fig. 2).

Currently, the only thoroughly studied α-*N*-acetylgalactosaminidase is that of the marine bacterium *Arenibacter latericius* of the family *Flavobacteriaceae* [21, 32].

Thus, the massive screening made it possible for the first time to characterize the profiles of distribution of glycosidases, the enzymes playing an important role in trophic links between the microorganisms and the host species, among the alga-associated marine *Bacteroidetes*.

The ability of marine *Bacteroidetes* associated with algae to produce various glycosidases evidences their high metabolic activity. The data obtained confirm our assumption that representatives of the natural microflora of algae are directly involved in the degradation of both matrix and structural polysaccharides of the macrophyte cell walls. Apparently, the presence of a wide spectrum of glycosidases in marine *Bacteroidetes* allows them to occupy various ecological niches and ensures there dominance in the competition with other microorganisms. The revealed significant differences in glycosidase production among the members of various taxonomic groups are important for directed search for the promising enzymes for biotechnology.

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REFERENCES

1. Kloareg, B. and Quatrano, R.S., Structure of Cell Walls of Marine Algae and Ecophysiological Functions of the Matrix Polysaccharides, *Oceanogr. Mar. Biol. Annu. Rev.*, 1988, vol. 26, pp. 259–315.
2. Kusaykin, M., Bakunina, I., Sova, V., Ermakova, S., Kuznetsova, T., Besednova, N., Zaporozhets, T., and Zvyagintseva, T., Structure, Biological Activity, and Enzymatic Transformation of Fucoidans from the Brown Seaweeds, *Biotechnol. J.*, 2008, vol. 3, no. 7, pp. 904–915.
3. Usov, A.I. and Bilan, M.I., Fucoidans, Sulfated Polysaccharides from Brown Algae, *Usp. Khim.*, 2009, vol. 78, no. 8, pp. 846–862.
4. Jiao, G., Yu, G., Zhang, J., and Ewart, H.S., Chemical Structures and Bioactivities of Sulfated Polysaccharides from Marine Algae, *Mar. Drugs*, 2011, vol. 9, no. 2, pp. 196–223.
5. Usov, A.I., Polysaccharides of the Red Algae, *Adv. Carbohydr. Chem. Biochem.*, 2011, vol. 65, pp. 115–217.
6. Zvyagintseva, T.N., Besednova, N.N., and Elyakova, L.A., *Struktura i immunotropnoe deistvie 1,3;1,6-β-D-glyukanov* (Structure and Immunotropic Activity of 1,3;1,6-β-D-Glucans), Vladivostok: Dal'nauka, 2002.

7. Yu, S., Blennow, A., Bojko, M., Madsen, F., Olsen, C.E., and Engelsen, S.B., Physico-Chemical Characterization of Floridean Starch of Red Algae, *Starch-Starke*, 2002, vol. 54, no. 2, pp. 66–74.
8. Shevchenko, N.M., Burtseva, Yu.V., Zvyagintseva, T.N., Makar'eva, T.N., Sergeeva, O.S., Zakharenko, A.M., Isakov, V.V., Nguyen Thi Linh, Nguen Xuan Hoa, Bui Minh Ly, and Pham Van Huyen, Polysaccharides and Sterols of Green Algae *Caulerpa lentillifera* and *C. sertularioides, Khimiya Prirod. Soed.*, 2009, no. 1, pp. 5–8.
9. Sawabe, T., Ohtsuka, M., and Ezura, Y., Novel Alginate Lyases from Marine Bacterium *Alteromonas* sp. Strain H-4, *Carbohydr. Res.*, 1997, vol. 304, no. 1, pp. 69–76.
10. Alekseeva, S.A., Bakunina, I.Yu., Nedashkovskaya, O.I., Isakov, V.V., Mikhailov, V.V., and Zvyagintseva, T.N., Intracellular Alginolytic Enzymes of the Marine Bacterium *Pseudoalteromonas citrea* KMM 3297, *Biochemistry (Moscow)*, 2004, vol. 69, no. 3, pp. 262–269.
11. Sova, V.V., Elyakova, L.A., Ivanova, E.P., Fedosov, Yu.V., and Mikhailov, V.V., Induced β-1,3-Glucanase from the Marine Bacterium *Alteromonas* sp., *Biokhimiya*, 1994, vol. 59, no. 9, pp. 1369–1377.
12. Giordano, A., Andreotti, G., Tramice, A., and Trincone, A., Marine Glycosyl Hydrolases in the Hydrolysis and Synthesis of Oligosaccharides, *Biotechnol. J.*, 2006, vol. 1, no. 5, pp. 511–530.
13. Bakunina, I.Yu., Nedashkovskaya, O.I., Alekseeva, S.A., Ivanova, E.P., Romanenko, L.A., Gorshkova, N.M., Isakov, V.V., Zvyagintseva, T.N., and Mikhailov, V.V., Degradation of Fucoidan by the Marine Proteobacterium *Pseudoalteromonas citrea, Microbiology*, 2002, vol. 71, no. 1, pp. 41–47.
14. Colin, S., Deniaud, E., Jam, M., Descamps, V., Chevolot, Y., Kervarec, N., Yvin, J.-C., Barbeyron, T., Michel, G., and Kloareg, B., Cloning and Biochemical Characterization of the Fucanase FcnA: Definition of a Novel Glycoside Hydrolase Family Specific for Sulfated Fucans, *Glycobiology*, 2006, vol. 16, no. 11, pp. 1021–1032.
15. Sakai, T., Kimura, H., and Kato, I., Purification of Sulfated Fucoglucuronomannan Lyase from Bacterial Strain of *Fucobacter marina* and Study of Appropriate Conditions for Its Enzyme Digestion, *Mar. Biotechnol.*, 2003, vol. 5, no. 4, pp. 380–387.
16. Sakai, T., Ishizuka, K., Shimanaka, K., Ikai, K., and Kato, I., Structures of Oligosaccharides Derived from *Cladosiphon okamuranus* Fucoidan by Digestion with Marine Bacterial Enzymes, *Mar. Biotechnol.*, 2003, vol. 5, no. 6, pp. 536–544.
17. Hehemann, J.-H., Correc, G., Barbeyron, T., Helbert, W., Czjzek, M., and Michel, G., Transfer of Carbohydrate-Active Enzymes from Marine Bacteria to Japanese Gut Microbiota, *Nature*, 2010, vol. 464, no. 7290, pp. 908–911.
18. Pohlon, E., Marxsen, J., and Küsel, K., Pioneering Bacterial and Algal Communities and Potential Extracellular Enzyme Activities of Stream Biofilms, *FEMS Microbiol. Ecol.*, 2010, vol. 71, no. 3, pp. 364–373.
19. Nedashkovskaya, O.I., Marine Aerobic Heterotrophic Bacteria of the Type *Bacteroidetes, Extended Abstract of Doctoral (Biol.) Dissertation*, Vladivostok, 2007.
20. Urvantseva, A.M., Bakunina, I.Yu., Nedashkovskaya, O.I., Kim, S.B., and Zvyagintseva, T.N., Distribution of Intracellular Fucoidan Hydrolases among Marine Bacteria of the Family Flavobacteriaceae, *Appl. Biochem. Microbiol.*, 2006, vol. 42, no. 5, pp. 484–491.
21. Bakunina, I.Yu., Kuhlmann, R.A., Likhosherstov, L.M., Martynova, M.D., Nedashkovskaya, O.I., Mikhailov, V.V., and Elyakova, L.A., α-*N*-Acetylgalactosaminidase from Marine Bacterium *Arenibacter latericius* KMM 426^T Removing Blood Type Specificity of A-Erythrocytes, *Biochemistry* (Moscow), 2002, vol. 67, no. 6, pp. 689–695.
22. Bakunina, I.Yu., Nedashkovskaya, O.I., Kim, S.B., Zvyagintseva, T.N., and Mikhailov, V.V., Distribution of α-*N*-Acetylgalactosaminidases among Marine Bacteria of the Phylum *Bacteroidetes*, Epiphytes of Marine Algae of the Seas of Okhotsk and Japan, *Microbiology*, 2012, vol. 81, no. 3, pp. 373–380.
23. Nedashkovskaya, O.I., Kim, S.B., Shin, D.S., Beleneva, I.A., Vancanneyt, M., and Mikhailov, V.V., *Echinicola vietnamensis* sp. nov., a New Bacterium of the Phylum *Bacteroidetes* Isolated from Sea Water, *Int. J. Syst. Evol. Microbiol.*, 2007, vol. 57, pp. 761–763.
24. Bakunina, I.Yu., Ivanova, E.P., Mikhailov, V.V., Nedashkovskaya, O.I., Gorshkova, N.M., and Parfenova, V.V., Occurrence of α-*N*-Acetylgalactosaminidases among Marine and Fershwater Microorganisms, *Mikrobiologiya*, 1994, vol. 63, no. 5, pp. 847–853.
25. Ivanova, E.P., Bakunina, I.Yu., Nedashkovskaya, O.I., Gorshkova, N.M., Mikhailov, V.V., and Elyakova, L.A., Incidence of Marine Microorganisms Producing β-*N*-Acetylglucosaminidases, α-Galactosidases, and α-*N*-Acetylgalactosaminidases, *Russ. J. Mar. Biol.*, 1998, vol. 24, no. 6, pp. 365–372.
26. Bakunina, I.Yu., Ivanova, E.P., Nedashkovskaya, O.I., Gorshkova, N.M., Elyakova, L.A., and Mikhailov, V.V., Screening for α-Galactosidase Producers among Marine Bacteria of the Genus *Alteromonas, Appl. Biochem. Microbiol.*, 1996, vol. 32, no. 6, pp. 624–628.
27. Lynd, L.R., Weimer, P.J., van Zyl, W.H., and Pretorius, I.S., Microbial Cellulose Utilization: Fundamentals and Biotechnology, *Microbiol. Mol. Biol. Rev.*, 2002, vol. 66, no. 3, pp. 506–577.
28. Pesentseva, M.S., Kusaykin, M.I., Anastyuk, S.D., Sova, V.V., and Zvyagintseva, T.N., Catalytic Properties and Mode of Action of Endo-(1 ⟶ 3)-Beta-D-Glucanase and Beta-D-Glucosidase from the Marine Mollusk *Littorina kurila, Carbohydr. Res.*, 2008, vol. 343, no. 14, pp. 2393–2400.
29. Balabanova, L.A., Bakunina, I.Y., Nedashkovskaya, O.I., Makarenkova, I.D., Zaporozhets, T.S., Besednova, N.N., Zvyagintseva, T.N., and Rasskazov, V.A., Molecular Characterization and Therapeutic Potential of a Marine Bacterium *Pseudoalteromonas* sp. KMM 701 α-Galactosidase, *Mar. Biotechnol.*, 2010, vol. 12, no. 1, pp. 111–120.
30. Rebuffet, E., Groisillier, A., Thompson, A., Jeudy, A., Barbeyron, T., Czjzek, M., and Michel, G., Discovery and Structural Characterization of a Novel Glycosidase Family of Marine Origin, *Environ. Microbiol.*, 2011, vol. 13, no. 5, pp. 1253–1270.
31. Romani, A.M. and Sabater, S., Influence of Algal Biomass on Extracellular Enzyme Activity in River Biofilms, *Microb. Ecol.*, 2000, vol. 41, no. 1, pp. 16–24.
32. Balabanova, L.A., Bakunina, I.Yu., Likhatskaya, G.N., Zvyagintseva, T.N., and Rasskazov, V.A., Glycoside Hydrolases of Marine Bacteria Are Promising Tools in Haemotherapy, *2nd Int. Conf. Bioenvironment, Biodiversity and Renewable Energies BIONATURE*, Venice, 2011, pp. 47–50.