Title	DIVERSITY OF MICROORGANISMS IN SOME FAMOUS BAYS OF VIETNAM AND THEIR ABILITY TO USE IN AQUACULTURE
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DIVERSITY OF MICROORGANISMS IN SOME FAMOUS BAYS OF VIETNAM AND THEIR ABILITY TO USE IN AQUACULTURE

Lai Thuy Hien[1], Hoang Hai[1], Vuong Thi Nga[1], Hiroko Kawasaki[2], Le Tran Binh[1]
[1]Institute of Biotechnology, Vietnamese Academy of Science and Technology
[2]ICBiotech, Osaka University

ABSTRACT

In Vietnam, the offshore beach is so large and shallow that it is favorable for developing and rearing marine product. However, some latest reports showed that only 1/3 coastal area was used to rear. In the other hand, ecological balance is broken, environment is polluted remarkably and productivity is declining. In some famous bays of Vietnam, we research diversity of microorganisms and the roles of indigenous microorganisms were in self-cleaning wastewater. From 66 samples of water and sediment in three bay, Ha Long, Dung Quat, Qui Nhon, beneficial, harmful and pathogenic microorganisms were isolated. Total of beneficial microorganisms (nitrate reducer and hydrocarbon degrader) reached to 10^4-10^6 CFU/ml. In feeding shrimp for along time or died shrimp samples, amount harmful bacteria (sulfate-reducing bacteria) were a bit high. Pathogenic microorganisms such as Salmonella, Shigella, E.coli were found in high-polluted areas. These results show that these environments have to treat immediately and research deeply nitrite and nitrate-reducing bacteria to find the strain that reduce pollutants.

INTRODUCTION

The oceans occupy 70,8% of earth's surface and marine microbiology is consequently an important area of study. In Vietnam, research of marine organisms started in the end of the 18th century. In particularly, when Institute of oceanography was established, marine organisms are more taxonomically studied. However, reports concerned about animal and plant but not pay attention on microorganism. In part, the role of microorganism in marine ecology is great. Microorganisms not only were the food of protozoa but also were the food of multicellular animals. Besides, they were determinant in material cycles such as nitrous fixation, nitrite oxidation, nitrate reduction, sulfur oxidation and sulfate reduction. Therefore, microorganism is the object that was studied deeply. Many bays of Vietnam were polluted by aquaculture, tourism and oil recovery. Thereby, we should study thoroughly on environment, especially indigenous microorganisms in order to apply reasonable these microbes and balance the ecology. Among Vietnamese famous bays, we focused on studying of microbial diversity in three bays and their role in environmental protection. These bays are Halong (the world heritage), Dung Quat (being built the 1st oil refinery plant) and Quinhon (huge aquaculture)

MATERIALSAND METHODS

Samples were taken from three bays Halong, Dung Quat and Quinhon.
Media: Aerobic bacteria medium 1.5%; Gost 1.5%; Basruda; Giltai; modified Posgate B 2%; Desoxycholate citrate agar; TCBS agar, LB agar.
Observing cell morphology under light microscope and electro microscope.
Identifying bacteria by morphological, biochemical and genetic methods.
Analyzing chemical constituents of marine water and of metabolism products by chromatography.

RESULTS

Analyzing samples taken from Halong bay

All of 30 samples taken from Halong bay existed microorganism. Aerobic bacteria number was quite high, up to 10^6 CFU/ml water. Nitrogenous compound-converting bacteria were observed

in any sample with the number of 10^1-10^5 CFU/ml (g). Sulfate-reducing bacteria were present in almost of samples except 4 samples 4a, 5a, 7a and 9a. This result indicated that Halong bay was polluted by tourism. Moreover, water from this heritage was threatened by some pathogenic bacteria such as coliform (0 - 3×10^3 CFU/ml), *Vibrio* (0 - 5.5×10^1 CFU/ml), Salmonella-*Shigella* (0 - 1.9×10^1 CFU/ml).

Table 1. Useful, harmful and pathogenic bacteria in Halong bay

Sample	Site	Depth (m)	pH	Eh (mV)	Sal. (‰)	T (°C)	NH_4	NO_2	NO_3	Aerobic bacteria	HC-utilizing	Bio-surfactant	*Lacto-bacillus*	SRB	Coli-form	Vibrio	S - S
HL1a	20⁰56'514'' 107⁰01'707	5	7.89	-64.2	28.4	21,4	10^3	10^2	10^5	10^5	10^5	10^5	10^5	10^1	30	0	0
HL1b			8.03	-75.1	28.8	21,2	10^3	10^2	10^3	10^4	10^3	10^4	10^3	10^1	5	0	19
HL1c							10^2	10^2	10^3	10^3	10^3	10^5	10^4	10^2	0	38	0
HL2a	20⁰54'917'' 107⁰00'903	10	8.13	-81.9	28.9	20.5	10^2	10^1	10^4	10^5	10^3	10^4	10^4	10^1	7	4	0
HL2b			8.18	-85.6	29.0	20.6	10^3	10^2	10^4	10^4	10^2	10^5	10^6	10^1	2	14	1
HL2c							10^2	10^1	10^3	10^4	10^2	10^4	10^4	10^3	0	1	0
HL3a	20⁰53'541'' 107⁰01'318	8	8.23	-88.4	29.1	20.4	10^2	10^2	10^2	10^5	10^3	10^4	10^6	10^1	0	4	0
HL3b			8.23	-88.2	29.2	20.5	10^2	10^2	10^4	10^4	10^3	10^5	10^4	10^1	10	0	0
HL3c							10^1	10^1	10^3	10^3	10^2	10^4	10^4	10^2	0	0	0
HL4a	20⁰51'598'' 107⁰03'691	15	8.25	-90.1	29.2	20.2	10^3	10^3	10^5	10^6	10^4	10^4	10^4	0	5	0	6
HL4b			8.25	-90.7	29.3	20.2	10^1	10^1	10^4	10^5	10^3	10^1	10^3	10^1	7	0	3
HL4c							10^2	10^2	10^5	10^4	10^2	10^4	10^4	10^2	0	0	0
HL5a	20⁰51'220'' 107⁰06266	16	8.26	-90.9	29.2	20.1	10^2	10^2	10^3	10^6	10^3	10^4	10^6	0	4	0	0
HL5b			8.26	-91.2	29.3	20.1	10^1	10^1	10^2	10^5	10^2	10^5	10^3	10^1	1	0	0
HL5c							10^2	10^2	10^2	10^4	10^2	10^4	10^6	10^3	0	2	0
HL6a	20⁰50'574'' 107⁰07'085	15	8.27	-91.4	29.3	20.0	10^2	10^2	10^2	10^6	10^3	10^2	10^4	10^1	0	0	0
HL6b			8.27	-92.0	29.3	20.0	10^4	10^3	10^1	10^5	10^3	10^4	10^4	10^1	0	0	5
HL6c							10^2	10^2	10^3	10^4	10^1	10^5	10^4	10^2	0	0	0
HL7a	20⁰51'709'' 107⁰06'839	16	8.27	-91.8	29.2	20.1	10^2	10^2	10^2	10^6	10^4	10^4	10^6	0	0	1	0
HL7b			8.27	-91.6	29.2	20.1	10^1	10^1	10^4	10^5	10^3	10^5	10^4	0	0	5	0
HL7c							10^2	10^2	10^5	10^4	10^2	10^4	10^4	10^5	0	7	0
HL8a	20⁰53'459'' 107⁰06'17	16	8.27	-91.6	29.2	20.3	10^3	10^3	10^3	10^5	10^3	10^4	10^4	10^1	3	5	0
HL8b			8.27	-91.6	29.2	20.3	10^2	10^2	10^3	10^5	10^2	10^4	10^3	10^1	6	0	0
HL8c							10^2	10^2	10^3	10^4	10^2	10^4	10^2	10^2	0	10	0
HL9a	20⁰55'607'' 107⁰05'288	5,5	8.25	-89.7	29.0	20.7	10^2	10^2	10^3	10^5	10^3	10^5	10^6	0	6	55	13
HL9b			8.24	-89.0	29.2	20.9	10^2	10^2	10^4	10^4	10^2	10^5	10^3	10^1	5	5	1
HL9c							10^1	10^1	10^4	10^3	10^2	10^5	10^5	10^4	0	0	0
HL10a	20⁰56'771'' 107⁰02'954	6	8.19	-86.1	28.9	21.2	10^1	10^1	10^3	10^5	10^4	10^4	10^6	10^1	10	6	3
HL10b			8.18	-85.2	29.0	21.3	10^1	10^1	10^4	10^5	10^3	10^5	10^2	10^2	2	0	10
HL10c							10^1	10^1	10^3	10^5	10^2	10^4	10^5	10^3	0	0	0

Analyzing samples taken from Dung Quat bay

Table 2. Useful and harmful bacteria in Dung Quat bay

Sample	Site	pH	Eh (mV)	Depth (m)	T (°C)	NaCl (‰)	Aerobic bacteria	HC-utilizing bacteria	Nitrate reduction	Nitrification	SRB
DQ 1a	15°24'803" 108°47'686"	8.04	-85	0	24.2	34.2	10^7	10^5	10^5	0	10^1
DQ 1b		8.15	-75	4	23.5	34.4	10^6	10^5	10^5	0	10^1
DQ 1c				TT			10^5	10^2	10^5	0	10^4
DQ 2a	15°24'212" 108°46'921"	8.09	-104	0	23.7	33.6	10^7	10^6	10^2	0	10^1
DQ 2b		8.14	-91	5.2	24.0	33.6	10^6	10^3	10^3	0	10^1
DQ 2c				TT			10^5	10^3	10^2	0	10^4
DQ 3a	15°23'426" 108°46'512"	8.21	-92	0	25.5	26.5	10^6	10^6	10^5	0	10^1
DQ 3b		8.32	-79	1.5	25.1	26.5	10^6	10^3	10^3	0	10^1
DQ 3c				TT			10^5	10^3	10^3	0	10^4
DQ 4a	15°23'572" 108°47'393"	8.10	-132	0	24.8	34.2	10^7	10^5	10^3	0	0
DQ 4b		8.35	-120	2	23.3	34.2	10^6	10^1	10^3	0	0
DQ 4c				TT			10^5	10^1	10^1	0	10^4
DQ 5a	15°24'346" 108°48'302"	8.28	-160	0	23.9	34.3	10^7	10^6	10^2	10^3	0
DQ 5b		8.36	-130	1	24.3	34.0	10^6	10^2	10^2	0	10^1
DQ 5c				TT			10^4	10^2	10^2	0	10^5
DQ 6a	15°24'424" 108°48'275"	8.39	-93	0	25.8	34.0	10^7	10^6	10^4	0	10^1
DQ 6b		8.40	-78	1.5	25.8	34.3	10^5	10^4	10^3	0	10^1
DQ 6c				TT			10^5	10^2	10^2	0	10^5

Table 3. Pathogenic bacteria in Dung Quat bay

Station	Site	Sample	Depth (m)	Unit	Coliform	*E.coli*	S-S*	*Vibrio*
DQ-1	15°24'803	DQ-1a	0m	CFU/ml	1.2×10^1	0	0	0.4×10^1
	108°47'686	DQ-1b	4m	CFU/ml	1.1×10^1	0	0	0.1×10^1
		DQ-1c	TT	CFU/g	0.9×10^1	0	0	0.2×10^1
DQ-2	15°24'212	DQ-2a	0m	CFU/ml	0.1×10^1	0	0	0.2×10^1
	108°46'921	DQ-2b	5m	CFU/ml	0.3×10^1	0.1×10^1	0	0
		DQ-2c	TT	CFU/g	1.0×10^1	0	0	0
DQ-3	15°23'426	DQ-3a	0m	CFU/ml	2.8×10^1	0.2×10^1	0	0
	108°46'512	DQ-3b	1.5m	CFU/ml	0	0	0	0
		DQ-3c	TT	CFU/g	1.2×10^1	0	0	2.6×10^1
DQ-4	15°23'572	DQ-4a	0m	CFU/ml	0.1×10^1	0	0	0.2×10^1
	108°47'393	DQ-4b	2m	CFU/ml	2.1×10^1	0	0	1.6×10^1
		DQ-4c	TT	CFU/g	0.4×10^1	0	0	0.0
DQ-5	15°24'247	DQ-5a	0m	CFU/ml	0.5×10^1	0	0	0.2×10^1
	108°48'374	DQ-5b	1m	CFU/ml	1.5×10^1	0	0	0.2×10^1
		DQ-5c	TT	CFU/g	9.9×10^1	0	0	1.0×10^1
DQ-6	15°24'346	DQ-6a	0m	CFU/ml	0.1×10^1	0	0	0.8×10^1
	108°48'302	DQ-6b	1.5m	CFU/ml	1.8×10^1	0	0	0.7×10^1
		DQ-6c	TT	CFU/g	1.6×10^1	0	0.1×10^1	2.7×10^1

Note: (S-S*): *Salmonella - Shigella*; TT: sediment

Dung Quat is also one of central bay in Vietnam where there are huge aquaculture areas. The first oil refinery plant is being built there and its operation is coming soon. Number of aerobic bacteria was higher than that of anaerobic (10^4-10^7 CFU/ml compared to 10^1-10^4CFU/ml), especially in the water samples. Number of useful bacteria (hydrocarbon-utilizing bacteria and nitrate reduction) was rather high, even in the sediment samples. Although SRB is only 10^1CFU/ml, it showed that water in this area started to be polluted. In addition, the presence of pathogenic bacteria also showed that water should be treated

Analyzing samples taken from Quinhon bay

The highest microbial number was of aerobic bacteria (10^4-10^7 CFU/ml); the lowest of that was of nitrification bacteria (0-10^2 CFU/ml). Numbers of harmful bacteria (SRB) were different in levels. In the same station, SRB in the surface water was less than that in floor. In contrast, aerobic and hydrocarbon-utilizing bacteria in the surface water were more than that in sediment. Within Quinhon bay, SRB number was higher than in the control sample, very far from beach where there were not SRB. This result showed that water in Quinhon bay was polluted. At the same time, this pollution was also documented by pathogenic bacteria analysis.

Analyzing 18 samples showed that QN-1, QN-2, QN-3, samples taken far from beach as control, had pathogenic bacteria quantity lower than that in QN-4, QN-5, QN-6, samples taken from aquaculture area. Coliform existed in all of samples with 10^1-10^2 CFU/ml (the highest is 1.4 x10^2 CFU/ml in sample QN-5c). Especially, *E.coli* and Salmonella-Shigella presented in almost samples taken within Quinhon bay, whereas they were rarely observed in samples near Culaoxanh, far from beach. Therefore, Quinhon bay is polluted not only by SRB but also by pathogenic bacteria.

Table 4. Pathogenic bacteria in Quinhon bay

Station	Site	Depth (m)	Sample	Layer	Unit	Coliform	*E.coli*	S-S*	*Vibrio*
QN-1	13°44'276	13m	QN-1a	0m	CFU/ml	1.3 x10^1	0	0	0
	109°15'881		QN-1b	6m	CFU/ml	0.2 x10^1	0	0	0
			QN-1c	TT	CFU/g	1.4 x10^1	0	0	3.5 x10^1
QN-2	13°45'378	8.3m	QN-2a	0m	CFU/ml	1.2 x10^1	0.1 x10^1	0	0.3 x10^1
	109°16'582		QN-2b	4m	CFU/ml	0.4 x10^1	0	0.2 x10^1	0.3 x10^1
			QN-2c	TT	CFU/g	5.9 x10^1	3.0 x10^1	4.2 x10^1	1.9 x10^2
QN-3	13°45'559	6m	QN-3a	0m	CFU/ml	0.7 x10^1	0	0	0.5 x10^1
	109°14'568		QN-3b	3m	CFU/ml	1.2 x10^1	0	0	0.3 x10^1
			QN-3c	TT	CFU/g	4.0 x10^1	0	0	2.7 x10^1
QN-4	13°46'287	8.5m	QN-4a	0m	CFU/ml	3.4 x10^1	0.2 x10^1	0.5 x10^1	1.5 x10^1
	109°15'158		QN-4b	4m	CFU/ml	3.2 x10^1	0.2 x10^1	0.2 x10^1	0.2 x10^1
			QN-4c	TT	CFU/g	5.6 x10^1	0.2 x10^1	0.7 x10^1	1.6 x10^2
QN-5	13°47'963	3m	QN-5a	0m	CFU/ml	6.8 x10^1	0.1 x10^1	0.1 x10^1	0.4 x10^1
	109°14'850		QN-5b	1.5m	CFU/ml	1.4 x10^2	0	0	0.6 x10^1
			QN-5c	TT	CFU/g	8.9 x10^1	0.1 x10^1	0.1 x10^1	1.9 x10^1
QN-6	13°50'581	2m	QN-6a	0m	CFU/ml	0.6 x10^1	0	0	1.3 x10^1
	109°13'630		QN-6b	1m	CFU/ml	0.5 x10^1	0.4 x10^1	0.5 x10^1	1.4 x10^1
			QN-6c	TT	CFU/g	3.1 x10^1	0.2 x10^1	0.7 x10^1	5.2 x10^1

Note: (S-S*): *Salmonella - Shigella*; TT: sediment

Table 5. Useful and harmful bacteria in Quinhon bay

Sample	Site	pH	Eh	Depth (m)	T (^{0}C)	NaCl (‰)	Aerobic bacteria	HC-utilizing bacteria	Nitrate reduction	Nitrification	SRB
QN 1a		8.16	171	0	29.5	39	10^6	10^4	10^3	0	0
QN 1b	$13^044'276''$ $109^015'881''$	8.2	160	6.5	28.8	38.8	10^5	10^2	10^3	0	0
QN 1c				TT			10^4	0	10^4	0	10^4
QN 2a		8.1	149	0	27.9	34.8	10^7	10^5	10^6	0	10^2
QN 2b	$13^045'378''$ $109^016'582''$	8.2	144	4	28	34.9	10^5	10^5	10^2	10^2	10^2
QN 2c				TT			10^4	0	10^4	0	10^5
QN 3a		8.06	143	0	29.2	34.3	10^5	10^3	10^2	10^2	10^1
QN 3b	$13^045'559''$ $109^014'568''$	8.2	139	3	27.7	34.6	10^4	10^2	10^3	0	10^1
QN 3c				TT			10^4	10^2	10^5	0	10^4
QN 4a		8.05	137	0	29.7	34	10^7	10^2	10^2	10^2	10^1
QN 4b	$13^046'287''$ $109^015'158''$	8.22	138	4	27.8	34.6	10^6	10^1	10^2	0	10^1
QN 4c				TT			10^6	10^2	10^6	0	10^2
QN 5a		8.13	-19	0	28.6	33.8	10^6	0	10^2	10^2	10^2
QN 5b	$13^047'693''$ $109^014'850''$	8.18	-52	1.5	29.2	34.1	10^6	10^3	10^5	0	10^2
QN 5c				TT			10^5	10^2	10^4	10^2	10^4
QN 6a		8.06	-45	0	29.1	27.9	10^5	10^3	10^3	10^2	10^1
QN 6b	$13^050'581''$ $109^013'630''$	8.1	-46	1	29.3	28.1	10^5	10^1	10^3	0	10^2
QN 6c				TT			10^4	10^3-10^4	10^4	10^1	10^7

The ability to use some useful isolates in aquaculture

Among bacteria strains isolated, some of them were able to convert nitrogenous compounds and to resist to pathogenic bacteria. These strains can be applied to make product using in aquaculture water treatment. Some predominant strains were classified as *Bacillus subtilis*, *Nitrosomonas* sp., *Nitrobacter* sp., *Nitrococcus* sp.
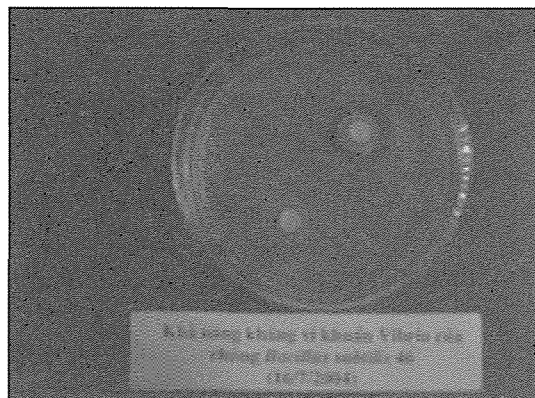

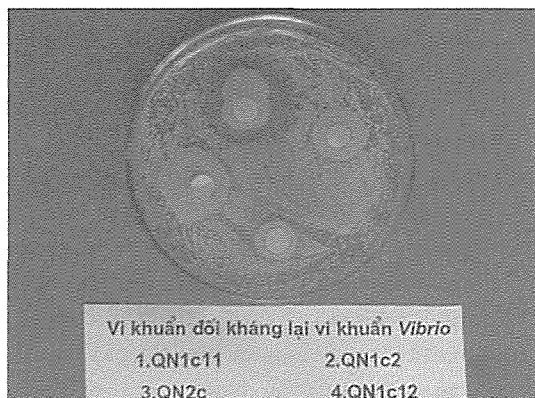Fig 1. *Bacillus subtilis* resisted *Vibrio*
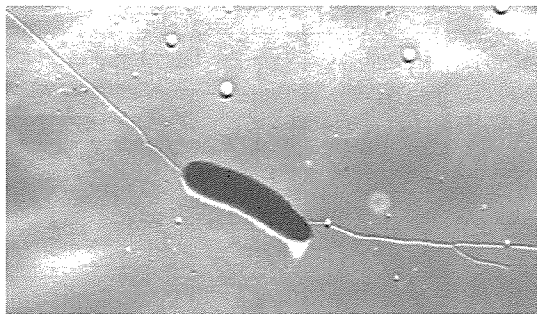

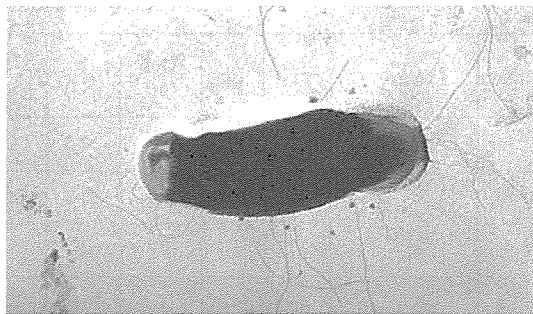Fig 2. *Bacillus cereus* resisted *Vibrio*

Fig 3. *Bacillus* sp.   Fig 4. *Bacillus cereus*

From the above bays, we isolated some biosurfactant-producing bacteria such as *Pseudomonas, Rhodotorula, and Bacillus*. These strains are needed to study more to apply in oil-contaminated treatment.

CONCLUSION

1. Analysis result of 66 samples taken from three bays Halong, Dung Quat and Quinhon showed that:

2. Number of useful bacteria was rather high, 10^5-10^6 CFU/ml (*Nitrosomonas, Nitrobacter, Nitrococcus, Bacillus, Lactobacillus, Pseudomonas, Sphingomonas, Rhodotorula, Candida*).

3. Number of sulfate-reducing bacteria increased in water samples taken from aquaculture area (10^4-10^7CFU/ml in sediment, 10^1-10^3 CFU/ml in surface water).

4. Pathogenic bacteria *Salmonella, Shigella, E.coli* and *Vibrio* also existed in polluted area. In some samples, number of *E.coli* was 3×10^1 CFU/ml; *Salmonella* and *Shigella* were $4,2.10^1$ CFU/ml; *Vibrio* was $5,5.10^1$ CFU/ml).

5. Isolated more than 50 SRB strains from three bays, most of them are rod.

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