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EXPERIMENTAL ARTICLES

Distribution of α-N-Acetylgalactosaminidases among Marine Bacteria of the Phylum *Bacteroidetes*, Epiphytes of Marine Algae of the Seas of Okhotsk and Japan

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Abstract—Occurrence of α-N-acetylgalactosaminidases among 177 strains of marine bacteria of the phylum *Bacteroidetes,* epiphytes of marine algae growing on the littoral of the Seas of Okhotsk and Japan, was studied. About 36% of the isolates studied contained α-N-acetylgalactosaminidase. All of the bacteria of the genus *Arenibacter* (species *A. latericius, A. certesii,* and *A. palladensis*), irrespective of the source of isolation, synthesized this enzyme. The greatest number of α-N-acetylgalactosaminidase producers was found among the isolates from the algae *Neosiphonia japonica, Acrosiphonia sonderi,* and *Ulva fenestrata* sampled in the Cove of Trinity, Posyet Bay, the Sea of Japan. These were mainly bacteria of the genera *Zobellia* (50%) and *Maribacter* (58%). Among the epibionts studied, the bacteria *Arenibacter latericius* KMM 3523, an epiphyte of the brown alga *Chorda filum* from the Sea of Okhotsk, and *Cellulophaga* sp. KMM 6488, an epiphyte of the green alga *Acrosiphonia sonderi* from the Sea of Japan, were marked as the most promising sources of the enzyme. The results of this study showed that aerobic nonpathogenic marine *Bacteroidetes*, algal associants not requiring special cultivation conditions, are the promising, economical, and ecologically pure sources of unique and biotechnologically significant α-N-acetylgalactosaminidases.

Keywords: α-N-acetylgalactosaminidase, marine bacteria of the phylum *Bacteroidetes: Arenibacter, Cellulophaga, Maribacter, Zobellia, Formosa*, marine algae.

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α-N-Acetylgalactosaminidase (EC 3.2.1.49) catalyzes the hydrolytic cleavage of the terminal α-O-glycoside-bonded residues of N-acetylgalactosamine from the nonreducing ends of various complex carbohydrates and glycoconjugates. Glycolipids, glycopeptides, and glycoproteins containing the structures with the O-glycoside core, oligosaccharides, and blood group A erythrocyte antigens are it's physiological substrates [1–3]. An increased interest in this enzyme was generated by the possibility of its use in biotechnology when the group 0 "universal blood" was obtained by enzymatic conversion of A and AB donor blood [4, 5]. The practical solution of the problem is hampered by the absence of economically advantageous sources of the enzymes, which act effectively under conditions where erythrocytes retain viability (neutral pH, temperature of 20–25°C, and low ionic strength). Therefore, the search for new sources of specific, stable, and efficient enzymes for the large-scale modification of A-erythrocytes is topical.

To date, α-N-acetylgalactosaminidases with the required properties were revealed in the anaerobic bacteria of terrestrial origin, *Clostridium perfringens* [6–9] and *Ruminococcus torques* [10, 11] of the phylum *Firmicutes* and *Elisabethkingia meningoseptica* of the phylum *Bacteroidetes* [12–14]. The evidence of α-N-acetylgalactosaminidases from marine bacteria is scarce.

Earlier, we investigated the distribution of these enzymes in the inhabitants of various ecotopes of the World Ocean and Lake Baikal [15, 16]. As result of thorough screening of over 800 strains, α-N-acetylgalactosaminidase, which inactivated the serological activity of A-erythrocytes at neutral pH, was isolated from the biomass of the obligate marine bacterium *Arenibacter latericius* KMM 426^T of the phylum *Bacteroidetes* and studied [17]. No *Bacteroidetes* epiphytic bacterial strains isolated from the algoplane of marine algae were among the isolates tested.

The aim of the present study was to investigate the distribution of α-N-acetylgalactosaminidases among the marine bacteria of the phylum *Bacteroidetes*, which are the epiphytes of the algae in the Seas of Okhotsk and Japan.

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Table 1. Occurrence of α-N-acetylgalactosaminidases among marine epiphyte bacteria isolated from different algae

Taxonomic position of the alga	Sampling area	Sampling date	Total number of strains	Number of active strains (% of those studied)
Brown algae (*Phaeophyta*)				
Chorda filum	Iturup Island, Sea of Okhotsk	October 1997	5	3 (60%)
Saccharina crassifolia	Iturup Island, Sea of Okhotsk	October 1997	3	0
Saccharina japonica	Cove of Trinity, Posyet Bay, Sea of Japan	June 2000	17	5 (29%)
Green algae (*Chlorophyta*)				
Acrosiphonia sonderi	Cove of Trinity, Posyet Bay, Sea of Japan	June 2000	83	30 (36%)
Ulva fenestrata	Cove of Trinity, Posyet Bay, Sea of Japan	August 1999	3	3 (100%)
Ulva fenestrata	Cove of Trinity, Posyet Bay, Sea of Japan	June 2000	50	18 (40%)
Red algae (*Rhodophyta*)				
Chondrus sp.	Paramushir Island, Sea of Okhotsk	August 1992	1	0
Neosiphonia japonica	Cove of Trinity, Posyet Bay, Sea of Japan	June 2000	13	5 (38%)
Polysiphonia sp.	Paramushir Island, Sea of Okhotsk	August 1992	2	0
Total:			**177**	**64 (36%)**

MATERIALS AND METHODS

The red algae *Chondrus* sp., *Polysiphonia* sp., and *Neosiphonia japonica* sp., the brown algae *Chorda filum, Saccharina crassifolia*, and *Saccharina japonica*, and the green algae *Acrosiphonia sonderi* and *Ulva fenestrata* were used in the present work. The habitat water area and the dates of algae harvesting are indicated in Table 1.

For primary screening of the strains, the brown, green, and red algae were sampled aseptically into sterile plastic bags treated with γ-rays. The algal tissue samples (1 g in 10 mL of sterile seawater) were decomposed in a glass homogenizer. The microorganisms were isolated by directly inoculating the petri dishes with marine agar (Difco, United States) with the algal tissue homogenate sample (0.1 mL per plate). The heterotrophic bacteria isolated from well-isolated colonies after incubation for seven days at 28°C were purified using sequential transfers. The pure bacterial cultures obtained were stored at –80°C in plastic test tubes containing artificial seawater and glycerol (30%) as a cryoprotector. In order to screen for glycosidases, the bacteria were cultivated on marine agar (Difco, United States) for 96 h at 28°C.

Identification of bacterial strains. The bacteria were identified based on the study of the standard phenotypic, chemotaxonomic, and genotypic characteristics as described earlier [18]. The genus and species identities were determined from the nucleotide sequences of the 16S rRNA gene using the GenBank BLAST databank (http://www.ncbi.nlm.nih.gov/BLAST) and by the set of the physiological and biochemical characteristics of the strains.

Preparation of the extracts from bacterial biomass. The bacterial cells were separated from the culture medium by centrifugation at 3000 *g*. The supernatant was discarded. The bacterial biomass was frozen at –20°C. The weighed portion of the frozen raw biom-

ass was resuspended in 0.01 M of Na^+ phosphate buffer solution, pH 7.3, on an ice bath. The biomass to buffer ratio did not exceed 0.2 g/mL. The cells were homogenized using an ultrasound sonicator at a frequency of 22 kHz and a current of 0.4 A by exposing them to ultrasound for 40 s four to five times at a 20-s intervals. The cell suspension was incubated at 4°C for 3 h; the homogenate was then centrifuged at 10000–15000*g* for 30 min. The pellet was discarded; the protein concentration and α-N-acetylgalactosaminidase activity were determined in the extract.

Determination of the α-N-acetylgalactosaminidase activity. In the first screening procedure, the α-N-acetylgalactosaminidase activity in bacteria was determined in plates for immunoenzyme assay. Each well contained 0.05 mL of the cell suspension containing 50 μg of the biomass of bacteria grown on solid medium and transferred into the wells with a platinum loop, as well as 0.05 mL of 0.025% substrate solution in 0.05 M Na^+ phosphate buffer, pH 7.3. The plates were incubated for 3 h at room temperature. *p*-Nitrophenyl-α-N-acetyl-D-galactosaminide (Sigma, United States) was used as the substrate for determining the α-N-acetylgalactosaminidase activity. The enzyme activity was assessed visually after 5, 10, 15, 30, 60, 120, and 180 min from the moment of the substrate addition by the appearance of the yellow color due to the *p*-nitrophenol releasing in the course of the enzymatic reaction. The glycoside solutions and the biomass suspensions were used as controls.

In order to measure the α-N-acetylgalactosaminidase activity in bacterial extracts, 0.05 mL of the extract solution and 0.35 mL of *p*-nitrophenyl-α-N-acetyl-D-galactosaminide solution (1 mg/mL) in 0.1 M Na^+ phosphate buffer, pH 7.3, was incubated for 15 to 180 min at 20°C. The reaction was stopped by addition of 0.6 mL of 1 M Na_2CO_3, and the optical density was measured at 400 nm. The initial reaction rate was determined from the linear segment of the time dependence of A_{400}. The substrate solution in the same buffer with 1 M of Na_2CO_3 served as the control.

The standard hydrolytic glycosidase activity was determined by the amount of *p*-nitrophenol formed during the enzymatic reaction. The amount of *p*-nitrophenol was determined spectrophotometrically at 400 nm ($\varepsilon_{400} = 18300\ mol^{-1}\ cm^{-1}$). One unit of enzyme activity was defined as the amount of the enzyme in 1 mL of the solution required to release 1 nmol of *p*-nitrophenol per minute. The specific enzyme activity in the extracts was calculated as the standard activity value to the protein concentration ratio (mg/mL).

Protein concentration in bacterial biomass extracts was determined according to the Lowry method [19] using bovine serum albumin (Sigma, United States) as the standard.

RESULTS AND DISCUSSION

The microbial communities colonizing the surfaces of marine macroalgae are primarily represented by the *Proteobacteria* and *Bacteroidetes* [20, 21]. According to one of the hypotheses, these bacterial phyla, especially the *Bacteroidetes*, play a key role in the biodegradation and mineralization of organic matter in the ocean [22, 23].

The polysaccharides of the marine environments are diverse in chemical composition and differ significantly in their composition and structure from compounds of this group occurring in terrestrial sources [24, 25]. Bacterial glycosidases, including α-N-acetylgalactosaminidases, are involved in the degradation of these biopolymers. However, the interactive CAZy (Carbohydrate-Active enZyme, http://www.cazy.org) database presently includes only several enzymes which exhibit specificity to the true marine polysaccharides, forming independent families of glycoside hydrolases [26].

In this work, we present the analysis of screening of the epiphytes of marine algae, which produce α-N-acetylgalactosaminidases, infrequently occurring glycosidases.

One hundred seventy-seven *Bacteroidetes* bacterial strains isolated from the red, green, and brown algae were analyzed (Table 1). An average of 36% of the isolates from marine algae contained α-N-acetylgalactosaminidase. Those were mainly the epiphytes of *Saccharina japonica* (29%), *Neosiphonia japonica* (38%), *Ulva fenestrata* (40%), and *Acrosiphonia sonderi* (36%). Sixty-four strains of epiphytic bacteria exhibited the α-N-acetylgalactosaminidase activity; of them, 16 strains had activity from 0.60 to 5.5 nmol/(min mg protein) (Table 2). It should be noted that our earlier large-scale screening revealed α-N-acetylgalactosaminidase to be a relatively rare enzyme, because only 24% of the bacterial strains isolated from different water areas of the World Ocean synthesized α-N-acetylgalactosaminidases [15]. Among animal associants, this enzyme had a higher frequency of occurrence than among free-living bacteria (12 and 6% of the total number of the strains studied, respectively) [16]. Among the Sea of Okhotsk red alga associants, 9.5% of active strains were discovered among the not numerous algal isolates.

Occurrence of α-N-acetylgalactosaminidases among the marine epiphytic bacteria is shown in Table 3. The bacteria of the genera *Arenibacter, Cellulophaga, Formosa, Maribacter, Zobellia*, and *Winogradskyella* were the prevailing taxa. Nine (90%) of the 10 *Arenibacter* isolates studied revealed the α-N-acetylgalactosaminidase activity. Fifty-eight percent of *Maribacter* isolates and 50% of *Zobellia* isolates contained α-N-acetylgalactosaminidase. In our previous study, we showed that the bacteria of the genera *Maribacter* and *Zobellia* were characterized by a broad range of glycoside hydrolases [27]. The bacteria synthesizing

Table 2. Strains of the marine bacteria synthesizing the most active α-N-acetylgalactosaminidases

Ordinal no.	Taxonomic position	Strain no.	Source of isolation	Activity, nmol/(min mg protein)
1	*Arenibacter latericius*	KMM 3523	*Chorda filum*	5.1
2	*A. latericius*	KMM 3528	*Chorda filum*	1.7
3	*A. palladensis*	KMM 3980	*Ulva fenestrata*	1.83
4	*A. palladensis*	KMM 3961^T	*Ulva fenestrata*	0.83
5	*Arenibacter* sp.	KMM 6041	*Acrosiphonia sonderi*	0.84
6	*Cellulophaga* sp.	KMM 6483	*Saccharina japonica*	1.80
7	*Cellulophaga* sp.	KMM 6488	*Acrosiphonia sonderi*	5.5
8	*Formosa* sp.	KMM 6408	*Saccharina japonica*	0.92
9	*Kriegella aquimaris*	KMM 3942	*Ulva fenestrata*	0.64
10	*Maribacter* sp.	KMM 6487	*Acrosiphonia sonderi*	0.68
11	*Maribacter* sp.	KMM 6485	*Acrosiphonia sonderi*	0.91
12	*Maribacter* sp.	KMM 6489	*Ulva fenestrata*	0.79
13	*Maribacter* sp.	KMM 6484	*Ulva fenestrata*	0.65
14	*Maribacter* sp.	KMM 6486	*Ulva fenestrata*	0.63
15	*Maribacter* sp.	KMM 3952	*Ulva fenestrata*	1.38
16	*Zobellia laminariae*	KMM 6205	*Saccharina japonica*	0.68

Table 3. Prevalence of α-N-acetylgalactosaminidases in marine epiphytic bacteria

Taxonomic position	Total number of strains	Number of active strains (% of those studied)
Algibacter	6	0
Algoriphagus	3	0
Aquimarina	1	0
Arenibacter	10	9 (90%)
Cellulophaga	26	4 (15%)
Gillisia	1	0
Formosa	17	3 (18%)
Kriegella aquimaris	1	1
Maribacter	41	24* (58%)
Mesonia	5	1
Olleya	1	0
Pseudozobellia	1	1
Roseivirga erhenbergii	2	2*
Salegentibacter	1	0
Tenacibaculum	2	0
Ulvibacter	5	0
Winogradskyella	14	0
Zobellia	40	20 (50%)
Total:	**177**	**64 (36%)**

Note: * The α-N-acetylgalactosaminidase activity in the extracts of these bacteria did not exceed 0.1 nmol/(min mg protein).

α-N-acetylgalactosaminidase were scarce among the representatives of the genera *Mesonia* and *Formosa*.

The epiphytic bacteria of the genera *Algibacter, Algoriphagus, Aquimarina, Gillisia, Olleya, Salegentibacter, Tenacibaculum, Ulvibacter*, and *Winogradskyella* revealed no α-N-acetylgalactosaminidase activity (Table 3).

Table 4 shows the occurrence of α-N-acetylgalactosaminidases among the numerically significant bacterial genera isolated from different algal species. Among the strains of the genus *Maribacter*, α-N-acetylgalactosaminidase was synthesized by 40% of *Neosiphonia japonica*, by 53% of *Acrosiphonia sonderi*, and 29% of *Ulva fenestrata* epiphytes. Acetylgalacosaminidase was found in 67% of *Zobellia* strains (epiphytes of *Neosiphonia japonica* and *Saccharina japonica*), as well as in 52 and 20% of the epiphytes of *Acrosiphonia sonderi* and *Ulva fenestrata*, respectively.

It was established that α-N-acetylgalactosaminidases occurred more often among the epibionts of green algae (80% of the number of active strains; Tables 1, 4). In this connection, it is interesting to note that the bacteria colonizing the surface of marine green algae play the main role in the development of normal morphogenesis and the attachment of the host zoospores [28, 29]. Moreover, it has been found recently that N-acetylgalactosamine enters into the composition of the plasma membranes of the zoospores of green algae *Enteromorpha flexuosa* and *Ulva fasciata* [29]. It is possible that the presence of bacterial α-N-acetylgalactosaminidases cleaving the terminal N-acetylgalactosamine in glycoconjugates

Table 4. Occurrence of α-N-acetylgalactosaminidases in the prevailing genera of bacteria isolated from different algae

Taxonomic position of bacterium	*Polysiphonia japonica*		*Acrosiphonia sonderi*		*Ulva fenestrata*		*Saccharina crassifolia*		*Saccharina japonica*		*Chorda filum*	
	all	active	all	active	all	active	all	active	all	active	all	active
Arenibacter	0	0	3	2	4	4	0	0	1	1	2	2
Cellulophaga	2*	0	7	2	8	0	2	0	6	1	1	1
Formosa	1	1	7	1	6	0	0	0	3	1	0	0
Maribacter	5	2 (40%)	19	10 (53%)	15	12 (29%)	0	0	1	0	0	0
Zobellia	3	2 (67%)	29	15 (52%)	5	1 (20%)	0	0	3	2 (67%)	0	0

Note: * The strains were isolated from *Polysiphonia* sp. (The Sea of Okhotsk).

exerts a direct influence on the morphological changes in the algae.

An information about the isolates containing a highly active α-N-acetylgalactosaminidase is summarized in Table 2. The highest specific activity of the enzyme was observed in *Cellulophaga* sp. KMM 6488 and *Arenibacter latericius* KMM 5523. This fact is of special interest because earlier we purified and characterized α-N-acetylgalactosaminidase capable to inactivate the serological activity of blood group A erythrocytes from the biomass of the *Arenibacter latericius* type strain KMM 426^T isolated from the bottom of the South China Sea [17]. Based on the homology of the amino acid sequence, this enzyme was attributed to the 109th family of glycoside hydrolases (GH) [30]. The closest related enzyme of this family is a single, comprehensively characterized α-N-acetylgalactosaminidase from the anaerobic pathogenic bacterium *Elisabethkingia meningoseptica* of the phylum *Bacteroidetes*, which has already found application in biotechnology [12]. The α-N-acetylgalactosaminidases of the family GH109 realize the mechanism of hydrolysis of the O-glycoside bond with the involvement of NAD^+ [12, 13] in contrast to the enzymes of the bacteria *Clostridium perfringens* [8] and *Ruminococcus torques* [10] of the phylum *Firmicutes*, which are included in the family GH36 and appear to be the classical glycoside hydrolases (http://www.cazy.org/GH36_bacteria.html).

Thus, the results of this investigation showed that aerobic nonpathogenic marine algal associants of the *Bacteroidetes* phylum, which do not require special conditions for cultivation, are the promising, economical, and ecologically pure sources of unique and biotechnologically significant α-N-acetylgalactosaminidases.

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REFERENCES

1. Clausen, H. and Hakomori, S.-i., ABH and Related Histo-Blood Group Antigens; Immunochemical Differences in Carrier Isotypes and Their Distribution, *Vox Sang*, 1989, vol. 56, no. 1, pp. 1–20.
2. Yamamoto, F., Clausen, H., White, T., Marken, J., and Hakomori, S., Molecular Genetic Basis of the Histo-Blood Group ABO System, *Nature*, 1990, vol. 345, no. 6272, pp. 229–233.
3. Wu, A.M., Wu, J.H., Chen, Y.Y., Tsai, M.S., and Herp, A., Forssman Pentasaccharide and Polyvalent Galβ1→4GlcNAc as Major Ligands with Affinity for *Caragana arborescens* Agglutinin, *FEBS Lett.*, 1999, vol. 463, no. 3, pp. 223–230.
4. Olsson, M.L., Hill, C.A., de la Vega, H., Liu, Q.P., Stroud, M.R., Valdinocci, J., Moon, S., Clausen, H., and Kruskall, M.S., Universal Red Blood Cells-Enzymatic Conversion of Blood Group A and B Antigens, *Transfus. Clin. Biol.*, 2004, vol. 11, no. 1, pp. 33–39.
5. Olsson, M.L. and Clausen, H., Modifying the Red Cell Surface: Towards an ABO-Universal Blood Supply, *Br. J. Haematol.*, 2007, vol. 140, no. 1, pp. 3–12.
6. Levy, G.N. and Aminoff, D., Purification and Properties of α-N-Acetylgalactosaminidase from *Clostridium perfringens, J. Biol. Chem.*, 1980, vol. 255, no. 24, pp. 11737–11742.
7. Hsieh, H.Y., Mitra, M., Wells, D.C., and Smith, D., Purification and Characterization of α-N-Acetylgalactosaminidase from *Clostridium perfringens, IUBMB Life*, 2000, vol. 50, no. 2, pp. 91–97.

8. Calcutt, M.J., Hsieh, H.Y., Chapman, L.F., and Smith, D.S., Identification, Molecular Cloning and Expression of an α-N-Acetylgalactosaminidase Gene from *Clostridium perfringens, FEMS Microbiol. Lett.*, 2002, vol. 214, no. 1, pp. 77–80.

9. Hsieh, H.Y. and Smith, D., *Clostridium perfringens* α-N-Acetylgalactosaminidase Blood Group A_2-Degrading Activity, *Biotechnol. Appl. Biochem.*, 2003, vol. 37, pp. 157–163.

10. Hoskins, L.C., Boulding, E.T., and Larson, G., Purification and Characterization of Blood Group A-Degrading Isoforms of α-N-Acetylgalactosaminidase from *Ruminococcus torques* Strain IX-70, *J. Biol. Chem.*, 1997, vol. 272, no. 12, pp. 7932–7939.

11. Hoskins, L.C. and Boulding, E.T., Changes in Immunologic Properties of Group A RBCs during Treatment with an A-Degrading Exo-α-N-Acetylgalactosaminidase, *Transfusion*, 2001, vol. 41, no. 7, pp. 908–916.

12. Liu, Q.P., Sulzenbacher, G., Yuan, H., Bennett, E.P., Pietz, G., Saunders, K., Spence, J., Nudelman, E., Levery, S.B., White, T., Neveu, J.M., Lane, W.S., Bourne, Y., Olsson, M.L., Henrissat, B., and Clausen, H., Bacterial Glycosidases for the Production of Universal Red Blood Cells, *Nat. Biotechnol.*, 2007, vol. 25, no. 4, pp. 454–464.

13. Sulzenbacher, G., Liu, Q.P., Bennett, E.P., Levery, S.B., Bourne, Y., Ponchel, G., Clausen, H., and Henrissat, B., A Novel α-N-Acetylgalactosaminidase Family with an NAD^+-Dependent Catalytic Mechanism Suitable for Enzymatic Removal of Blood Group A Antigens, *Biocatal. Biotrans.*, 2010, vol. 28, no. 1, pp. 22–32.

14. Yu, C.Y., Xu, H., Wang, L.S., Zhang, J.G., and Zhang, Y.P., Human RBCs Blood Group Conversion from A to O Using a Novel α-N-Acetylgalactosaminidase of High Specific Activity, *Chinese Sci. Bull.*, 2008, vol. 53, no. 13, pp. 2008–2016.

15. 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 Freshwater Microorganisms, *Mikrobiologiya*, 1994, vol. 63, no. 5, pp. 847–853.

16. Ivanova, E.P., Bakunina, I.Yu., Nedashkovskaya, O.I, Gorshkova, N.M., Mikhailov, V.V., and Elyakova, L.A., Occurrence of Marine Microorganisms Producing β-N-Acetylglucosaminidases, α-Galactosidases, and α-N-Acetylgalactosaminidases, *Biol. Morya*, 1998, vol. 24, no. 6, pp. 351–358.

17. Bakunina, I.Yu., Kul'man, 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 426T Removing Blood Type Specificity of A-Erythrocytes, *Biochemistry (Moscow)*, 2002, vol. 67, no. 6, pp. 689–695.

18. Nedashkovskaya, O.I., Kim, S.B., Shin, D.S., Beleneva, I.A., Vancanneyt, M., and Mikhailov, V.V., *Echinicola vietnamencis* sp. nov., a New Bacterium of the Phylum *Bacteroidetes* Isolated from Sea Water, *Int. J. Syst. Evol. Microbiol.*, 2007, vol. 57, pp. 761–763.

19. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Rahdall, R.J., Protein Measurement with the Folin Phenol Reagent, *J. Biol. Chem.*, 1951, vol. 193, no. 1, pp. 265–275.

20. Staufenberger, T., Thiel, V., Wiese, J., and Imhoff, J.F., Phylogenetic Analysis of Bacteria Associated with *Saccharina saccharina, FEMS Microbiol. Ecol.*, 2008, vol. 64, no. 1, pp. 65–77.

21. Crump, B.C. and Koch, E.W., Attached Bacterial Populations Shared by Four Species of Aquatic Angiosperms, *Appl. Environ. Microbiol.*, 2008, vol. 74, no. 19, pp. 5948–5957.

22. Kirchman, D.L., The Ecology of *Cytophaga–Flavobacteria* in Aquatic Environments, *FEMS Microbiol. Ecol.,* 2002, vol. 39, no. 2, pp. 91–100.

23. Azam, F. and Malfatti, F., Microbial Structuring of Marine Ecosystems, *Nat. Rev. Microbiol.*, 2007, vol. 5, no. 10, pp. 782–791.

24. 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.

25. Komandrova, N.A., Nazarenko, E.L., Tomshich, S.V., and Gorshkova, R.P., Structure of O-Antigenic Polysaccharides of Marine Proteobacteria *Pseudoalteromonas* and *Shewanella, Vestnik DVO RAN*, 2004, no. 3, pp. 54–65.

26. 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.

27. 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.

28. Marshall, K., Joint, I., Callow, M.E., and Callow, J.A., Effect of Marine Bacterial Isolates on the Growth and Morphology of Axenic Plantlets of the Green Alga *Ulva linza, Microb. Ecol.*, 2006, vol. 52, no. 2, pp. 302–310.

29. Michael, T.S., Glycoconjugate Organization of *Enteromorpha* (=*Ulva*) *flexuosaand* and *Ulva fasciata* (*Chlorophita*) Zoospores, *J. Phycol.*, 2009, vol. 45, no. 7, pp. 660–677.

30. 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*, 2011, pp. 47–50.