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Diversity and Antimicrobial Activity of Hydrobionts Associated Microorganisms from the Sea of Japan with the Occurrence of Tropodithietic Acid Producing Bacteria

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Authors' contributions

This work was carried out in collaboration between all authors. Author LAR designed the study, isolated and tested bacteria, analyzed the results, and wrote the first draft of the manuscript. Authors VVK and NYC performed the molecular studies and literature searches. Authors NIK, PSD and RSP performed extraction and spectral analyses of antimicrobial compounds. Author VVM managed the analyses of the study and analyzed the results. All authors read and approved the final manuscript.

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ABSTRACT

The study was undertaken to survey microorganisms associated with colonial fouling hydrozoans and red alga *Polysiphonia* sp. collected from the Sea of Japan seashore and to screen them for antimicrobial effects. On the basis of 16S rRNA gene sequences the isolates were assigned to 21 genera of the *Alphaproteobacteria*, *Gammaproteobacteria*, *Actinobacteria* as the dominant followed by *Firmicutes*, *Bacteroidetes* and *Betaproteobacteria*; the most shared 98-99% sequence similarity to recognized species recovered from marine sources. Hydrozoan's and red alga microbial associations were different in their taxonomic compositions at the generic level. Members of the genera *Shewanella, Labrenzia* and *Streptomyces* occured in both specimens. Antimicrobial

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screening revealed 46 strains capable to inhibit growth of two to seven indicatory cultures. Active hydrozoan's strains were phylogenetically close to *Phaeobacter inhibens.* Active isolates from red alga were similar to *Pseudovibrio ascidiaceicola*, and in addition to *Paenibacillus xylanexedens* and *Bacillus murimartini*. *Streptomyces* strains with antimicrobial activity were found in both specimens. Strains *Phaeobacter* sp. H8 and *Pseudovibrio* sp. rh17 were selected to be examined for the production of tropodithietic acid, TDA, which is the known antimicrobial metabolite produced by *Phaeobacter* and *Pseudovibrio* bacteria. On the basis of spectral analyses both strains were found to produce TDA, which could be responsible for their antimicrobial activity. Our findings demonstrated that hydrozoans and red alga inhabitating the Peter the Great Bay of the Sea of Japan harbor diverse microbial communities with a high proportion of bacteria capable of antimicrobials production, including TDA-producing strains which are conceivable to be of importance for chemical protection of their host-hydrobionts and bacterial relationships in marine shallow environments.

Keywords: Bacterial diversity; hydrobionts; antimicrobial activity; Phaeobacter; Pseudovibrio; tropodithietic acid.

1. INTRODUCTION

There has been increasing interest in the study of microbial communities that are associated with invertebrates and plants dwelling in marine shallow habitats. Coastal ecosystems are known to be of biological significance, harboring a lot of hydrobionts species and providing favourable conditions for marine biota nursery and bioremediation activities. Marine invertebrates and plants serve as unique natural accumulators of specific microbial communities. External envelops and internal tissues of hydrobionts enriched by nutrient compounds and adhesive substances, polysaccharides, proteins, glycolipids can be considered to provide auspicious conditions for colonization, attachment and activity of associated microorganisms. Microbial associants of marine biota have proven to be a rich source of biologically active substances with antimicrobial, cytotoxic, or antineoplastic activities which have biotechnological and pharmaceutical application [1,2,3,4]. At the same time, our knowledge of the diversity of marine hydrobionts associated bacteria and their biological activity, interrelations between bacteria and host organism is still limited. It is becoming important to study microbial diversity from unexplored or known marine resources, and to search potential microorganisms producing bioactive metabolites. It should be noted that some bacteria play an increasingly significant role as applied to the biological control of aquacultures. This study is focused on the cultivable heterotrophic bacteria isolated from colonial hydrozoan's and red alga *Polysiphonia* sp. specimens collected from the Sea of Japan seashore where being common and abundant they form the fouling on the rocks, mussels, clams and other underwater substrates. The study was aimed to evaluate potential of associated bacteria for the production of biologically active metabolites.

2. MATERIALS AND METHODS

2.1 Isolation and Cultivation of Microorganisms

Fifty-five strains were isolated from colonial unidentified hydrozoan's (class *Hydrozoa*) and eighty strains were isolated from red alga *Polysiphonia* sp. (*Polysiphonia* Greville, 1823, family *Rhodomelaceae*) specimens, which were collected from two different shallow locations of the Peter the Great Bay, the Sea of Japan, Russia, in October 2011 and September 2015, respectively. Isolation and phenotypic characterization of microorganisms were carried out as described previously [5]. The strains were stored at –80°C in Marine Broth 2216 supplemented with 30% glycerol and deposited to the Collection of Marine Microorganisms, KMM, G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Vladivostok, Russia.

2.2 PCR Amplification of 16S rRNA Genes and Sequencing

Single colonies of isolates were added to 50 µl of autoclaved ultra purified water, and incubated for 15 min at 98ºC. The mixture was centrifuged at 13000 g for 3 min, and 3 µl of the crude extracts was used in the PCR. Partial 16S rRNA gene sequences were amplified using the universal primers 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 15r (5'-AAGGAGGTGATCCARCCGCA-

3'), generating a PCR product of c. 1300-1400 bp. The following PCR conditions were applied: initial denaturation at 95°C for 15 min, followed by 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 2 min, and a final elongation step of 72°C for 10 min. The reaction mixture (50 µl) contained DreamTaq™ Green PCR Master Mix (2X) 25 µl (Fermentas), 27f forward primer 5 µl (5 µmol l^{-1}) (Evrogen, Russia), 15 r reverse primer 5 µl (5 µmol l^{-1}) (Evrogen, Russia), 12 µl molecular biology grade water and 3 µl DNA. The PCR products were analysed by agarose gel electrophoresis. The purified PCR fragments were sequenced using the ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems) and by the Big Dye v. 3.1 sequencing kit (Applied Biosystems).

2.3 Phylogenetic Analysis

Phylogenetic and molecular evolutionary analyses were conducted using Molecular Evolutionary Genetics Analysis (MEGA, version 6.0.) [6]. Twenty five nucleotide sequences generated in this study have been deposited in the DDBJ/EMBL/GenBank database under the accession numbers from LC230094 to LC230118 as indicated in the phylogenetic trees (Figs. 1,2).

2.4 Extraction and Isolation of Antimicrobial Compounds

Two strains H8 and rh17 were grown in Marine Broth 2216 with shaking for 30 h. Bacterial cells were removed by centrifugation and the pH of the supernatants was adjusted to 3.5. Equal volume ethyl acetate with 1% formic acid were added twice to the cell-free supernatant (CFS) and incubated shaking at 200 rpm for 30 min followed by centrifugation. Subsequently, the upper layer was removed, washed with H_2O, dried over Na_2SO_4 and vacuum concentrated at 40°C until dry. The extracts obtained were examined for the presence of antimicrobial activities at a concentration of 1 mg/ml by an agar diffusion method as described previously [7].

2.5 Instrumental Analysis

UV spectra were recorded on a UV spectrophotometer CECIL, CE 7250, 7000 series (England) in methanol. Liquid chromatography-mass spectrometry, including high resolution, (LC-HR-MS) were recorded on an HPLC Agilent 1200 Series coupled to a maXis impact II (Bruker) spectrometer equipped with electrospray source. HPLC analysis was conducted using a Zorbax Eclipse XDB C18 column (1.0 mm ID, 150 mm, 3.5 µm). The system was operated at a flow rate of 0.1 mL/min with solvents A/B. The gradient was started at 10% B and increased to 95% B within 25.0 min, then to 100% in 1.0 min, keeping this for 10.0 min, returning to 10% B in 2.0 min, and equilibrating for the next sample in 3.0 min (total runtime 41.0 min).

2.6 Antimicrobial Assay

The inhibitory activity against indicatory bacteria, *Escherichia coli* K-12 CL588, *Enterococcus faecium* CIP 104105, *Staphylococcus aureus* CIP 65.8^T, *Staphylococcus epidermidis* CIP 81.55^T, *Bacillus subtilis* CIP 52.65^T, *Xanthomonas* sp. pv. *badrii* LMG 546, *Candida albicans* KMM 455 obtained from the respective Culture Collections was tested by an agar diffusion method as described previously [5]. All tests were carried out in three independent experiments.

3. RESULTS AND DISCUSSION

3.1 Diversity of *Hydrozoa* and Red Alga *Polysiphonia* sp. Associated Bacterial Isolates

Fifty-five strains were isolated from colonial hydrozoan's (class *Hydrozoa*) specimen and investigated phenotypically, and selected strains were studied phylogenetically. On the basis of 16S rRNA gene sequence analysis the hydrozoan's isolates were affiliated to twelve genera *Pseudoalteromonas*, *Shewanella,* (class *Gammaproteobacteria*); *Phaeobacter, Sphingorhabdus, Aurantimonas, Labrenzia* (class *Alphaproteobacteria*); *Hydrogenophaga* (class *Betaproteobacteria*); *Streptomyces, Demequina, Cellulomonas* (phylum *Actinobacteria*); *Kriegella*, *Maribacter* (phylum *Bacteroidetes*) (Fig. 1).

Eighty strains were isolated from red alga *Polysiphonia* sp. specimens. All isolates were investigated phenotypically, and selected strains were studied phylogenetically. Bacteria associated with red alga *Polysiphonia* sp. were assigned to twelve genera *Pseudovibrio, Labrenzia*, *Sphingomonas* (class *Alphaproteobacteria*); *Cobetia, Shewanella, Vibrio* (class *Gammaproteobacteria*); *Microbacterium, Dietzia, Agrococcus, Streptomyces* (phylum *Actinobacteria*); *Bacillus, Paenibacillus* (phylum *Firmicutes*) (Fig. 2).

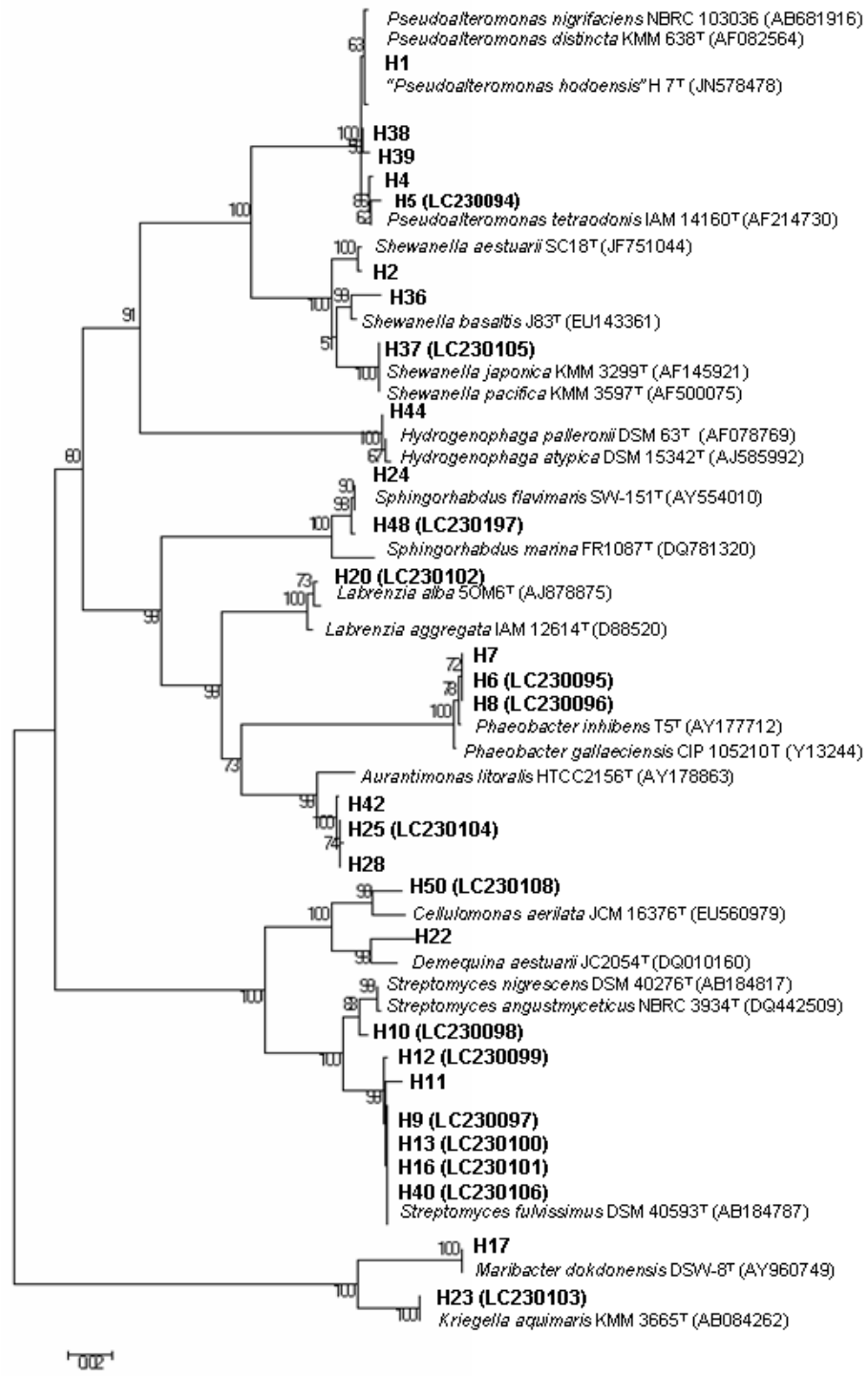


Fig. 1. A neighbor-joining phylogenetic tree based on the 16S rRNA gene sequences available from the GenBank/EMBL/DDBJ databases (accession numbers are given in parentheses) showing relationship of *Hydrozoa* associated isolates and related bacteria. Bootstrap values based on 1000 replications are given as percentages at the branching points. Numbers indicate percentages greater than 50%. Bar, 0.02 substitutions per nucleotide position

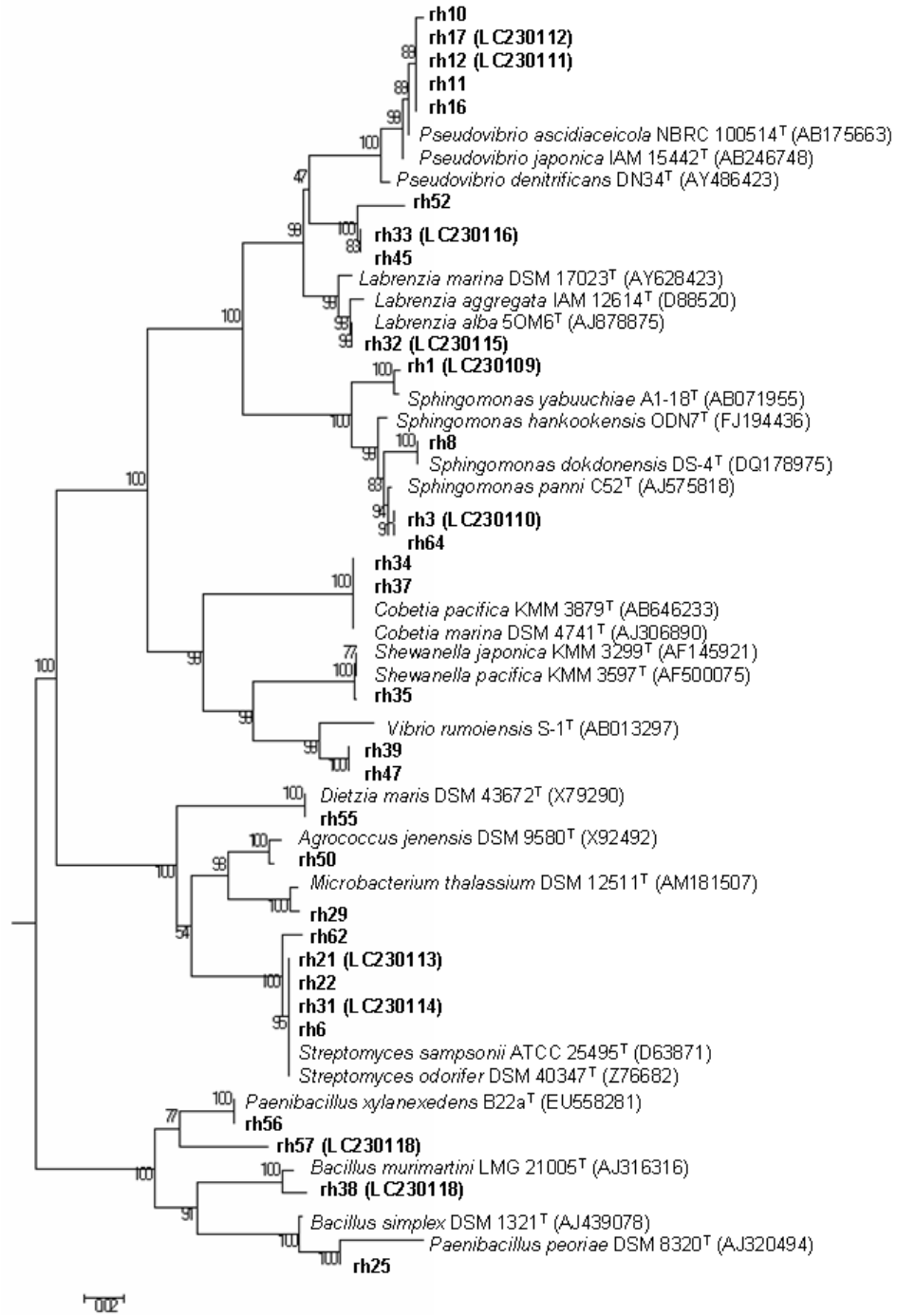


Fig. 2. A neighbor-joining phylogenetic tree based on the 16S rRNA gene sequences available from the GenBank/EMBL/DDBJ databases (accession numbers are given in parentheses) showing relationship of isolates associated with red alga *Polysiphonia* sp. and related bacteria. Bootstrap values based on 1000 replications are given as percentages at the branching points. Numbers indicate percentages greater than 50%. Bar, 0.02 substitutions per nucleotide position

Phylogenetic analysis revealed most isolates from both specimens shared 98-99% sequence similarity to recognized species, including those recovered previously from marine sources.

Bacterial compositions associated with hydrozoans and red algae were diverse and different to each other at the generic level. Strains of three genera *Shewanella, Labrenzia* and *Streptomyces* occurred in both specimens (Figs. 1,2), and some of *Shewanella* and *Labrenzia* strains belonged to the same phyletic groups whereas *Streptomyces* strains clustered with different phylogenetic lineages of the genus *Streptomyces* separately for each of hydrobionts. Hydrozoan associated bacterial community comprised *Pseudoalteromonas* and *Shewanella* members as abundant and phylogenetically diverse at the species level whereas the isolates within each of the genera *Phaeobacter, Sphingorhabdus, Aurantimonas, Labrenzia* were represented by the homogeneous 16S rRNA gene sequence types. *Hydrogenophaga* (class *Betaproteobacteria*) and *Bacteroidetes* members were found among bacteria isolated from the hydrozoan's specimen.

Red alga associated bacteria belonging to the genera *Sphingomonas, Bacillus, Paenibacillus* and *Labrenzia* were characterized by the phylogenetic diversity. A group of strains rh45, rh52, rh44, and strain rh62 showed 16S rRNA gene sequence identities less than 97-98% to the members of the genera *Labrenzia* and *Streptomyces*, respectively, and therefore, they may represent novel species according to the criteria stated by Stackebrandt and Ebers [8].

The results obtained for the taxonomic compositions are mainly corroborated with the occurrence of many genera found in microbial communities associated with marine algae [9,10,11], hydrozoans [12,13], bryozoans [14], sponges [15,16,17], corals [18] collected from various sea habitats. It was summarized in the review of Goecke et al. [10] that bacteria isolated from *Polysiphonia* spp. and unidentified red algae belonged to the genera *Shewanella* (*Proteobacteria*), *Zobellia*, *Lacinutrix*, *Maribacter* (phylum *Bacteroidetes*), *Luteolibacter* (class *Verrucomicrobiae*); and *Alphaproteobacteria* and *Bacteroidetes* members in microbial associations with macroalgae have being observed as constant components. Interrelations between macroalgae and some species of associated bacteria were noted to be highly specific [10]. At the same time our knowledge regarding microbial communities of hydrozoans is very restricted. Stabili et al. [12] have reported luminous bacteria of the genus *Vibrio* in associations with hydrozoan and *bryozoan* species. Di Camillo et al. [13] found that epibiontic bacteria of *Ectopleura crocea* (*Cnidaria, Hydrozoa*) were dominated by members of *Comamonadaceae* and *Flavobacteriaceae*.

3.2 Antimicrobial Activity of *Hydrozoa* and Red Alga *Polysiphonia* sp. Bacterial Isolates

All isolates were tested by the agar diffusion method for their antimicrobial activity. Screening has resulted in 22 hydrozoan's strains and 24 those from red alga (40% and 30% of total, respectively) with inhibitory activity against from two to seven indicatory strains, mainly, Gram-positive *Staphylococcus aureus*, *Enterococcus faecium*, *Bacillus subtilis*, *Candida albicans* and Gram-negative phytopathogenic *Xanthomonas* sp. pv. *badrii*, and less against *Staphylococcus epidermidis* and *Escherichia coli* (Tables. 1,2). Most active hydrozoan's strains were phylogenetically close to *Phaeobacter* and *Streptomyces* members. Streptomycetes constituting about half of active strains contributed significantly to the overall antimicrobial activity. They grouped with recognized species of the genus *Streptomyces*, *S. fulvissimus* and *S. angustmyceticus* which have been reported to be producers of antibiotics, valinomycin and angustmycin, respectively. Strains having moderate or weak activity were found among representatives of the genera *Pseudoalteromonas*, *Shewanella*, *Sphingorhabdus*, *Cellulomonas*. Strains belonging to *Phaeobacter, Pseudoalteromonas*, *Shewanella* and *Streptomyces* are well-known antagonists, whereas *Sphingorhabdus* or *Cellulomonas* are rarely mentioned bacteria in this context. Hydrozoan's active strains belonging to the genus *Phaeobacter* clustered all together with *Phaeobacter inhibens* sharing gene sequence similarity of 99%. The genus *Phaeobacter* belongs to the *Roseobacter* lineage (class *Alphaproteobacteria*) which has been reported to be the essential component of microbial communities colonizing marine eukaryotes [19,20]. The genus *Phaeobacter* was created by Martens et al. [21] as a result of reclassification of the *Roseobacter gallaeciensis* isolated from a rearing of the scallop *Pecten maximus* [22], and description of a new species P. inhibens from surface water of a tidal mud flat in the German Wadden Sea. During recent years Phaeobacter strains have attracted attention due to their ability to produce biologically active compounds [23,24,25], being of importance as probiotic agents in marine aquaculture [26] and as causative bleaching diseases in marine red algae [27].

Table 1. Antimicrobial activity of *Hydrozoa* associated strains

Strain	Nearest phylogenetic relative	Similarity (%)	Inhibitory zone (mm)						
			B. subtilis	***S. aureus***	***S. epidermidis***	***E. faecium***	***E. coli***	***Xanthomonas* sp. pt. *badrii***	***C. albicans***
H1	*Pseudoalteromonas hodoensis*	99	W*	-	-	W	-	-	12
H2	*Shewanella aestuarii*	98	-	-	W	-	-	-	12
H4	*Pseudoalteromonas tetraodonis*	99	W	-	-	W	-	-	12
H5	*Pseudoalteromonas tetraodonis*	99	-	-	-	W	-	-	12
H6	*Phaeobacter inhibens*	99	20	13	15	16	-	20	15
H7	*Phaeobacter inhibens*	99	17	15	17	16	-	21	15
H8	*Phaeobacter inhibens*	99	14	12	15	12	12	15	w
H9	*Streptomyces fulvissimus*	99	20	20	-	25	-	14	20
H10	*Streptomyces angustmyceticus*	98	15	22	-	12	-	15	20
H11	*Streptomyces fulvissimus*	98	18	22	-	18	-	18	21
H12	*Streptomyces fulvissimus*	98	25	16	21	15	-	15	17
H13	*Streptomyces fulvissimus*	100	20	22	-	-	-	15	20
H14	*Streptomyces fulvissimus*		15	16	12	W	15	12	21
H15	*Streptomyces fulvissimus*		14	15	20	15	12	12	20
H16	*Streptomyces fulvissimus*	100	15	16	15	W	12	20	21
H33	*Streptomyces fulvissimus*		W	12	13	25	12	12	20
H36	*Shewanella basaltis*	98	-	-	-	-	-	W	20
H38	*Pseudoalteromonas hodoensis*	99	-	-	W	-	-	12	18
H40	*Streptomyces fulvissimus*	99	W	-	12	-	12	12	22
H41	*Streptomyces fulvissimus*		W	-	12	-	12	12	20
H48	*Sphingorhabdus flavimaris*	99	W	W	12	-	-	-	W
H50	*Cellulomonas aerilata*	98	-	W	W	-	-	-	-

**W, weak activity, inhibition zones were observed as zones with reduced growth*

Table 2. Antimicrobial activity of strains associated with red alga *Polysiphonia* sp.

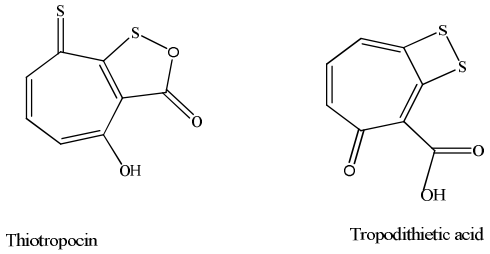
Strain	Nearest phylogenetic relative	Similarity (%)	Inhibitory zone (mm)						
			B. subtilis	***S. aureus***	***S. epidermidis***	***E. faecium***	***E. coli***	***Xanthomonas* sp. pt. *badrii***	***C. albicans***
rh6	*Streptomyces albidoflavus*	99	18	15	-	15	13	14	12
rh10	*Pseudovibrio ascidiaceicola*	99	-	-	-	W*	12	-	-
rh11	*Pseudovibrio ascidiaceicola*	99	W	W	-	-	12	15	-
rh12	*Pseudovibrio ascidiaceicola*	99	15	16	-	15	W	28	14
rh16	*Pseudovibrio ascidiaceicola*	99	15	W	-	w	W	15	12
rh17	*Pseudovibrio ascidiaceicola*	99	15	W	-	15	W	15	12
rh20	*Streptomyces albidoflavus*	99	15	15	W	w	W	12	17
rh21	*Streptomyces albidoflavus*	99	16	15	12	15	12	15	18
rh22	*Streptomyces albidoflavus*	99	18	15	W	w	12	12	18
rh23	*Streptomyces albidoflavus*	99	-	-	W	15	-	-	12
rh30	*Bacillus murimartini*		-	12	12	14	-	-	-
rh31	*Streptomyces albidoflavus*	99	14	16	12	18	12	13	12
rh62	*Streptomyces intermedius*	93	14	15	W	18	12	14	15
rh63	*Streptomyces intermedius*		18	15	-	20	-	14	12
rh44	*Labrenzia marina*		-	12	-	15	-	15	-
rh45	*Labrenzia marina*	97	-	12	W	-	W	15	-
rh50	*Agrococcus jenensis*	99	-	14	-	15	11	12	12
rh56	*Paenibacillus xylanexedens*	99	15	20	-	20	-	12	-
rh57	*Paenibacillus terrae*	99	-	15	-	20	-	12	12
rh74	*Paenibacillus xylanexedens*		18	20	-	22	-	18	W
rh77	*Paenibacillus xylanexedens*		W	14	-	20	-	15	18
rh35	*Shewanella pacifica*	99	-	-	-	w	W	-	12
rh38	*Bacillus murimartini*	98	-	15	-	-	-	15	-
rh58	*Bacillus murimartini*		-	15	-	-	-	15	-

**W, weak activity, inhibition zones were observed as zones with reduced growth*

Most active strains-associants of red alga *Polysiphonia* sp. were placed within the genera *Pseudovibrio, Paenibacillus, Bacillus* and *Streptomyces* sharing 99% gene sequence similarity to previous recognized species. *Streptomyces* strains occurred abundantly in both hydrobionts specimens and showed remarkable antibacterial properties. Unlike hydrozoan's bacterial consortium most *Streptomyces* strains from red alga were phylogenetically related to *S. albidoflavus* which also includes strains producing bioactive compounds [28]. Members of the genera *Bacillus*, *Paenibacillus*, *Streptomyces* from marine sources have been reported to be of importance as producers of bioactive compounds [3,29,30]. In previous studies we have reported bioactive substances produced by *Bacillus, Paenibacillus*, and *Saccharothrix* strains associated with ascidian *Halocynthia aurantium* and mollusk *Anadara broughtoni* inhabiting in the Sea of Japan seashore [5,31,32,33].

Bacteria of the genus *Pseudovibrio* (family *Rhodobacteriaceae,* class *Alpharoteobacteria*), to which red alga active isolates belonged, have been reported to be abundant and ubiquitous bacteria associated with diverse marine dwellers, sponges [15,16,17,34], ascidians [35], corals [18] or red alga [36]. In the present study *Pseudovibrio* strains with antimicrobial activity sharing 99% gene sequence similarity positioned all on the line of *Pseudovibrio ascidiaceicola*, strains of which were isolated from ascidians *Polycitor proliferus* and *Botryllidae* sp. collected from a Pacific Ocean beach, the Boso peninsula, Japan [35]. *Pseudovibrio* strains have been reported to produce antimicrobial compounds, tropodithietic acid, TDA, [36], the red pigment heptylprodigiosin [37], polypeptide pseudovibrocin [38].

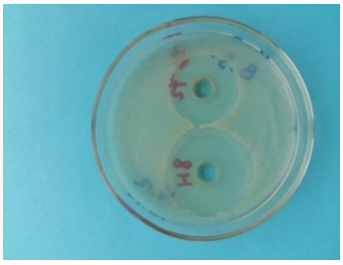
Comparing microbial associations of hydrozoans and red alga specimens it can be concluded that each of hydrobionts harbored a certain bacterial composition. Despite the taxonomic differences they are united by the occurrence of representatives of *Phaeobacter* and *Pseudovibrio* which are known TDA-producing bacteria. TDA is a disulfide tropolone, which can exist in a tautomeric form with thiotropocin (Fig. 3) and exhibits antimicrobial activity against a broad spectrum of microorganisms [23,20,39]. TDA was firstly isolated from a soil bacterium *Pseudomonas* species [40], and subsequently from marine *Caulobacter* sp. [41] and from the *Roseobacter* clade bacteria including *Ruegeria* sp. and *Phaeobacter inhibens* [23,26,42,43].



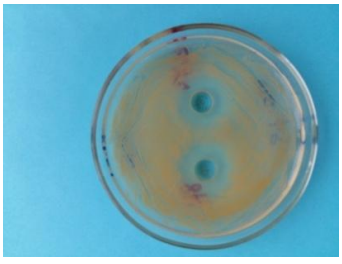
Thiotropocin Tropodithietic acid

Fig. 3. The chemical structure of TDA

Strains *Phaeobacter* sp. H8 and *Pseudovibrio* sp. rh17 were selected to be examined for the production of TDA, which could contribute their antimicrobial activity. Antimicrobial activities against Gram-positive indicatory strains *B. subtilis*, *S. aureus* and *E. faecium* were revealed in ethyl acetate extracts of CFS of these strains (Fig. 4); very weak activity was observed towards Gram-negative *E. coli* and *Xanthomonas* sp. pv. *badrii*, and no activity against *S. epidermidis* and *C. albicans*.



(a)



(b)

Fig. 4. *In vitro* antimicrobial activity assay of ethyl acetate extracts obtained from strains *Phaeobacter* sp. H8 and *Pseudovibrio* sp. rh17 against *Bacillus subtilis* (a) and *Staphylococcus aureus* (b)

The UV spectra of active ethyl acetate extracts exhibited broad strong UV absorption bands at

303 nm, a shoulder at 356 nm and a weak broad absorption around 450 nm, that could be superimposed on the spectrum of thiotropocin or tropodithietic acid [24]. To further confirm the TDA production by strains H8 and rh17, active ethyl acetate extracts were redissolved in 10 mL 85% acetonitrile/15% MilliQ water and analyzed by liquid chromatography-mass spectrometry, including high resolution, (LC-HR-MS). Separation was performed on a Zorbax Eclipse XDB C18 column using a water-acetonitrile gradient solvent system, with both water (solvent A) and acetonitrile (solvent B) containing 0.1% formic acid. The peaks representing the TDA in the extracts of strains H8 and rh17 were t_R =17.8 and 17.2 min, respectively (Figs. 5,6).

LC-HR-MS analysis confirmed the presence of TDA in the extracts of strains H8 and rh17 based on the unique accurate mass of the $[M+H]^+$ ion at *m/z* 212.9671 and 212.9678, respectively, (calcd. for $C_8H_4O_3S_2$ *m/z* 212.9675). To our knowledge, this is the first report concerning TDA production by bacteria associated with hydrozoans and red alga from the Peter the Great Bay, the Sea of Japan.

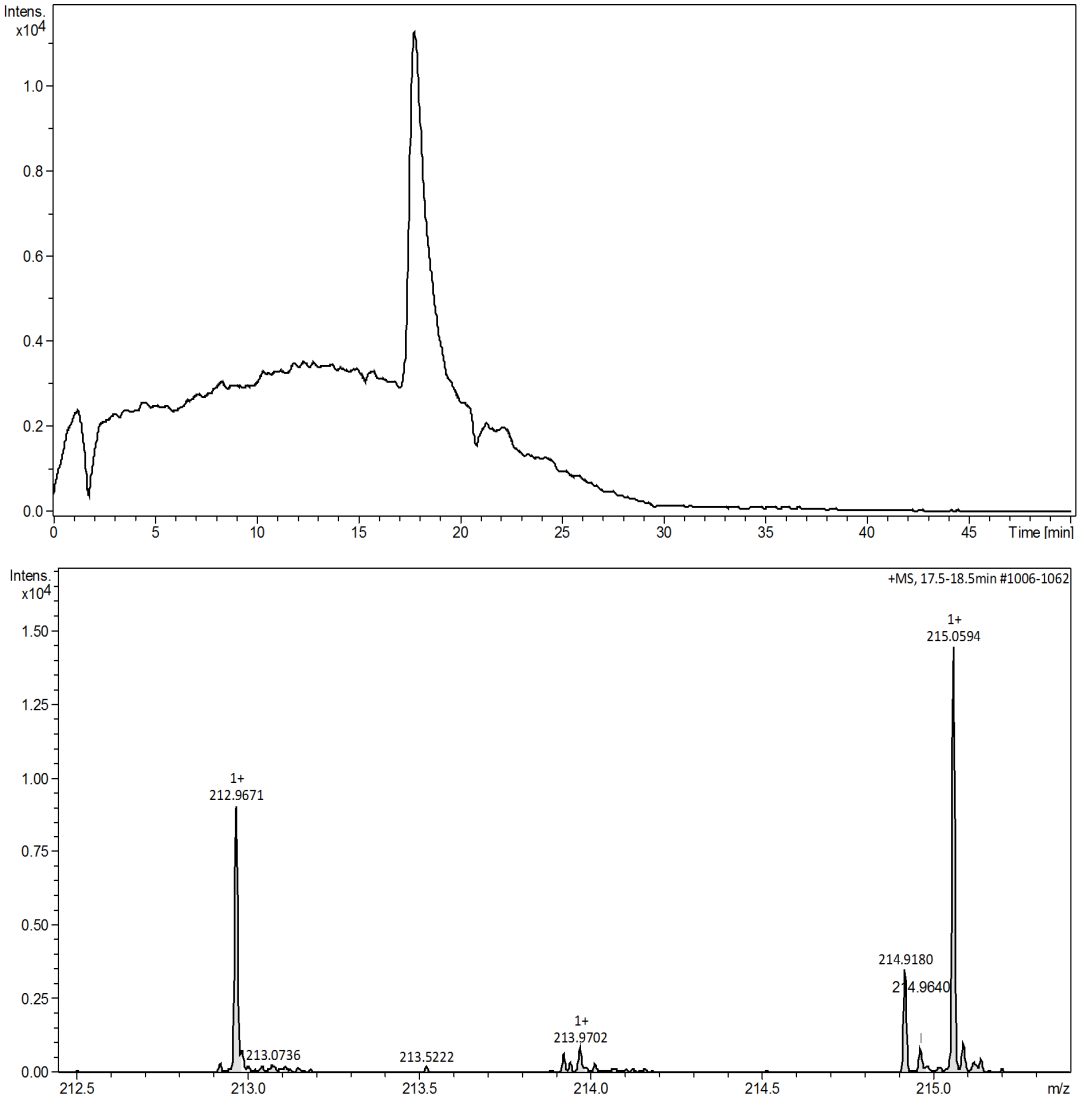


Fig. 5. LC-MS analysis of TDA produced by the strain *Phaeobacter* sp. H8: chromatographic profile (at the top) and corresponding MS spectra in positive ion mode (at the bottom)

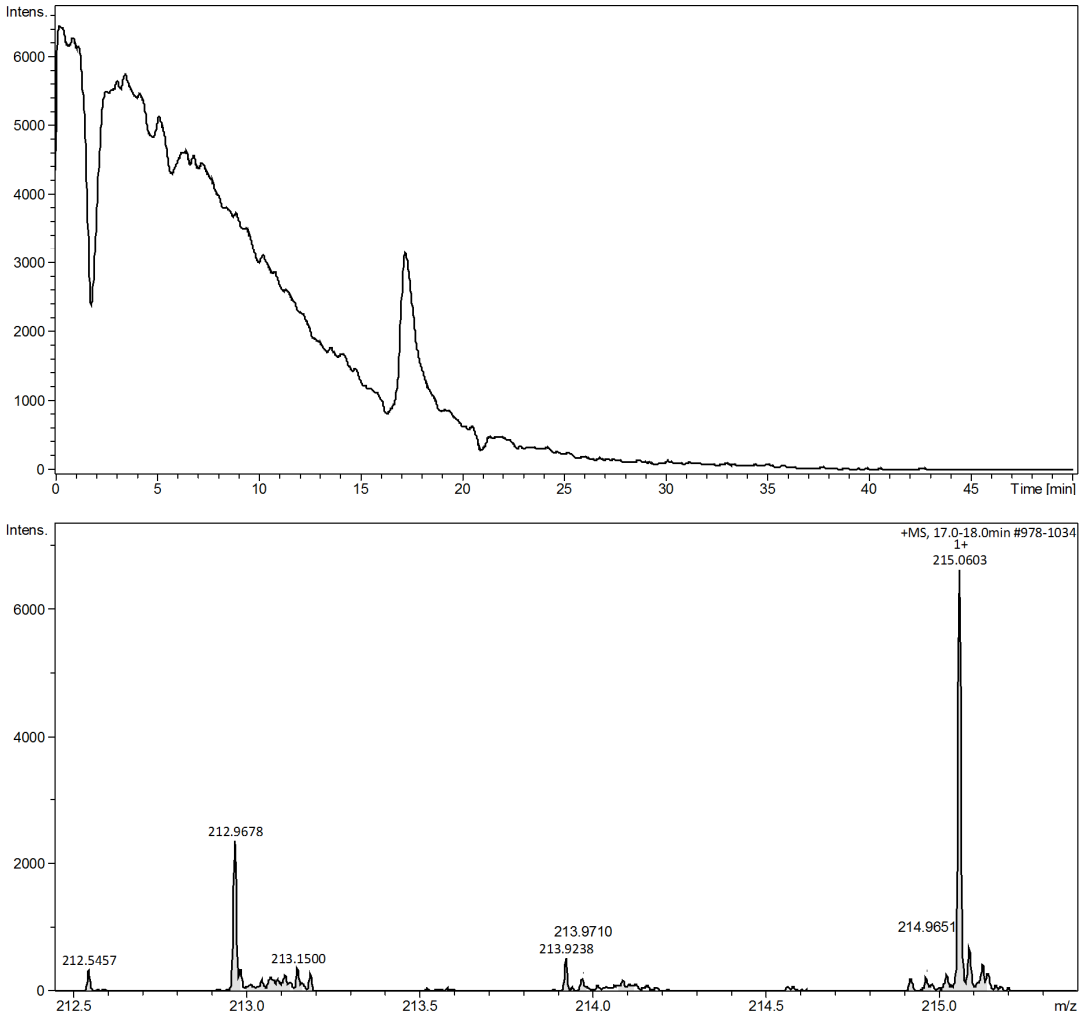


Fig. 6. LC-MS analysis of TDA produced by the strain *Pseudovibrio* sp. rh17: chromatographic profile (at the top) and corresponding MS spectra in positive ion mode (at the bottom)

4. CONCLUSION

Overall our findings showed that hydrobionts studied harbored taxonomically diverse microbial communities with specific composition for each of them. A large proportion of associated bacteria was found to exhibit antimicrobial activities and can be considered as a potential source of bioactive compounds. This study provides evidence that TDA-producing strains belonging probably to *Phaeobacter inhibens* and *Pseudovibrio ascidiaceicola* occurred abundantly in microbial associations colonizing hydrozoans and red alga *Polysiphonia* sp., respectively, in the Sea of Japan seashore. TDA and other antimicrobials produced by bacteria-associants are conceivable to provide chemical protection of their host-organisms and serve for bacterial relationships in marine shallow environments.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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