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EXPERIMENTAL ARTICLES

Characterization of Communities of Heterotrophic Bacteria Associated with Healthy and Diseased Corals in Nha Trang Bay (Vietnam)

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Abstract—A comparative investigation of the heterotrophic microflora of 11 species of healthy corals and of white-band-diseased and yellow-band-diseased corals inhabiting the reefs of Nha Trang Bay (Vietnam), which has been exposed to anthropogenic impact, was performed. Fifty-nine strains of heterotrophic bacteria isolated on Y/K and Endo media were investigated and characterized. All the isolates were identified at the genus level by consideration of the results of analysis of their phenotypic properties, determination of the molar percent of G+C bases in their DNA, and the composition of the fatty acids of lipids. In the composition of the microflora of tissues of healthy corals, γ-proteobacteria prevailed, with halomonads being dominant among them. In addition, the gram-negative bacteria included *Pseudomonas* and *Vibrio* spp., members of the *Cyto-phaga–Flavobacterium–Bacteroides* (CFB) phylogenetic cluster, and *Moraxella* sp. The gram-positive bacteria revealed included *Bacillus, Staphylococcus, Halococcus*, and *Micrococcus* spp., and coryneform bacteria. In the composition of the microflora of the tissues of affected corals, bacteria of the family *Enterobacteriaceae* and of the genera *Planococcus* and *Arthrobacter*, which were not revealed in healthy hydrobionts, were found. The anthropogenic impact is not the sole factor determining the infection of corals.

Key words: heterotrophic bacteria, associated bacteria, corals.

The attention of investigators was first attracted to the problems of coral reefs in the 1970s, when previously unknown diseases destroyed colonies of reef-forming corals over enormous areas [1]. In the same period, attempts were made to identify the pathogens responsible [2]. A group of gram-negative bacteria was discovered to be associated with the healthy corals *Acropora palmata* and corals affected with white band disease in the Caribbean Sea. Over a period of five years, 95% of the coral colonies in the investigated reef perished. Attempts to isolate and cultivate the pathogens failed or yielded controversial results. Precise diagnosis and prevention of outbreaks of the infection required fundamental knowledge of the composition and distribution of the microbial communities associated both with healthy and diseased corals. Studies of the interaction between corals and microbes were started in the 1970s [3]. The results of these investigations demonstrated that the surface of corals and probably their tissues are populated by microorganisms. However, it is still unknown whether they play any specific role in the biology of corals or whether these are just opportunistic interactions between animals and the bacterial population of the water column. The presence of specific microbial associations in the corals that include nitrogen-fixing bacteria has been reported [4]. For better understanding of the physiology and ecology of bacterial species, their isolation in pure cultures remains the principal method employed [5]. The currently widely applied molecular methods, including those using 16S rRNA genes as molecular markers, have certain limitations. Thus, fragments of the rRNA genes of microorganisms present in low numbers may not be represented in the product of the polymerase chain reaction [6]. It should be kept in mind that, in aquatic ecosystems, only a small part of the community is active at a given moment [7]; therefore, only culture-based methods can yield precise information on living and active cells. At the present time, there is no sufficient information either on the associated microflora of corals or on changes in the composition of the microflora in case of various infections of hydrobionts.

Our study was aimed at comparative investigation of the bacterial communities of various species of healthy and affected corals inhabiting ecotopes differing in their level of anthropogenic impact in Nha Trang Bay (Vietnam). It should be stressed that the focus of the present investigation is a highly complex multifactor system of macro- and microorganisms, so our results should be viewed as preliminary.

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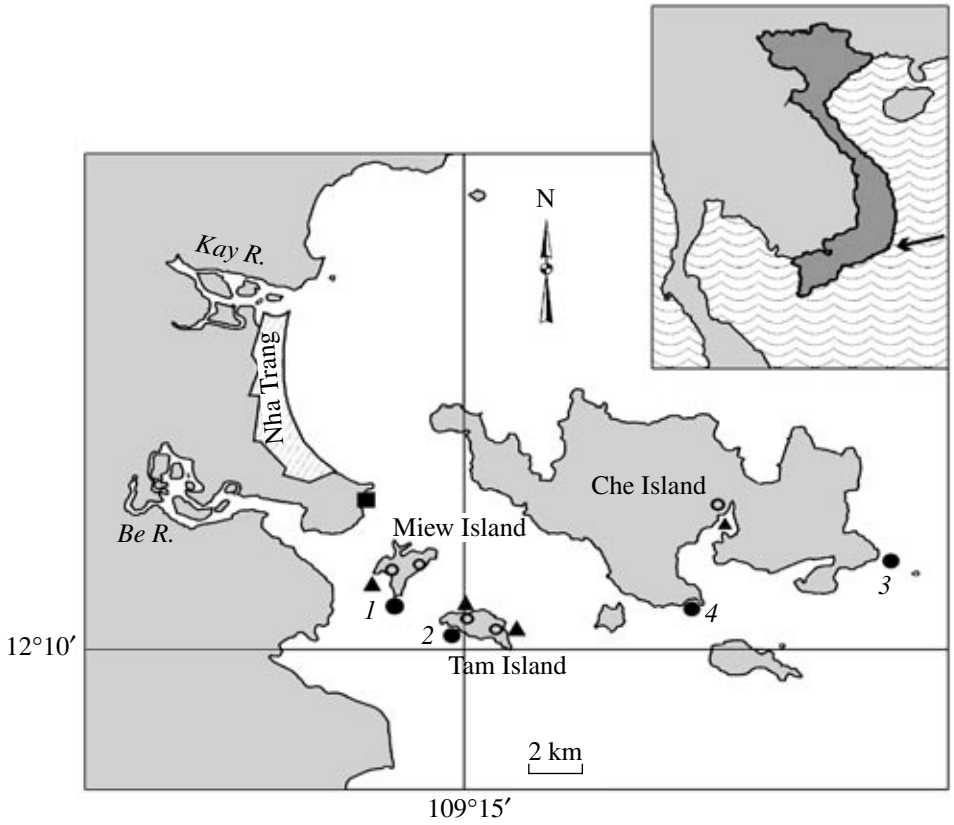

Schematic map of the area under investigation (October and November 2003). The numerals designate sampling stations. ▲ indicates a mariculture farm; ○, a settlement; and ■, port.

MATERIAL AND METHODS

The corals (*Montipora aequituberculata, M. digitata, M. foliosa, Porites cylindrica, P. nigriscens, P. lobata, P. australiensis, Acropora hyacinthus, A. nobilis, A. cyntherea*, and *Hydnophora rigida*) were collected in October and November 2003 from reefs in Nha Trang Bay, South Vietnam, by means of standard diving equipment at the sites marked on the map (see the figure). The water temperature was 30°C. Samples of healthy and affected corals 5 cm^2 in size were cut from the colony using a chisel and placed separately in 50-ml flasks with sterile seawater. Once on the shore, the samples were twice washed with sterile seawater. The bacteria associated with the coral tissue were isolated according to [8] on solid Y/K medium [9]. For this purpose, coral pieces were ground with a pestle in 5 ml of seawater in a mortar, observing the rules of antiseptics, and transferred to 10-ml tubes. After 3 min, the upper part of the liquid phase was taken with a pipette (particles of the coral skeleton and soft tissues settled on the bottom) and diluted from 10^{-1} to 10^{-5} with sterile seawater; 0.1-ml aliquots were then plated onto dishes. The inoculated dishes were incubated for 14 days at 30°C and periodically examined under a binocular microscope. Colonies that differed morphologically were transferred to new dishes with the same medium. As it was not possible to identify each of the colonies grown on the medium used for isolation (their number reached a hundred per dish), one colony of each morphotype was taken for identification. In order to obtain a pure culture, each isolate was subcultured three times on a fresh medium. The isolates were maintained in test tubes with semisolid Y/K agar at a temperature of 8–10°C. Bacteria of the *Escherichia coli* group were isolated on the Endo medium. When identifying the marine isolates, the cultural, morphological, physiological, and biochemical properties were studied according to the 2nd edition of *The Prokaryotes* [10] and, also, using the modern electronic version of *The Prokaryotes* (http://141.150.157.117:8080/proPUB/index.htm). Isolation of DNA from the cells, determination of the DNA G+C content, and analysis of the fatty acids of bacterial lipids were performed by the methods described in [11]. Methyl esters of fatty acids were analyzed using a Shimadzu GC-14A chromatograph equipped with a flame-ionization detector and a capillary quartz column (30 m × 0.25 mm) with Supelcowax-10. The temperature of separation was 200°C. Helium was used as the carrier gas. The fatty acids were identified from their relative retention times and from the values of the equivalent chain lengths. Additionally, fatty acid esters were identified using a GCMS-QP5050A chro-

matograph–mass spectrometer (Shimadzu) equipped with an MDS-5 column (30 m × 0.25 mm). The temperature was programmed to rise from 160 to 240°C at 2°C per min; the temperature of 240°C was maintained for 20 min. The energy of the electronic impact was 70 eV. Identification was carried out by comparing the mass spectra obtained with spectra from an electronic library (www\lipid\co.uk).

RESULTS AND DISCUSSION

From three species of the coral *Montipora*, four species of *Acropora*, and one species of *Hydnophora*, 59 strains of heterotrophic bacteria were isolated and characterized (Table 1). Bacteria of the *E. coli* group were isolated only from sample no. 3M, which was taken from the bleaching-affected coral *Montipora foliosa.* Inoculations of Endo medium with the other samples gave negative results. When identifying the bacterial isolates, their morphological, biochemical, and other properties were taken into consideration (Table 2). Thus, halomonads were distinguished by their halotolerance: their strains grew on media containing 12.5% NaCl, were unable to grow without the addition of NaCl, and had a high DNA G+C content. The composition of the fatty acids of strains 350 and 369 was typical of representatives of the genus *Halomonas* [12] (Table 3). In both strains, the components 18:1 (*n*-7), 16:1 (*n*-7), and 16:0 dominated, making up 92–97% of the total fatty acids. Such a set of acids makes halomonads similar to pseudomonads; however, bacteria of the genus *Halomonas* can be differentiated from pseudomonads by the absence of the cyclopropane acids cyclo 17:0 and cyclo 19:0.

The pseudomonads were characterized by growth without NaCl and the presence of arginine dihydrolase. Three isolates (344, 368, and 374) were attributed to *Pseudomonas* according to the profile of their fatty acids. The strains of this genus are characterized by four major fatty acids: 16:0, 16:1 (*n*-7), cyclo 17:0, and 18:1 (*n*-7). The fatty acids of *Pseudomonas* have been thoroughly studied. Strains 344 and 368 demonstrated a high similarity in relation to the composition of their fatty acids, which suggests a close taxonomic relationship between these isolates. Strain 374 differed in that it had a comparatively low level of 16:1 (*n*-7) and cyclo 19:0 was a major component. *Pseudomonas* species are known to exhibit noticeable variations in the proportions of the major fatty acids [13].

Bacteria of the *E. coli* group formed red colonies with a metal luster on Endo medium, fermented glucose at 43°C in 24 h with the evolution of gas, and were oxidase-negative.

The vibrios fermented glucose in 24 h, were sensitive to the pteridine derivative O-129 at a dose of 10 μg per disk, possessed a characteristic form of curved rods, and exhibited certain other features. *Vibrio* sp. 366 contained diverse fatty acids, mainly with straight chains, that were both saturated and monounsaturated; over 40% of them were 16:1 (*n*-7), which is a distinctive feature of vibrios [14].

Gram-negative gliding bacteria with a low DNA G+C content were attributed to the *Cytophaga–Flavobacterium–Bacteroides* (CFB) phylogenetic cluster.

The cells of *Arthrobacter* sp. differed by their pleomorphism; they were irregular rods with outgrowths and ramifications. In the process of growth, the rods were transformed into cocci, which occurred singly, in pairs, and in aggregates. The immotile *Arthrobacter* cells possessed an oxidative type of metabolism and had a high G+C content in their DNA (62.9 mol %). In the composition of the strain 357 fatty acids, *anteiso* 15:0 and *anteiso* 17:0 were found to be major components, which is a trait characteristic of bacilli; however, the contribution of the C16 acids was significantly lower than in *Bacillus.* Strain 357 differed from the closely related genus *Micrococcus* by its high content of *anteiso* 17:0 acid. Taking into account these traits, strain 357 was identified as a representative of the genus *Arthrobacter.*

The bacilli were regular gram-positive rods exhibiting a tendency toward gram-variable staining, especially in old cultures. Peritrichous flagellation, absence of growth on Mac Conkey agar, and low G+C content in its DNA were the reasons for attributing an isolate to the genus *Bacillus.* Strain 356 was distinguished by a relatively simple set of fatty acids characteristic of *Bacillus.* Among the cellular fatty acids of the bacilli, branched acids were dominant: *anteiso* 15:0, making up over half of the total fatty acids; *iso* 15:0; *iso* 16:0; and *iso* 17:0. Of the straight-chain fatty acids, the acid 16:0 was present.

Planococci, which also belong to the family *Bacillaceae*, were differentiated by their inability to form spores and the absence of nitrate reductase. A remarkable feature of the fatty acid profile of strain 362 was the prevalence of branched C14–C17 fatty acids, both saturated and monounsaturated.

Bacteria of the genus *Micrococcus* formed tetrads and irregular aggregates of spherical immotile cells, had a yellow pigment, and showed good growth on media both without NaCl and with 9% of NaCl. The micrococci differed from *Planococcus* spp. in the absence of C17 acids and a high concentration of the *anteiso* 15:0 acid. The content of the latter in strain 370 made up 75% of the total fatty acids. These features allowed strain 370 to be identified as a representative of the genus *Micrococcus*.

Strains 356, 357, 362, and 370 had a similar composition of fatty acids, the major components being *iso* and *anteiso* methyl-branched fatty acids. However, taking into account the contribution of the other components to the fatty acid spectrum, these strains were attributed to four different genera: *Bacillus, Arthrobacter, Planococcus*, and *Micrococcus*, respectively.

Table 1. Origin of the strains of heterotrophic bacteria isolated from the coral reefs of Nha Trang Bay (Vietnam)

Sample number	*N*	Bacterial taxon	Coral species	State of coral	Notes on reefs
14	*	*Vibrio* sp.	*Montipora digitata*	normal	Station no. 1. Mew Island. Maximum anthropogenic impact: sewage, tourism, settlement, mariculture farm, 4 km from port
	5.5×10^2	*Pseudomonas* sp.			
3M	1.8×10^3	*Planococcus* sp.	*Montipora foliosa*	bleaching	
	1.2×10^2	*Halomonas* sp.			
	1.0×10^2	*Staphylococcus* sp.			
	1.0×10^4	Enterobacteriaceae			
	1.1×10^2	*Staphylococcus* sp.			
	*	*Pseudomonas* sp.			
	1.3×10^4	Enterobacteriaceae			
	2.0×10^4	Enterobacteriaceae			
1	3.0×10^5	*Arthrobacter* sp.	*Montipora aequituberculata*	yellow bands	Station no. 3. Maximum distance from the city and port (20 km). No nearby settlements
	1.3×10^2	*Bacillus* sp.			
17	3.5×10^5	*Vibrio* sp.		bleaching	
	3.1×10^2	*Pseudomonas* sp.			
	1.5×10^2	*Halomonas* sp.			
	2.3×10^2	*Staphylococcus* sp.			
	*	*Planococcus* sp.			
	*	*Halococcus* sp.			
	*	*Arthrobacter* sp.			
	1.1×10^2	*Pseudomonas* sp.			
16	2.6×10^4	*Halomonas* sp.	*Porites cylindrica*	normal	Station no. 1. Mew Island. Maximum anthropogenic impact: sewage, tourism, settlement, mariculture farm, 4 km from port
	*	*Halococcus* sp.			
	4.0×10^2	*Pseudomonas* sp.			
	1.3×10^4	*Halomonas* sp.			
18	5.7×10^4	*Halomonas* sp.			
	*	*Bacillus* sp.			
4M	8.5×10^3	*Halomonas* sp.			
	9.0×10^3	*Halomonas* sp.			
5	1.3×10^2	*Vibrio* sp.			Station no 2. 8 km from port. Tourism, settlement, mariculture farm
	2.1×10^2	*Vibrio* sp.			
	1.0×10^2	*Vibrio* sp.			
2	*	*Moraxella* sp.	*Porites nigrescens*	normal	Station no 2. 8 km from port. Tourism, settlement, mariculture farm
	3.2×10^2	*Pseudomonas* sp.			
	2.3×10^4	*Halomonas* sp.			
	2.1×10^4	*Halomonas* sp.			
9	2.0×10^2	*Pseudomonas* sp.			
	3.1×10^2	*Staphylococcus* sp.			
	*	CFB cluster			
	2.7×10^4	*Halomonas* sp.			
11	5.5×10^3	*Halomonas* sp.			
	*	CFB cluster			
	*	Not identified			
13	3.6×10^2	Not identified			
6	3.2×10^2	*Pseudomonas* sp.	*Porites australiensis*	normal	Station no. 4. 13 km from the port. Settlement, mariculture farm
	7.3×10^3	*Halomonas* sp.			
	*	*Micrococcus* sp.			
19	4.8×10^4	*Halomonas* sp.			
	*	CFB cluster			
20	5.3×10^4	*Halomonas* sp.			
7	1.7×10^4	*Halomonas* sp.	*Porites lobata*	normal	
8	*	*Staphylococcus* sp.	*Acropora cyntherea*	normal	
	4.8×10^2	*Pseudomonas* sp.			
10	2×10^4	*Halomonas* sp.	*Acropora hyacinthus*	normal	Station no 2. 8 km from port. Tourism, settlement, mariculture farm
	1.5×10^4	*Halomonas* sp.			
	1.3×10^4	*Halomonas* sp.			
12	6.1×10^3	*Halomonas* sp.	*Acropora nobilis*	normal	
	1.1×10^2	*Vibrio* sp.			
15	*	Coryneformes	*Hydnophora rigida*	normal	Station no 2. 8 km from port. Tourism, settlement, mariculture farm
	3.7×10^4	*Halomonas* sp.			

Note: *N* is the abundance of bacteria in a sample. An asterisk means occasional detection in the course of examination of colonies.

Table 2. Phenotypic properties of heterotrophic bacteria associated with different coral species

Character	Microorganisms											
	Halomonas spp.	*Pseudomonas* spp.	Enterobacteriaceae	*Vibrio* spp.	CFB cluster	*Moraxella* sp.	Coryneformes	*Planococcus* spp.	*Micrococcus* sp.	*Staphylococcus* spp.	*Halococcus* spp.	Coryne–formes
Gram staining	–	–	–	–	–	–	+	+	+	+	+	+
Cell morphology*	r, c	r, c	rr	ir	r, f	c	rr	c	c	c	c	ir
Motility	+	+	+	+	–	–	+	+	–	–	–	+
Pigment**	w, y	y, w	–	–	–	w	w, b	y, w, o	y	y, w, o	y	–
Oxidation/Fermentation***	O	O	F	F	F	O	O	O	O	F	O	O
	+/–, –/–	+/–, –/–	+/+	+/+	+/+	–/–	–/–	–/–	–/–	+/+	–/–	–/–
Oxidase	+, –	+, –	–	+	–	+	–	–	–	–	–	–
Catalase	+	+	+	+	+ weak	+	+	+	+	+	+	+
Growth without NaCl	–	+	+	–	+	+	+	+	+	+	–	+
Growth with	+	+	+	+	+	+	+	+	+	+	+	+
3% NaCl	+	+	+	+	+	–	+	+	+	+	+	+
6% NaCl												
9% NaCl	+	+	–	+	+	–	–	+	+	+	+	–
12.5% NaCl	+	+, –	–	–	+	–	–	+	–	+	–	–
NO_3-reductase	+, –	+	+	+	–	+	+	–	–	+	–	+
Arginine dihydrolase	–, +	+	–	–	ND****	ND	ND	–	–	+, –	ND	–
Lysine decarboxylase	+	ND	+	+	ND	ND	ND	–	ND	ND	ND	ND
Ornithine decarboxylase	ND	ND	+, –	–	ND	ND	ND	–	ND	ND	ND	ND
Indole	ND	ND	+	+, –	ND	ND	–	ND	ND	ND	ND	–
Urease	ND	ND	–	ND	ND	–	ND	–	ND	ND	+	ND
Sensitivity:												
to O-129	ND	–	–	+	–	ND	–	ND	ND	ND	–	–
polymyxin	–	ND	ND	+	+	ND	ND	ND	ND	ND	ND	ND
benzylpenicillin	–	ND	ND	–	+	+	+	+	ND	ND	+	ND
ampicillin	–	ND	ND	+, –	+	ND	ND	ND	ND	ND	ND	ND
oxycilline	–	ND	ND	+, –	+	ND	ND	ND	ND	ND	ND	ND
streptomycin	–	ND	ND	+, –	+	ND	ND	ND	ND	ND	ND	ND
Gelatine	–, +	+	ND	+	+	–	–	+	ND	+	+	–
Amylase	+	+	ND	+	+	–	ND	–	ND	–	ND	ND
Acid from:												
glucose	+, –	+	+, ga	+	+	–	–	–	–	+	–	–
mannose	ND	ND	+	+	ND	–	ND	ND	–	+	ND	–
arabinose	ND	ND	+	+, –	ND	–	ND	ND	ND	–	ND	–
sucrose	ND	ND	+	+, –	ND	–	ND	–	ND	+, –	ND	–
lactose	ND	ND	+	–	ND	–	ND	ND	–	+, –	ND	–
G+C content in DNA, mol %	61.2–66.6	57.3–67.1	47.0–50.0	40.9–45.2	33.2–41.9	44.2	41.9–50.0	45.0–47.0	68.5	33.2–34.8	67.1–70.6	68.7

* r stands for rods; c, cocci; rr, regular rods; cr, curved rods; ir, irregular rods; and f, filaments.

** w indicates white; y, yellow; b, beige; o, orange; and "–", nonpigmented.

*** O means oxidative metabolism; F, fermentative metabolism; and +/+, oxidation/fermentation of glucose on Hugh–Leifson medium.

**** ND stands for not determined.

Table 3. Fatty acid profiles of heterotrophic bacteria associated with corals

Fatty acids	*Pseudomonas*			*Halomonas*		*Bacillus*	*Planococcus*	*Micrococcus*	*Vibrio*	*Moraxella*	*Arthrobacter*	*Halococcus*	*Coryneform*
	344	368	374	350	369	356	362	370	366	361	357	348	345
10:0	3.2	–	0.7	2.8	1.1	–	–	–	–	1.4			
11:0	–	–	–	–	–	–	–	–	–	0.4			
12:0	4.6	3.4	1.5	3.6	1.1	–	–	–	3.9	1.9			
iso 13:0	–	–	–	–	–	–	–	0.6	0.5			0.9	
anteiso 13:0	–	–	–	–	–	–	–	2.5	–				
13:0	–	–	–	–	–	–	–		0.3				
iso 14:0	–	–	–	–	–	0.9	2.4	4.3	–		2.2	3.9	
14:0	0.1		0.1	0.3	0.2	3.2	0.5	0.5	5.1		0.7		4.4
14:1	–	–	–	–	–	–	–	–	0.3				6.6
iso 15:0	–	–	–	–	–	6.3	12.1	8.5	–		9.0	6.0	
anteiso 15:0	–	–	–	–	–	60.0	44.9	75.3	0.6		55.4	67.0	
15:0	0.1	–	0.1	–	–	0.6	2.4	2.3	3.2	0.5		16.0	2.0
15:1 (*n*-8)	–	–	–	–	–	–	–	0.8	1.3	0.3			
iso 16:0	–	–	–	–	–	5.0	1.9	1.5	0.9		6.1	3.5	
16:0	22.2	23.1	18.4	20.7	21.8	12.8	0.7	0.4	14.2	1.5	3.5		41.1
iso 16:1	–	–	–	–	–	–	6.5	0.8	–			0.7	
16:1 (*n*-7)	44.1	42.3	9.7	33.2	17.5	–	–	0.9	42.0	9.8			23.7
16:1 (*n*-9)	–	–	–	–	–	–	3.1	–	–				
iso 17:0	–	–	–	–	–	–	3.2	0.9	–	0.4	2.1		
anteiso 17:0	–	–	–	–	–	10.6	11.5	–	–		19.4	2.1	
17:0	0.1	–	0.1	–	–	–	0.8	–	1.6	1.5			
iso 17:1	–	–	–	–	–	–	2.8	–	–				
anteiso 17:1	–	–	–	–	–	–	4.9	–	–				
17:1 (*n*-8)	0.2	–	–	–	–	–	–	0.8	2.5	25.0			
cyclo 17:0	4.2	3.3	6.2		–	–	–	–	0.5				
iso 18:0	–	–	–	–	–	–	0.5	–	–				
18:0	–	–	0.1	0.4	–	–	0.3	–	–	1.5	1.5		5.9
18:1 (*n*-9)	–	–	–	–	–	–	–	–	–	55.7			8.3
18:1 (*n*-7)	20.1	24.8	36.4	38.2	57.8		1.1		23.2				8.0
19:0							0.3						
cyclo 19:0			25.8										
3OH-11:0	0.9		0.9	0.2									
3OH-12:0		0.7		0.5	0.5								
3OH-14:0						0.7							

Identification of strains of the genus *Staphylococcus* was not difficult. On Y/K agar, these bacteria formed characteristic small opaque brightly pigmented colonies. In Gram-stained smears, spherical cells formed irregular aggregates. These properties, as well as their halotolerance, the absence of oxidase, their lack of motility, and low G+C content in their DNA, meant that we should attribute five bacterial isolates to the genus *Staphylococcus*.

In contrast to *Micrococcus* spp., halococci did not grow on media devoid of NaCl. One of the simplest with respect to its set of fatty acids was strain 348: about 90% of its total fatty acids were C15 acids. These traits defined this strain as a representative of archaea of the genus *Halococcus*.

Coryneform bacteria were identified by taking into account the specificity of their cell morphology, fatty acid profile, and the high G+C content in DNA.

The *Moraxella* isolates formed small white crumbling colonies on Y/K agar. They were highly sensitive to penicillin. Strain 361, identified as *Moraxella* sp., occupies a separate position among the strains investigated. 12 fatty acids were identified in this strain, and over 90% of them were 18:1 (*n*-9) and 16:1 (*n*-7) fatty acids; the 18:1 (*n*-9) acid was dominant, which is rare for bacteria.

In investigations of the isolates, the data obtained from analysis of the fatty acids of cell envelope lipids were in good agreement with the results of morphological and biochemical analyses. Thus, application of the aforementioned methods for identification of natural isolates of heterotrophic bacteria considerably facilitates identification of microorganisms and increases its accuracy.

Gram-negative bacteria prevailed in the tissues of the corals investigated, making up 74.6% of the total number of isolates. Among the gram-negative microorganisms, the most numerous were representatives of the genus *Halomonas* (45.5%). The gram-positive flora was diverse, consisting of *Planococcus, Bacillus, Micrococcus, Staphylococcus, Arthrobacter, Halococcus* spp., and coryneform bacteria. Analysis of the phenotypic traits of the isolates presented in Table 2 demonstrated that the bacteria associated with corals may have both oxidative and fermentative types of metabolism. This indicated the existence in the body of the hydrobionts of both of aerobic and anaerobic conditions. This allows microorganisms to occupy appropriate ecological microniches, forming specific communities.

The tissues of healthy corals contained an insignificant variety of heterotrophic bacteria. Each sample contained representatives of one to four genera, with two bacterial genera being average. The majority of the colonies grown in the course of isolation on Petri dishes (80 and, sometimes, 100%) belonged to a morphotype later identified, from the results of biochemical tests, as *Halomonas* sp. Their cell number ranged from 7.3×10^3 to 5.7×10^4 cells per ml, depending on the sample (Table 1). In addition to halomonads, pseudomonads, vibrios, and staphylococci were rather frequent; however, the abundance of bacteria belonging to each of these genera did not exceed 5.6×10^2 cells/ml. Ritchie and Smith [15] believe that the slimy substance excreted by corals is attractive to certain microorganisms closely associated with the superficial mucus of hydrobionts. It seems that the mucus secreted by healthy corals is attractive for representatives of heterotrophic bacteria from the genera *Halomonas, Pseudomonas, Vibrio*, and some others (Table 1). *Moraxella, Bacillus, Halococcus, Micrococcus* spp., and members of the CFB bacterial cluster were rarely recorded and occurred as solitary colonies in the course of isolation.

In the tissues of four species of healthy scrleractinia of the genus *Porites*, nine genera of heterotrophic bacteria were identified. Halomonads were present in the microflora of all four species of these corals; in some cases, only *Halomonas* spp. could be isolated from the samples (samples nos. 4, 7, and 29) (Table 1). However, usually, different samples from a coral of the same species contained different bacteria in addition to halomonads; i.e., the samples were not identical in their microflora. No regular distribution of bacterial taxa by species of corals was found. For two species of corals, *P. nigriscens* and *P. australis*, the associated bacterial community included pseudomonads and members of the CFB phylogenetic cluster.

In *Hydnophora rigida*, similarly to *Acropora* spp., halomonads also prevailed. Thus, from *A. hyacinthus*, only *Halomonas* spp., most probably belonging to different species, were isolated, and, in *A. nobilis*, some vibrios were also present. The community of heterotrophic bacteria in *A. cyntherea* differed from the bacterial communities of other scleractinias by the absence of *Halomonas* spp. This special trait was also noted only in *Montipora digitata.* The microflora of *A. cyntherea* and *M. digitata* was represented by *Pseudomonas* sp., *Staphylococcus* sp., and *Vibrio* sp.; pseudomonads prevailed in numbers, while vibrios and staphylococci grew on the isolation medium as solitary colonies. Our results do not contradict the published data. In papers on the microflora of healthy corals, we did not find any data on the bacterial communities of hydrobiont species that we investigated. Such studies have been performed with other reef-building scleractinias. Cooney *et al.* [16] found that representatives of α-, β-, and γ-proteobacteria and members of the *Bacillus–Clostridium* group and the CFB cluster occur in the healthy corals *Diploria strigosa, Montastrea annularis*, and *Colpophyllia natans.* In healthy tissues of *Montastrea cavernosa* and *Diploria strigosa,* γ-proteobacteria were found to prevail (*Pseudomonas, Pseudoalteromonas, Shewanella* spp., etc.) (62 and 81%, respectively). In the composition of the microflora of *M. annularis*, green sulfur bacteria (19%), α-proteobacteria (16%), firmicutes (16%), and planktomycetes were found to dominate [17]. It should be noted that, in the cited studies, molecular bacteriological methods were used for identification of the microorganisms associated with corals, including sequencing of 16S rDNA, which allows considerably more members of the bacterial community to be revealed than with culture-based methods. Rohwer *et al.* compared the results of investigations of the composition of microflora of the same samples of *M. franksi* using molecular methods and cultivation of bacteria. In the former case, cyanobacteria (36%) and various α-proteobacteria were found to prevail in the bacterial community; in the second case, *Pseudoalteromonas* (39%) and *Vibrio* (38%) apparently predominated, with proteobacteria making up 23% [18]. Thus, special care should be taken when comparing data on the composition of hydrobiont microflora obtained by different methods.

Generally, we did not find any significant differences between the compositions of the microflora of the analyzed species of healthy corals. A distinctive trait was the presence and prevalence of bacteria of the genus *Halomonas* in all of the investigated samples except the samples of *Acropora cyntherea* and *Montipora digitata.* As to the taxonomic diversity of bacteria associated with the tissues of healthy corals, 66% of the isolated strains were representatives of γ-proteobacteria, 13.6% were members of the family *Micrococcaceae*, and 5% were members of the CFB cluster. *Bacillus* sp., *Moraxella* sp., and *Halococcus* sp. occurred occasionally. The prevalence of γ-proteobacteria in the culturable microflora associated with corals has also been recorded by other authors [17, 18]. However, among the γ-proteobacteria populating the healthy tissues of corals investigated by these authors, pseudomonads and pseudoalteromonads prevailed, while, in our studies, the leading place belonged to halomonads. Such a difference may point to the specificity of bacterial communities formed on certain species of corals and probably in certain reefs.

More strains were isolated from the samples taken from corals affected by bleaching than from healthy corals, and the composition of the microflora was more diverse. Enterobacteria were found only in the coral *Montipora foliosa*, which was affected by bleaching (Table 1). The abundance of red colonies with a metal luster on the Endo medium corresponded to 4.3×10^4 cells/ml. It has been reported that bacteria associated with sewage, including bacteria of the *E. coli* group, can be found only in affected corals and not in healthy corals [17]. In addition to enterobacteria, the above-mentioned sample yielded planococci (1.8×10^3 cells/ml), which were not found in the samples of healthy hydrobionts. Halomonads were present in affected corals in a considerably lower quantity than in healthy samples, specifically, 1.2×10^2 and 1.5×10^2 cells/ml in *M. foliosa* and *M. aequituberculata*, respectively. In the latter coral, enterobacteria were absent; however, the sample contained numerous vibrios and a few other bacteria. It is probable that, in this case, the vibrios caused white band disease of this hydrobiont, as has also been suggested by other authors [8]. From tissues of *M. aequituberculata* that had numerous yellow spots on the surface, we isolated strains of *Arthrobacter* sp. and *Bacillus* sp. No other heterotrophic bacteria were found in this sample. The colonies of *Arthrobacter*, characteristic in their form, were present on the isolation medium as a monoculture; the abundance of arthrobacters was 3.7×10^5 cells/ml, while the abundance of bacilli was low, specifically, 1.3×10^2 cells/ml. The bright yellow pigment characteristic of the *Arthrobacter* isolates was obviously the cause of the yellow coloration of the surface of the affected corals. For comparison, it may be mentioned that, in the healthy coral *M. digitata*, enterobacteria, bacilli, and arthrobacters were absent. The associative microflora was represented by *Pseudomonas* sp. and *Vibrio* sp. Our data indicate that, in tissues affected either with yellow or white band disease, the composition of the microflora changes. It seems that the equilibrium between the associated bacteria and the coral is disturbed, a part of the normal flora is eliminated, and its place is occupied by microorganisms from the surrounding water normally not characteristic of the bacterial community of the coral.

The published information on the composition of the microflora of healthy and affected corals is controversial. Richardson *et al.* [19] reported that, from a coral with white band disease, they isolated bacteria similar in colony form, color, structure, and size, whereas healthy samples yielded mixed populations. Other authors stress lower abundance and lower species diversity of the associated bacteria in healthy corals in comparison with diseased corals [16]. The controversies in the literature point to an insufficient amount of accumulated data and to insufficient knowledge of the composition of the microflora of healthy and diseased corals.

We considered the anthropogenic impacts, namely domestic and industrial sewage; tourism; and the presence of mariculture farms in the immediate proximity of the reefs, of a port, of agricultural lands, and of settlements (Table 1) when trying to identify the potential causes of coral diseases. It should be noted that two of the three affected colonies of corals occurred on a reef remote from the city and port and exposed to a comparatively small anthropogenic load in comparison with the other reefs. On Miew Island, situated in immediate proximity to the mouth of the Be River (Fig. 1), the city, and the port, a third site of corals affected with bleaching was revealed. The distribution of bleaching and yellow band disease in corals on the reefs exposed to a strong anthropogenic impact was not a mass phenomenon during the period of observation. In the samples taken from healthy hydrobionts inhabiting areas strongly affected by economic activities, there were no bacteria of the *E. coli* group. It seems that healthy corals possess sufficiently strong mechanisms to support homeostasis and the associated microflora. A surface microlayer of corals with the constituent zooxanthellae protects them from solar radiation and sedimentation and forms the first line of protection against infections [20]. We believe that the anthropogenic impact undoubtedly contributed to the development of the process of infection of corals but is not its sole controlling factor. The probable causes of diseases in corals may depend on fluctuations of natural processes, and their investigation has just started. In an integrated investigation of the biology of coral reefs, identification of the bacteria associated with corals remains the main method. Identification of the microbiota of healthy corals and corals exposed to stress and infections is also a method of monitoring the state of reefs.

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