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EXPERIMENTAL ARTICLES

Distribution of the Enzymes of Carbohydrate Metabolism among Marine Microorganisms in the Sea of Japan and the South Chinese Sea

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Abstract—The ability to produce extracellular enzymes of carbon metabolism was studied in 55 strains related to 18 taxa and isolated from various habitats. Production of a number of enzymes was found to depend on the source from which the strains were isolated. Among the strains associated with algae and marine invertebrates, the highest number of producers were found in the seawater of Primorski krai. The strains isolated from this temperate climatic zone possessed higher enzymatic activity than those isolated from the tropical zone. The results obtained in the current research are useful for further directed searching for the producers of specific O-glycoside hydrolases.

Keywords: marine bacteria, producers, galactosidase, glucosidase, fucosidase, fucoidanase, mannosidase, laminarinase, pustulanase, pullulanase.

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Recent interest of researchers in marine microorganisms, especially bacteria, results from the peculiarities of their metabolism caused by environmental conditions. The metabolism of marine bacteria is affected significantly by such factors as the content of dissolved oxygen, temperature fluctuations in the ocean waters, and hydrostatic pressure. Marine microorganisms possess a wide range of enzymes of carbohydrate metabolism different from those of terrestrial microbes. These enzymes are involved in various biochemical reactions and in microbial degradation of protein molecules, nucleic acids, and the polysaccharides specific for marine environments, such as agar, chitin, fucoidan, laminaran, etc. [1]. Notably, by enzymatic transformation of polysaccharides, novel compounds can be obtained with higher biological activity than the natural compounds (RF patent no. 2095417). Enzymes with known specificity play an important role as tools in studies of structural peculiarities of polysaccharides and their biological role.

O-glycoside hydrolases (EC 3.2.1) catalyze hydrolysis of glycoside bonds in polysaccharides and carbohydrate-containing biopolymers and are the key enzymes of carbohydrate metabolism. These enzymes participate in various biochemical reactions in the cell and are found in the strains of marine bacteria isolated from diverse habitats (Sea of Okhotsk, Bering Sea, Sea of Japan) [2, 3]. Some hydrolases are detected more frequently in marine bacteria associated with multicellular organisms than in free-living bacteria [4]. To increase the potential of application of the enzymes in raw material processing, enzymes possessing new properties are required. Microorganisms are the cheapest source of any enzyme. Detection of the enzymes that hydrolyze natural compounds in the ocean and their further investigation is scientifically and practically significant, making it possible to elucidate the mechanisms of the transformation of organic substances in marine ecosystems and to fulfill the requirements of modern biotechnology.

In the Zhirmunsky Institute of Marine Biology (IMB), Far Eastern Division, Russian Academy of Sciences, a collection of marine heterotrophic bacteria was created and maintained, a small part of which served as a basis for this study. In the Pacific Institute of Bioorganic Chemistry (PIBOC), Far Eastern Division, Russian Academy of Sciences, a scientific program was developed devoted to detection of marine microorganisms producing unique enzymes of various specificity and to the study of enzyme biosynthesis in microorganisms isolated from different areas of the World Ocean.

The objective of this work was to analyze the characteristics of distribution of marine bacteria producing glycoside hydrolases isolated from different sources in the Sea of Japan and South Chinese Sea and to select promising strains.

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MATERIALS AND METHODS

Strains of marine bacteria isolated from Nha Trang Bay (Vietnam) at a depth of 1–10 m in June to August and December 2008 and in January 2009, as well as strains isolated from Peter the Great Bay (Sea of Japan, Russia) at a depth of 5–10 m in August 2008, were used in the study. In Nha Trang Bay, strains were isolated from the microflora of periphyton from metal plates (stainless steel, aluminum, aluminum alloy, brass, zinc, and copper); from the thallomes of brown algae *Padina* spp., *Turbullaria* spp., *Sargassum* spp.; from the sea squirt *Didemnum molle*; from undefined species of sponges (three samples); and from seawater (SW). In Peter the Great Bay, the strains were obtained from the algae *Laminaria japonica* and *Sargassum pallidum*, sea grass *Zostera marina*, sea urchins *Strongylocentrotus nudus*, trepangs *Apostichopus japonicus*, oysters *Crassostrea gigas*, sipunculoids *Sipuncula* spp., and soil samples.

All samples were delivered to the laboratory in separate sterile plastic packs and glass vials not later than 2 h after recovery, washed three times with sterile seawater, and dissected; the averaged samples were collected under aseptic conditions. Bacteria were isolated from the inner tissues of sea squirts and sponges; from digestive tracts of sea urchins, sipunculoids, and holothurians; and from shell surface periphyton of oysters. The tissues of animals and algae were then homogenized. Homogenates of the tissues of invertebrates and algae, suspensions of "microperiphyton" after serial dilutions, and seawater (0.1 ml) were plated on solid Youschimizu-Kimura medium [5] and incubated at room temperature for up to 5 days. The grown colonies were transferred to new plates of the same medium to obtain pure bacterial cultures. Identification of bacterial strains was performed using their morphological, cultural, and biochemical characteristics as described earlier [6]. To confirm the determined taxonomical status, some randomly selected strains were identified by 16S rRNA gene sequence analysis (tables, marked with *). The total DNA was extracted from 1 ml of a bacterial culture, lyzed in a solution containing SDS and proteinase K, and deproteinized with phenol and chloroform [7]. For amplification of the fragments of 16S rRNA gene, primers 5'-GTTTGATCMTGGCTCAG-3' and 5'-TACGGYTACCTTGTTACGACTT-3' were used [8]. The PCR reaction mixture (25 μl) consisted of 2.5 μl 10 × PCR buffer, 2 μl of 10 mM mixture of dNTPs (2.5 mM each), 2.5 μl of each 2.5 μM primer, 10 ng of DNA, and 1 U of *Taq* DNA polymerase (Fermentas, Lithuania). Amplification conditions were as follows: initial denaturing at 95°C for 3 min, 30 cycles at 94°C for 1 min, 56°C for 30 sec, and 72°C for 1 min 45 sec, and the final cycle at 72°C for 5 min (PerkinElmer GeneAmp PCR System 9600). The PCR products were separated on a 1% agarose gel, and resultant amplification products served as templates for DNA sequencing. For this purpose, the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) was used. The purified sequence products were analyzed by electrophoresis in an ABI Prism 3130 genetic analyzer with 50-cm capillaries. The sequences were determined on both strands. The obtained sequences for each species were aligned using the SeqScape v2.5 software package (Applied Biosystems). The consensus sequences were compared with microbial 16S rRNA gene sequences from the GenBank database.

To detect the enzymes, bacteria were grown in Youschimizu-Kimura medium on a rotary shaker at 24–26°C for 24 h. The culture liquid was concentrated by ultrafiltration through a PM-30 membrane (Amicon, United Kingdom). The filtrate was dialyzed at 4°C for 24 h with phosphate buffer (pH 7.2), fractionated on a G-25 Sephadex column (25 ± 1.5 cm), and eluted with 0.01 M phosphate buffer (pH 7.2). The volume of a fraction was 0.5 ml. Bacterial pellet (0.5 g) was suspended in 1 ml of 0.05 M phosphate buffer (pH 7.2) and sonicated in an UZDN-2 sonicator (22 kHz, 0.4 A). The obtained suspension was centrifuged at 10000 *g* for 30 min in a K-24 centrifuge. The extracts were fractionated on a G-25 Sephadex column (25 ± 1.5 cm), eluted with 0.01 M phosphate buffer (pH 7.2), and collected (0.5 ml). The obtained fractions were studied for containing of glycanase and glycosidase activities as described below.

To determine β-1,3-glucanase activity, laminaran (1,3;1,6-α-D-glucan) from the brown algae *L. cichorioides* [9] was used; to determine fucoidan-hydrolase activity, fucoidan (sulfated 1,3-α-L-fucan) from the brown algae *L. cichorioides* [10] was used; for β-1,6-glucanase activity, pustulan (1,6-β-D-glucan) from lichens [11]; and for pullulanase activity, pullulan (1,4;1,6-α-D-glucan) from the collection of polysaccharides of PIBOC FEB RAS Laboratory of Enzyme Chemistry.

Glycosidase activities were tested using *p*-nitrophenyl (Np-) derivatives of the relevant monosaccharides (Sigma): Np-β-D-glucopyranoside, Np-β-D-galactopyranoside, Np-α-D-mannopyranoside, and Np-α-D-fucopyranoside. Formation of *p*-nitrophenol was registered by absorption at 410 nm.

Glycanase activities were evaluated according to the increase in reducing capacity of the mixture: 400 μl of substrate solution (1 mg/ml in 0.05 M of phosphate buffer, pH 7.2) and 100 μl of the analyzed extract were incubated at 37°C for 1–24 h. For each enzyme, a control incubation mixture was developed containing 100 μl of the buffer and 400 μl of the polyisaccharide or 400 μl of the buffer and 100 μl of the extract. The reducing capacity was measured as described by Nelson [12].

The glycosidase activity unit was defined as the amount of the enzyme capable of producing 1 nmol of *p*-nitrophenol per 1 h under experimental conditions. The glycanase activity unit was defined as the amount of the enzyme capable of producing 1 nmol of reducing sugars per 1 h. Specific activity was expressed in

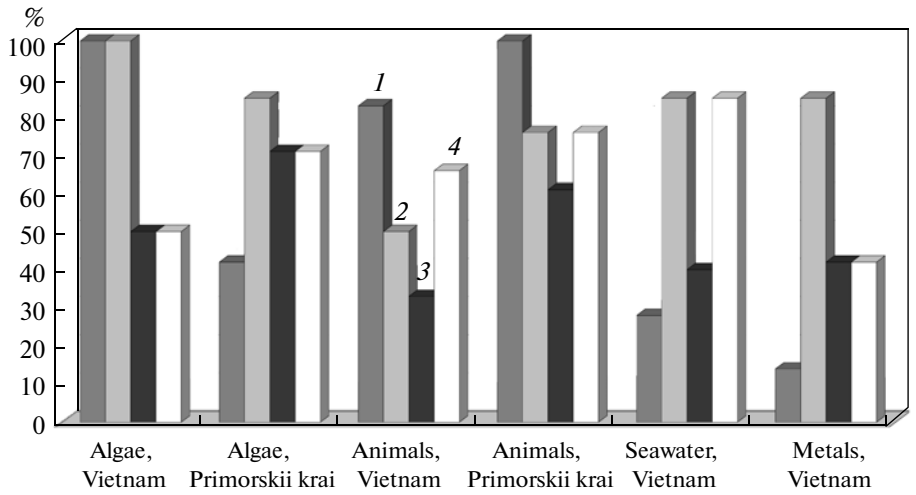

Fig. 1. Distribution of the galactosidase-producing microorganisms associated with algae, invertebrates, metals, and seawater isolated from Nha Trang Bay (Vietnam) and Peter the Great Bay (Primorski krai, Russia). β-D-galactosidase (*1*), β-D-glucosidase (*2*), α-L-fucosidase (*3*), and α-D-mannosidase (*4*). In Figs. 1–3, the *Y* axis indicates the percent of the active strains of the total amount of strains isolated from a specific object.

units per 1 mg of protein. The concentration of proteins was determined according to Lowry et al. [13].

The data shown in the tables were obtained in three repetitions. Statistical analysis was carried out using the Student criteria. The values of $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Distribution of glycanases and glycosidases was studied in 55 marine bacterial strains isolated from Nha Trang Bay (Vietnam) and Peter the Great Bay (Sea of Japan, Russia). The culture liquid of the analyzed strains contained almost no O-glycoside hydrolases. The study of the enzyme activity of the biomass of marine bacteria indicated that 63% of the strains produced β-D-galactosidase (52% of Vietnamese strains and 75% of strains isolated in Primorski krai). In 75% of the strains, β-D-glucosidase was found (75% of strains from Vietnam and 75% of strains from Primorski krai), while α-L-fucosidase was detected in 51% of isolates (42% from Vietnam amd 60% from Primorski krai) and α-D-mannosidase in 70% of strains (66% from Vietnam and 75% from Primorski krai). As shown in Fig. 1, most producers among the strains isolated from algae and marine invertebrates were found in Peter the Great Bay. These strains also demonstrated the highest activities of the investigated enzymes (Table 1). The highest activities of β-D-galactosidase, β-D-glucosidase, α-L-fucosidase and α-D-mannosidase were registered in strains associated with oyster shells, sargassum, and black sea urchin (Peter the Great Bay). Of the isolates from Nha Trang Bay, the highest activity was demonstrated by the strains from the microflora of a brass plate periphyton, *Padina* spp. algae, sponges, and free-living strains.

All the strains, except one Vietnamese isolate, possessed the ability to synthesize laminarinase (Tables 2, 3). Fucoidanases hydrolyzing fucoidan isolated from the brown seaweed *L. cichorioides* were found in 28% of the Vietnamese strains and in 44% of the strains from Peter the Great Bay. However, most of the strains possessed low fucoidanase activity. Pustulanase and pullulanase activities were detected in 78 and 59% of the Vietnamese and 64 and 56% of the Primorski krai

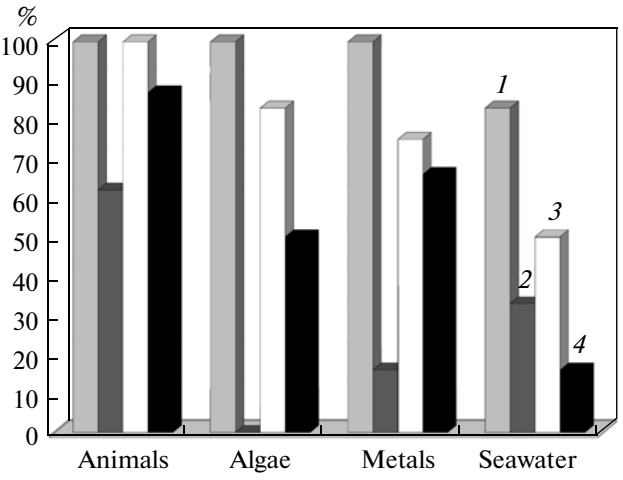

Fig. 2. Distribution of the glycanase-producing microorganisms associated with algae, invertebrates, metals, and seawater isolated from Nha Trang Bay (Vietnam). Laminarinase (*1*), fucoidanase (*2*), pustulanase (*3*), and pullulanase (*4*).

Table 1. Specific activity of glycosidases from marine microorganisms isolated from Nha Trang Bay and Peter the Great Bay (nmole/mg/h($\times 10^{-3}$))

Strain	Taxon	Source of isolation	Substrate			
			Np-β-D-galactopyranoside	Np-β-D-glucopyranoside	Np-α-L-fucopyranoside	Np-α-D-fucopyranoside
1380	*Acinetobacter* sp.	SW	339	1269	135	0
1399	*Arthrobacter* sp.	Sea squirt	0	1290	172	139
1403	*Arthrobacter* sp.	Sponge	740	0	387	398
1237	*Bacillus* sp.	Turbullaria	1321	0	0	231
1238	*Bacillus* sp.	*Padina* sp.	276	1502	0	321
1331	*Bacillus* sp.	Trepang, CF	6	0	1021	81
1402	*Bacillus* sp.	Sponge	519	1621	0	0
1404	*Bacillus* sp.	Sponge	1121	0	0	224
1405	*Bacillus megaterium**	Sponge	956	1143	0	243
1364	*Brevibacillus* sp.*	Cu	0	811	162	140
1326	@ CFB	Sea urchin	214	6091	0	743
1377	@ CFB	SW	0	121	173	154
1376	*Enterobacter* sp.	SW	0	1841	0	471
1226	*Micrococcus* sp.	*Padina* sp.	451	2581	112	140
1333	*Planococcus maritimus**	Shell	551	939	0	0
1314	*Pseudoalteromonas piscicida**	Soil	237	0	0	0
1242	*Pseudomonas aeruginosa**	B63	195	3276	1408	1923
1318	*Pseudomonas* sp.	Sipunculoid	27	1536	1374	43
1323	*Pseudomonas* sp.	Trepang, w/c	282	0	0	973
1307	*Pseudomonas* sp.	Sargassum	0	3236	2312	237
1303	*Pseudomonas* sp.	Laminaria	0	1385	1734	733
1322	*Pseudomonas* sp.	Sargassum	976	2252	528	0
1319	*Pseudomonas* sp.	Sipunculoid	164	1753	0	1159
1321	*Pseudomonas* sp.	Sargassum	0	0	7633	1276
1338	*Pseudomonas* sp.	Shell	0	1976	6732	1393
1386	*Pseudomonas* sp.	Al	0	362	0	109
1387	*Serratia marcescens*	Al	0	226	0	182
1359	*Serratia marcescens*	12H18N10T	0	128	0	0
1410	*Serratia marcescens*	AlMg3	0	0	251	0
1335	*Shewanella oneidensis**	Shell	1175	1796	5241	105
1336	*Shewanella oneidensis**	Shell	931	1941	2392	0
1392	*Sphingobacterium* sp.*	B63	0	1156	0	0
1382	*Stenotrophomonas* sp.*	Al	481	571	141	0
1298	*Vibrio parahaemolyticus**	Sea urchin, CF	374	0	0	603
1309	*Vibrio* sp.	Zostera	0	283	1944	257
1311	*Vibrio* sp.	Zostera	316	1825	0	175
1312	*Vibrio* sp.	Zostera	605	1674	0	0
1328	*Vibrio* sp.	Trepang, int.	161	1725	344	703
1371	*Vibrio* sp.	SW	182	293	0	173
1374	*Vibrio alginolyticus**	SW	0	0	0	0
1317	*Xanthomonas* sp.	Sipunculoid	244	1876	3284	197

Note: Numbers of strains isolated from Nha Trang Bay are shown in bold. In Tables 1–3, the following designations are used: *, Taxonomic position is confirmed by the analysis of 16S rRNA gene sequences; CF, coelomic fluid; shell, oyster shells; int., intestines; w/c, intestines without contents; Cu, copper; B63, brass; Al, aluminum; AlMg3, aluminum alloy; 12X18H10T, stainless steel; Zn, zinc.

Table 2. Specific activity of glycanases from microorganisms isolated from Nha Trang Bay, South China Sea (nmole/mg · h($\times 10^{-3}$))

Strain	Taxon	Source of isolation	Substrate			
			laminaran (1,3; 1,6-β-D-glucan)	fucoidan (1,3-α-L-fucan)	pustulan (1,6-β-D-glucan)	pullulan (1,4; 1,6-α-D-glucan)
1380	*Acinetobacter* sp.	SW	650	0	0	119
1399	*Arthrobacter* sp.	Sea squirt	11269	501	135	0
1403	*Arthrobacter* sp.	Sponge	21490	976	424	253
1402	*Bacillus* sp.	Sponge	21456	39	1341	120
1404	*Bacillus* sp.	Sponge	1236	84	218	165
1405	*Bacillus megaterium**	Sponge	16572	0	113	327
1396	*Bacillus* sp.	Sea squirt	11920	0	1347	106
1397	*Bacillus* sp.	Sea squirt	5939	81	658	284
1237	*Bacillus* sp.	Turbullaria	1423	0	233	249
1238	*Bacillus* sp.	*Padina* sp.	2830	0	2314	0
1250	*Bacillus* sp.	Zn	2321	0	223	700
1255	*Bacillus* sp.	Cu	3571	76	110	218
1364	*Brevibacterium* sp.*	Cu	3276	0	187	399
1377	@ CFB	SW	0	0	141	0
1376	*Enterobacter* sp.	SW	859	179	0	0
1226	*Micrococcus* sp.	*Padina* sp.	13161	0	446	822
1239	*Photobacterium* sp.	*Padina* sp.	2602	0	1241	254
1242	*Pseudomonas aeruginosa**	B63	3031	297	0	0
1386	*Pseudomonas* sp.	Al	5674	0	162	205
1389	*Pseudomonas fluorescens**	Al	4370	0	125	142
1372	*Pseudomonas* sp.	SW	2190	0	376	0
1387	*Serratia marcescens*	Al	2197	0	263	298
1410	*Serratia marcescens*	AlMg3	13161	0	446	822
1359	*Serratia marcescens*	12H18N10T	1976	0	517	0
1392	*Sphingobacterium* sp.	B63	11306	0	247	263
1248	*Staphylococcus aureus*	B63	1673	0	0	0
1257	*Staphylococcus* sp.	B63	11302	0	140	0
1382	*Stenotrophomonas* sp.	Al	6349	0	0	173
1374	*Vibrio alginolyticus**	SW	753	189	0	0
1371	*Vibrio* sp.	SW	1376	0	387	0
1240	*Vibrio* sp.	*Padina* sp.	1029	0	936	0
1241	*Vibrio* sp.	Sargassum	902	0	0	0

strains, respectively. Most producers of pustulanase from both regions represented the strains associated with animals (Figs. 2, 3). The highest activities of pustulanase and pullulanase were demonstrated by the strains isolated from algae *Padina* sp., sea squirt (Vietnam), soil, and oyster shells (Peter the Great Bay) (Tables 2, 3).

A search for bacteria producing fucoidan hydrolases has been undertaken previously in the Sea of Japan and Sea of Okhotsk. The sources of the enzymes were shown to be in equal parts bacteria associated with the brown algae and the holothurian *A. japonicus* [3]. In this study, the producers of fucoidanase isolated from Peter the Great Bay were shown to be the strains associated both with invertebrates and algae. Among the strains isolated from Nha Trang Bay, no producers of fucoidanase were found (Fig. 2).

In Vietnam, laminarinase producers were detected mainly among the microflora of algae, animals, and seawater; in Primorski krai, in the microflora of algae and animals (Figs. 2, 3). The most active producers in both regions were found in associations with animals.

Table 3. Specific activity of glycanases from microorganisms isolated from Peter the Great Bay, Sea of Japan (nmole/mg/h($\times 10^{-3}$))

Strain	Taxon	Source of isolation	Substrate			
			laminaran (1,3; 1,6-β-D-glucan)	fucoidan (1,3; 1,4-α-L-fucan)	pustulan (1,6-β-D-glucan)	pullulan (1,4; 1,6-α-D-glucan)
1331	*Bacillus* sp.	Trepang, CF	14331	363	504	1295
1326	@ CFB	Sea urchin	21092	0	0	184
1333	*Planococcus maritimus**	Oyster, shell	8181	0	923	1853
1314	*Pseudoalteromonas piscicida**	Soil	12706	0	4951	584
1310	*Pseudomonas* sp.	Zostera	2490	0	0	0
1307	*Pseudomonas* sp.	Sargassum	1836	0	0	0
1303	*Pseudomonas* sp.	Laminaria	2315	132	0	0
1321	*Pseudomonas* sp.	Sargassum	3116	0	3761	0
1322	*Pseudomonas* sp.	Sargassum	5767	665	2487	499
1318	*Pseudomonas* sp.	Sipunculoid	1219	0	0	0
1319	*Pseudomonas* sp.	Sipunculoid	5971	243	171	105
1323	*Pseudomonas* sp.	Trepang, w/c	110	0	0	0
1337	*Pseudomonas* sp.	Oyster, shell	5124	0	583	0
1338	*Pseudomonas* sp.	Oyster, shell	11620	55	101	0
1335	*Shewanella oneidensis**	Oyster, shell	5138	89	267	0
1336	*Shewanella oneidensis**	Oyster, shell	1869	0	88	0
1298	*Vibrio parahaemolyticus**	Sea urchin, CF	21247	551	100	215
1306	*Vibrio parahaemolyticus**	Laminaria	3232	0	372	316
1328	*Vibrio* sp.	Trepang, int.	26688	650	363	582
1311	*Vibrio* sp.	Zostera	1596	0	745	1355
1308	*Vibrio* sp.	Sargassum	1745	164	0	537
1309	*Vibrio* sp.	Zostera	1287	235	0	295
1313	*Vibrio* sp.	Zostera	5488	0	0	0
1312	*Vibrio* sp.	Zostera	2178	0	658	518
1317	*Xanthomonas* sp.	Sipunculoid	5437	78	441	768

Collectively, the ability to synthesize the enzymes of carbohydrate metabolism was studied in 55 strains belonging to 18 taxa and isolated from different habitats. The strains isolated from Peter the Great Bay showed a higher activity of all the enzymes compared to those isolated from Nha Trang Bay. The strains possessing high activity of several enzymes were also recovered from the temperate zone waters as well. These active strains were isolated from soil, coelomic fluid of trepang and periphyton of oyster shells. Among the Vietnamese strains, the most active ones were isolated from seawater and the seaweed *Padina* spp. (Tables 2, 3).

Among the isolates from Vietnam, gram-positive bacteria of the genera *Bacillus* were predominating; among the isolates from Primorski krai, gram-negative bacteria of the genera *Pseudomonas* and *Vibrio* did. Halotolerant strains of bacilli are known to be frequently isolated from seawater [14], as well as the cultured representatives of *Pseudomonas* and *Vibrio* [15]. In the tropical zone, gram-positive bacteria were found mainly in the periphyton of copper-containing plates and in sea squirt and sponges, which were not analyzed in the temperate zone. We reported recently the predominance of gram-positive microflora in copper-containing materials in seawater (in press), while the wide dissemination of this group of microorganisms in these animals has been previously described [16].

Almost all of the isolated strains of bacilli actively produced the wide range of investigated enzymes, except for α-L-fucosidase (33% of strains) and fucoidanase (60%), irrespective of the isolation site (Tables 1–3). Among vibrios, β-D-galactosidase, β-D-glucosidase, and α-D-mannosidase were produced by 71% of the strains; pustulonase, by 72%, pullulanase, by 63%; fucoidanase, by 54%; and α-L-fucosidase, by 57%. Among pseudomonads, 53% synthesized fucoidanase and pustulanase and 61% pullulanase. Most pseudomonad strains produced β-D-glucosidase, α-L-fucosidase, and α-D-mannosidase,

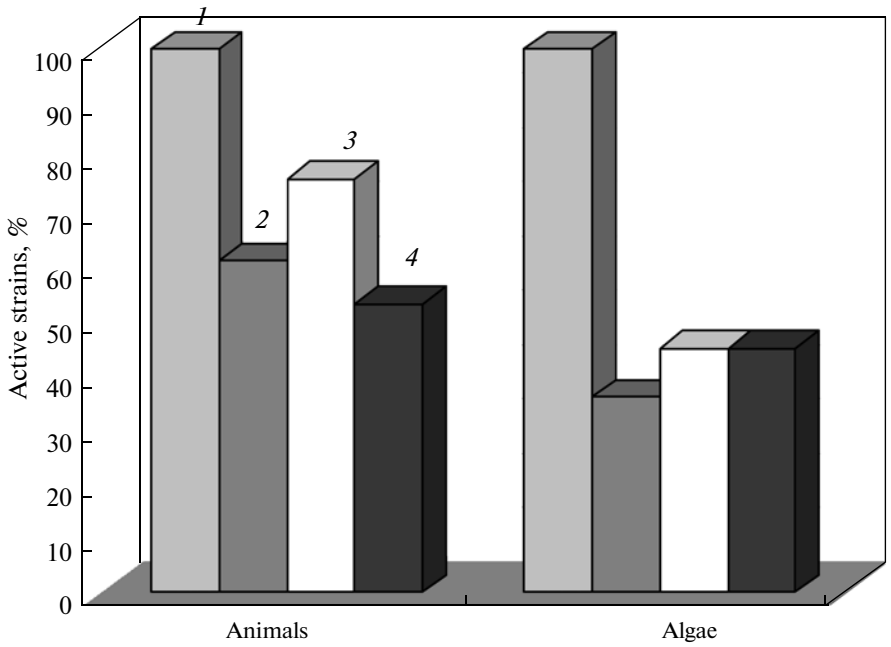

Fig. 3. Distribution of the glycanase-producing microorganisms associated with algae and invertebrates isolated from Peter the Great Bay (Primorskii krai). Laminarinase (*1*), fucoidanase (*2*), pustulanase (*3*), and pullulanase (*4*).

whereas β-D-galactosidase was synthesized by 50% of them. Enzyme activity of *Pseudomonas* and *Vibrio* isolates from temperate waters was higher than that of the strains from the tropical zone.

The *Vibrio* strains isolated from trepang intestines and black sea urchin coelomic fluid in Peter the Great Bay were shown to be perspective producers of laminarinase (Table 3). Among the Vietnamese isolates, gram-positive bacteria *Bacillus* sp. and *Arthrobacter* sp. were most active in laminarinase synthesis (Table 2). The strain 1326 attributing to the CFB branch of the *Bacteria* domain produced β-D-glucosidase effectively (Table 1). In accordance with the literature data, *Pseudomonas* strains were shown to be active producers of α-L-fucosidase, and the levels of enzyme activities were comparable to those of the strains used as the producers of these enzymes [2]. The possible sources of overproducers of β-D-galactosidase, α-D-mannosidase, pustulanase, and pullulanase are not found yet. As for the strains possessing high activity of several enzymes simultaneously, they belonged to diverse taxa. Among gram-positive bacteria, *Planococcus maritimus* (strain 1333), *Bacillus* spp. (1331, 1238), and *Micrococcus* sp. (1226) could be noted, while among gram-negative bacteria, *Pseudoalteromonas piscicida* (1314), *Shewanella oneidensis* (1335, 1336), and *Vibrio* spp. could be indicated(1371, 1240). Fifty percent of the strains of gram-positive bacteria possessing activity of several enzymes were isolated from the temperate zone (1333, 1331), while the vibrios were isolated from the water in the tropics (Tables 1–3).

Thus, this study revealed the production of a set of enzymes to depend on the source from which a bacterial strain was isolated. This will allow organization of the directed search for the producers of specific glycoside hydrolases. The highest enzymatic activity was observed in the strains isolated from the temperate zone. Interestingly, all the detected O-glycoside hydrolase activities were found to be cell-associated. Producers of extracellular hydrolases of polysaccharides probably also exist in the studied natural habitats. Since such organisms are more promising for biotechnological application, the study will be continued in this direction.

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