Hydrobiologia (2009) 632:107–126
DOI 10.1007/s10750-009-9831-8

PRIMARY RESEARCH PAPER

Comparison of bacterioplankton communities in three mariculture ponds farming different commercial animals in subtropical Chinese coast

Chaoling Wei · Yonghui Zeng · Kunxian Tang · Nianzhi Jiao

Received: 18 June 2008 / Revised: 3 April 2009 / Accepted: 23 May 2009 / Published online: 15 June 2009

Abstract In order to explore the responses of the bacterioplankton community to different types of aquaculture environments, three mariculture ponds comprised of groupers (*Epinephelus diacanthus*, ED), prawns (*Penaeus vannamei*, PV), and abalone (*Haliotis diversicolor* supertexta, HDS) in southeast, coastal China were investigated. The free-living bacterial diversity was analyzed through the construction of 16S rDNA clone library. A total of 203 16S rDNA sequences from three clone libraries were classified into 118 operational taxonomic units (OTUs), of which 51, 31, and 42 OTUs were distributed in the ED, PV, and HDS pond, respectively, with *Bacteroidetes* (30.6%), *Actinobacteria* (55.2%), and *Cyanobacteria* (32.8%) as the dominant division in the respective ponds. Meanwhile, each pond occupied some unique OTUs that were affiliated with uncommon (sub-)phyla, such as candidate OP11 division, *Acidobacteria*, *Deltaproteobacteria*, *Planctomycetes*, and *Verrucomicrobia*. Bacterial diversity in the ED pond was the richest, followed by the HDS and the PV pond. OTUs of 61.9% and 94.9% have less than 90% and 97% similarity to their nearest neighbors in public databases, respectively. All OTUs were grouped into 67 clusters, covering 11 (sub-)phyla. The OTUs only from single pond distributed in 53 clusters (79.1%), the OTUs shared by two ponds were affiliated with 14 clusters (20.9%), and none of clusters was formed by the OTUs which commonly originated from the three pond libraries, suggesting that the composition of bacterial populations in these ponds were significantly different. These results indicate that the aquatic environment created by different mariculture animals may foster very special and complex bacterial communities.

Keywords Bacterial community composition · Free-living · Diversity · 16S rDNA · Clone library · Mariculture pond · Coastal environment

Handling editor: David Philip Hamilton

C. Wei
School of Resources and Environment, Anhui Agricultural University, 230036 Hefei, Anhui Province, People's Republic of China
e-mail: weichl@ahau.edu.cn

Y. Zeng
Guangdong Provincial Key Laboratory of Pathogenic Biology and Epidemiology for Aquatic Economic Animals, Guangdong Ocean University, 524025 Zhanjiang, Guangdong Province, People's Republic of China
e-mail: marinezeng@gmail.com

K. Tang · N. Jiao (✉)
State Key Laboratory of Marine Environmental Science, Xiamen University, 361005 Xiamen, People's Republic of China
e-mail: jiao@xmu.edu.cn

Introduction

With the rapid expansion of mariculture in coastal areas throughout the world, various ponds and enclosures used in mariculture are changing the coastal environments. The ecological consequences of mariculture have become an important research area (La Rosa et al., 2002; Feng et al., 2004). Due to increased nutrient loads, especially organic and inorganic phosphorus and nitrogen that sometimes induce eutrophication, mariculture has a profound impact on the biogeochemistry of the water column and sediment in mariculture zones (Qian et al., 2001; Souchu et al., 2001; La Rosa et al., 2002; Mantzavrakos et al., 2007; Sarà, 2007). However, up to now, information on the impact of mariculture on the bacterial community composition in the water column is scant, except for studies concerned with the effects on bacterial physiological characteristics (Caruso et al., 2003) and density (Pitta et al., 2006).

Bacterial communities play a significant role in carbon and nutrient cycling of aquatic ecosystems. Aquatic bacteria redistribute dissolved organic matter, increase nutrient availability, and affect many other ecological processes (Gonzalez et al., 2000; Cotner & Biddanda, 2002). At the same time, these bacteria are sensitive to changes in environmental conditions. Their community structure and diversity vary with food sources, substrate availability, and other biochemical parameters. Based on previous comparative analysis focusing on bacterial communities in lakes (Haukka et al., 2006) and controlled mesocosm experiments in seawater (Schäfer et al., 2001; Carlson et al., 2002; Øvreås et al., 2003), certain taxa of bacteria out-compete other species under the prevailing specific physical and environmental conditions. The factors that determine bacterial composition in aquaculture are likely to include cultured animal species, culture methods, feed composition, fecal excretion habits, and feeding techniques. Amongst other factors, fish, shrimp, and shellfish culture lead to different bacterial co-habitants. For example, a comparative study of fish farming and non-farming environments demonstrated that the bacteria in fish-farm sediments are predominantly Gram-negative, while the bacteria in the non-farming reference site of the same study are largely Gram-positive (Vezzulli et al., 2002). It is known that there are specific bacterial populations associated with cultured shrimps, scallop larvae, and halibut (Lau et al., 2002; Sandaa et al., 2003; Jensen et al., 2004). Therefore it is likely that there will be differences in bacterial community structure for ponds farming with different mariculture animal species. In addition, previous reports selected culture ponds from different locations, thus, the bacterial communities investigated are under the influence of both culture and environmental conditions. Investigations of bacterial communities in the same geological locations but with different aquaculture species have not previously been carried out. This information is important to understand the establishment of a bacterial community and its environmental impacts, and to shed light on the consequences of mariculture on ecosystems, and the intricate interactions between the bacteria and cultured animals.

This study was designed to investigate the effects of different mariculture species on the composition of bacterial communities in three adjacent ponds where grouper, prawn, and abalone, respectively, have been farmed for over 5 years. We aim to reveal whether there are differences in marine planktonic bacterial composition after their long-time adaptation in these ponds. Various physicochemical parameters, e.g., nutrients, chlorophyll *a*, and bacterial abundance were measured to examine relationships between bacterial composition and environmental variables in each mariculture pond.

Materials and methods

Sample collection

All the three mariculture ponds are located in the Dongshan mariculture zone, north of Dongshan Island in the southeast coastal region of China (23°45′ N, 117°22′ E; southwest of the Taiwan Strait) (Fig. 1). Each pond is approximately 100 m long, 85 m wide with a mean depth of 1.5 m. The dominant ocean current around the ponds is via Dongshan Bay which belongs to the southeast coastal water system of China. This region has over 5 years of commercial marine culture history, including groupers (*Epinephelus diacanthus*, ED), prawns (*Penaeus vannameI*, PV), and abalones (*Haliotis diversicolor* supertexta, HDS).

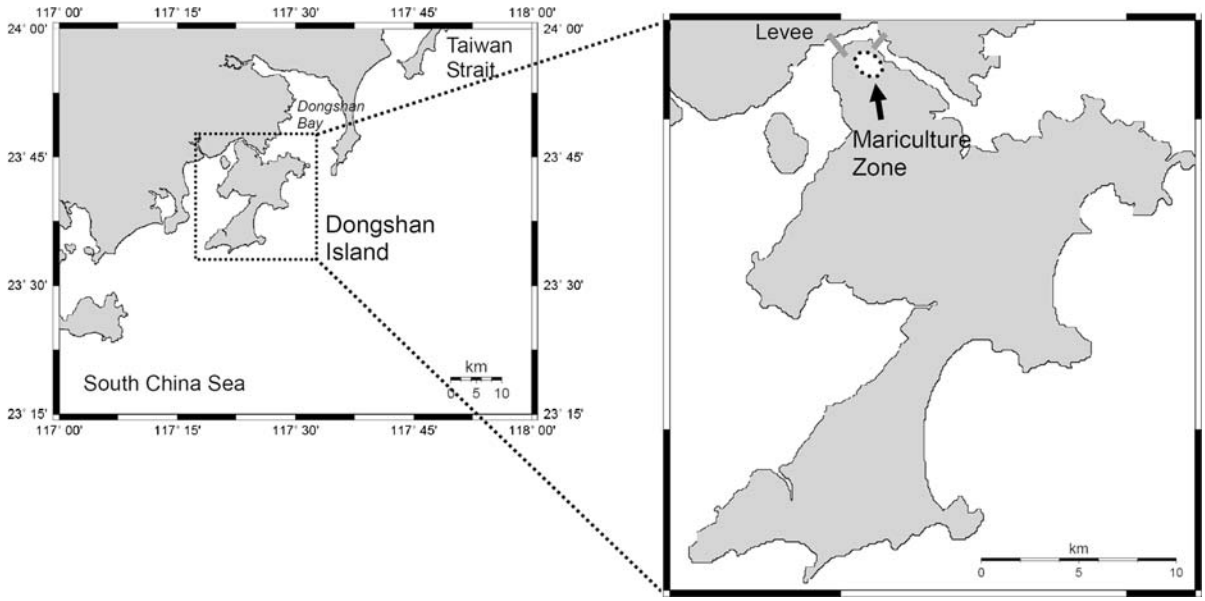


Fig. 1 Location of study area and sampling site

Food for groupers is composed of fish powder (60%), corn powder (15%), fish oil (5%), and the other remaining fraction is made up of vitamins, ash, and pigments. The feed for prawns and abalone are dry fish and streptomycin (final concentration 2 ppm), and phytoplankton (*Gracilaria*), respectively.

Triplicate samples of surface water were collected from an approximate depth of 30 cm at each pond on October 12, 2004. Samples were collected in polycarbonate bottles which had been rinsed in acid once, in distilled water three times, and autoclaved. Samples were returned immediately to the lab and kept in the dark until analysis.

Bacterial counts and water quality parameters

Water for microscopic counts of bacterial abundance was preserved with buffered formaldehyde (2%). Cells were stained with 4′,6′-diamidino-2-phenylindole (DAPI, 1 μg ml^{-1} final concentration) for 10 min, and filtered onto black 0.2 μm-pore-size polycarbonate membrane filters, then analyzed by epifluorescence microscopy. At least 200 cells or a minimum of ten fields of view were counted.

Water quality parameters that were measured include pH, salinity, chlorophyll *a*, and nutrients' concentrations (ammonium, inorganic nitrogen, and inorganic phosphorus). The pH and salinity of the water samples were measured in situ with portable meters during sampling. Samples for chlorophyll *a* analysis were filtered onto 0.7-μm pore-size duplicate glass fiber filters (GF/F, Whatman) and frozen. Chlorophyll *a* was extracted with 96% ethanol and quantified spectrophotometrically following a published protocol (Jespersen & Christoffersen, 1987). Ammonium, inorganic nitrogen, and inorganic phosphorus concentrations, and chemical oxygen demand (COD) were measured using standard methods (National Oceanographic Bureau in China, 1999).

DNA extraction

Approximately 3 l of water samples for DNA extraction were pre-filtered through a nylon mesh (100-μm pore size). Samples were subsequently filtered onto a 0.22-μm pore size nylon filter (Gelman Sciences Inc). Filter samples were immediately frozen in liquid nitrogen and stored at −80°C until extraction. DNA extraction was performed according to the methods of Fuhrman et al. (1988) and Massana et al. (1997) with minor modifications. In brief, the filters were cut into pieces by a sterilized scissors, and put into a 15-ml centrifuge tube, vortexed with 2.0 ml of lysis buffer (40 mM EDTA, 50 mM Tris–HCl, 0.75 M sucrose), and incubated at −80°C for 15 min, and transferred to a 37°C incubation bath for 6 min.

Then, an additional 2.0 ml of lysis buffer with lysozyme (1.0 mg ml^{-1}) was added. Samples were then incubated at 37°C for 30 min. Proteinase K (0.2 mg ml^{-1}) and 1% of sodium dodecyl sulfate were added, followed by incubation at 55°C for 2 h. The lysate recovered by centrifugation was poured into a sterile 15-ml centrifuge tube, and 1.5 ml of 10.5 M ammonium acetate plus 10 ml of ice-cold 100% ethanol on ice were added to precipitate the DNA at −20°C for 2 h. The DNA pellets were rinsed once with phenol–chloroform–isoamyl alcohol (25:24:1), once with chloroform–isoamyl alcohol (24:1), and twice with pre-chilled 70% ethanol. The pellets were dried under vacuum, gently suspended in 40 µl of TE buffer (10 mM Tris, 1 mM EDTA, pH 7.4), and stored at −20°C. DNA quality was checked by electrophoresis on 1% agarose gel.

PCR amplification

The 16S rDNA gene fragments for the clone library were amplified using the following primers: 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). The PCR conditions included an initial denaturation at 94°C for 3 min, followed by 20 cycles of 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 3 min, a final extension at 72°C for 7 min. PCR products were separated in a 1.2% agarose gel, purified by Agarose Gel DNA Purification Kit Ver.2.0. (TaKaRa Inc., Dalian, China), and quantified using a Biophotometer spectrophotometer (Eppendorf, Germany) at 260 nm.

Construction and sequencing of 16S rDNA clone library

The purified PCR products were ligated into the pMD-18T vector (TaKaRa Inc., Dalian, China) according to the manufacturer's protocol. The ligated mixtures were transformed into *Escherichia coli* DH5α competent cells (TaKaRa Inc., Dalian, China), and plated onto LB agar plates containing ampicillin. White colonies were picked randomly. Positive clones were identified based on expected sizes (ca. 1400 bp) on 1.2% agarose gel.

A total of 226 clones were sequenced with an ABI 3700 sequencer (Applied Biosystems, USA) using the 27F primer. These clones generated 600–900 bp readable sequences. Nine of the sequences were ambiguous, and six of the sequences belonged to chloroplasts and were discarded. The remaining sequences were analyzed by the CHIMERA_CHECK program (version 2.7) from the Ribosomal Database Project II (RDP II; http://rdp.cme.msu.edu/). Eight of these clones were identified as likely chimeric sequences and were removed from subsequent analysis. For phylogenetic analyses, the remaining 203 cloned sequences were grouped into different OTUs in each library based on similarity of more than 97%.

In order to assess the clone libraries, several statistical indexes are calculated. *Coverage*, derived from the equation $Coverage = 1 - (N/Individuals)$, where N is the number of clones that occurred only once and *Individuals* is the number of total positive clones screened out in each library; the Chao1 richness estimator (Chao, 1984); the reciprocal Simpson index (S) was calculated to take both the abundance and the richness of OTUs into account (Hill et al., 2003): $S = 1/D$ and $D = \sum [n_i (n_i - 1)/N(N - 1)]$, where n_i is the number of clones in the ith OTU and N is the total number of clones in the sample; *Shannon* index, is a measure of the structural diversity of the microbial community and was calculated from the number and relative abundance of OTUs in the libraries (Odum, 1971). In order to determine if these libraries are significantly different from one another, LIBSHUFF analysis was performed (http://schloss.micro.umass.edu/software/slibshuff; Schloss et al., 2004).

Phylogenetic analysis

The processed sequences identified as above were used to estimate the degree of similarity to other 16S rDNA sequences in GenBank and to select reference sequences using BLASTN. Alignments of the sequences, together with the most similar references were calculated using CLUSTAL_X 1.8 (Thompson et al., 1997). The multiple alignments were inspected manually to remove the regions containing many gaps before phylogenetic analysis. Phylogenetic relationships were inferred by a neighbour-joining method with a Jukes-Cantor distance correction (Kuhner & Felsenstein, 1994). The trees were constructed using the MEGA4.0 package (Molecular Evolutionary Genetics Analysis, Version 4.0, available at http://www.megasoftware.net), with bootstrap analysis (500 replicates) (Tamura et al., 2007).

Nucleotide sequence accession numbers

The 16S rDNA sequences described in this study were deposited into GenBank under the accession numbers EU283111–EU283317.

Results

Chlorophyll *a*, nutrient concentrations, and bacterial abundance in the three ponds

The general indicators of water quality were analyzed from all the three ponds. Chlorophyll *a*, COD, nutrients (ammonium, inorganic nitrogen, and inorganic phosphorus), pH, and salinity are shown in Table 1. The chlorophyll *a* concentration in the PV pond was the highest (9.14 μg l^{-1}) and the lowest in the HDS pond (1.22 μg l^{-1}). Similarly, the PV pond had the highest COD concentration and the HDS pond had the lowest; however, the difference in COD between the ED and the PV pond was less significant than that of chlorophyll *a*. The maximum concentrations of ammonium and inorganic nitrogen were in the HDS pond. The maximum concentrations of inorganic nitrogen and inorganic phosphorus were in the ED pond, while the minimum concentrations of ammonium, inorganic nitrogen, and inorganic phosphorus occurred in the PV pond (Table 1). These data suggested that the trophic level was the highest in the PV pond, followed by the ED and HDS ponds. Abalones in the HDS pond were fed with big algae, such as *Gracilaria verrucosa*. Other algae in the pond can also be consumed by abalones and, therefore, chlorophyll *a* concentration in this pond is much lower than the other two ponds though there were more available nitrate and phosphates. Since the HDS pond was not added with artificial feed, the organic matter in this pond is low relative to the other two ponds and therefore, COD was accordingly low (Table 1). As expected, the salinity, pH, and temperature did not display significant differences among the three ponds (Table 1). Total numbers of bacteria in the three ponds were obtained by counting DAPI-stained cells. The bacterial abundance varied with 2.86×10^7 cells ml^{-1} in the HDS pond, 2.0×10^7 ml^{-1} in the PV pond, and 1.11×10^7 ml^{-1} in the ED pond (Table 1). No significant differences were observed on the bacterial abundance among the three ponds.

Table 1 Water chemical and biological parameters in the three mariculture ponds

	ED pond	PV pond	HDS pond
Chlorophyll *a* (μg l^{-1})	6.38	9.14	1.22
COD (mg l^{-1})	2.37	2.48	0.25
NH_3-N (mg l^{-1})	0.024	0.021	0.049
Inorganic nitrogen (mg l^{-1})	0.077	0.050	0.208
Inorganic phosphorus (mg l^{-1})	0.068	0.004	0.024
Salinity (‰)	28.7	28.4	29.6
pH	8.42	8.40	8.2
Temperature (°C)	24.7	24.6	24.5
Bacterial abundance ($\times 10^7$ cells ml^{-1})	1.11	2.0	2.89

Bacterial community composition and distribution

In order to reveal the detailed distribution and composition of the bacterial communities, the 16S rDNA was amplified by PCR with a set of bacterial general primers, and the PCR products were cloned into a sequencing vector. A total of 226 clones (81 from the grouper pond, 74 from the prawn pond, and 71 from the abalone pond) were randomly selected and sequenced. After sequence alignment and BLAST search, a total of 203 valid sequences (72 from the ED pond, 67 from the PV pond, and 64 from the HDS pond) (Table 2) were identified and grouped into 118 OTUs. Three common OTUs appeared in the ED and PV ponds, two in the ED and HDS ponds, and one in the PV and HDS ponds. None was present in all three ponds. The remaining 118 OTUs were unique to a single pond, suggesting that large differences in bacterial community composition existed among the three ponds.

The taxonomic grouping and the relative abundances of the above libraries for each pond are given in Table 2. Most clones identified belonged to the divisions of *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, *Cyanobacteria*, or *Firmicutes*. However, the libraries differed markedly at the phylum and subphylum levels. The clones representing the highest number or percentage of the ED, PV, and HDS

Table 2 Statistical analysis of clones distribution

Phylum	No. of clones			Frequencies (%)		
	ED pond	PV pond	HDS pond	ED pond	PV pond	HDS pond
Actinobacteria	10	37	2	13.9	55.2	3.0
Bacteroidetes	22	1	6	30.6	1.5	9.4
Alphaproteobacteria	20	7	19	27.8	10.4	29.7
Gammaproteobacteria	2	4	8	2.8	6.0	12.5
Cyanobacteria	15	17	21	20.8	25.4	32.8
Firmicutes	0	0	6	0	0	9.4
Planctomycetes	2	0	0	2.8	0	0
Deltaproteobacteria	1	0	0	1.4	0	0
Verrucomicrobia	0	1	0	0	1.5	0
Candidate division OP11	0	0	1	0	0	1.6
Acidobacteria	0	0	1	0	0	1.6
Total	72	67	64			

pond library were affiliated with *Bacteroidetes* (30.6%), *Actinobacteria* (55.2%), and *Cyanobacteria* (32.8%), respectively. In comparison to the high percentage of *Bacterioidetes* in the ED pond, only 1.5% and 9.4% of the clones in the PV and HDS ponds, respectively, were *Bacteroidetes*. Similarly, only 13.9% and 12.5% of the clones from ED and HDS ponds, respectively were *Actinobacteria*; whereas only 20.8% and 25.4% of the clones obtained from ED pond and PV pond, respectively, were *Cyanobacteria*.

Clones affiliated with *Alphaproteobacteria* were least abundant in the PV pond and clones affiliated with *Gammaproteobacteria* were least abundant in the ED pond. In addition, several clones were found exclusively in one of the ponds. For example, clones affiliated with *Deltaproteobacteria* and *Planctomycetes* were found only in the ED pond, *Verrucomicrobia* only in the PV pond; and OP11, *Firmicutes* and *Acidobacteria* were only found in the HDS pond (Table 2).

Richness, evenness, and diversity of bacterial communities

The richness and diversity of the bacterial communities were calculated using Chao1 estimate and reciprocal Simpson index. The Chao1 estimate, used to predict the total number of OTUs (richness) showed that the richness of species was the highest in the ED pond and the lowest in the PV pond (Table 3). The reciprocal Simpson index ranged from 8.6 to 69.1 (Table 3). Once again, the PV pond had the lowest value. Also, compared to other aquatic systems, the reciprocal Simpson index was high in the ED and HDS ponds (Table 3), suggesting an even distribution of species in these two ponds. The bacterial diversities within the main divisions were different among the three mariculture ponds. The greatest diversity was found in *Bacteroidetes* and *Alphaproteobacteria* of the ED pond, *Actinobacteria* and *Gammaproteobacteri*a of the PV pond, and *Cyanobacteria* of the HDS pond (Table 4).

Table 3 Statistical analysis of the clone libraries

	Chao1 estimate	OTUs detected	Total clones analyzed	Reciprocal Simpson index	Shannon index	Coverage
ED pond	141.2	51	72	69.1	3.78	0.57
PV pond	86.2	31	67	8.6	2.81	0.79
HDS pond	110.7	41	64	53.0	3.57	0.60

Table 4 Comparison of the diversity of bacterial sequences from the three ponds based on sequence divergence

	ED pond	PV pond	HDS pond
Actinobacteria	0.0721	0.0793	0.0011
Bacteroidetes	0.1793	–	0.1435
Alphaproteobacteria	0.2775	0.1437	0.1318
Gammaproteobacteria	0.1716	0.1943	0.1789
Cyanobacteria	0.1315	0.0724	0.2525

Clone library analysis

Different patterns of diversity in clone libraries were displayed using coverage curves (Fig. 2). Based on the coverage curves, the ED and HDS libraries were similar but more diverse than the PV library, similar to the results of the Shannon index based on the analyses of clone libraries (Table 3). Based on the LIBSHUFF analysis, these libraries were significantly different from each other (P-values < 0.001). In addition, in a pair-wise comparison, the Delta-C values along the evolutionary distance (<0.3) were much higher than the values predicted for coverage curves ($P = 0.05$) (Fig. 3), indicating a low overlap between any two libraries.

Phylogenetic analysis

The distribution of the bacteria in the three ponds was analyzed by phylogenetic analysis. Thirty-two OTUs affiliated with the *Alphaproteobacteria* could be assigned to 18 clusters (Fig. 4A). Out of these 32, the alpha-a, c, h, and k clusters were the only groups containing OTUs from the ED pond. The three clusters contained sequences from seawater or coastal water. Interestingly, a relative of *Loktanella* isolate (*Rhodobacteraceae*) was found in the alpha-h cluster which has been shown previously to be able to grow on marine agar (Ivanova et al., 2005). Alpha-b, e, f, and m–r clusters contained OTUs retrieved only from the HDS pond. In the alpha-q cluster, a sequence (GenBank accession no. FJ203135) originating from black band diseased coral tissues was found. Alpha-j cluster with OTUs from the ED and PV ponds contained a sequence affiliated with *Roseobacteraceae*; whereas another cluster alpha-g containing clones from the ED and PV pond clustered with a sequence affiliated with *Erythrobacteracea*e. The majority of clusters, including OTUs shared by each two ponds (ED and HDS, ED and PV, or PV and HDS ponds), consisted of sequences from different environments (seawater, salt marsh, and coastal sediment).

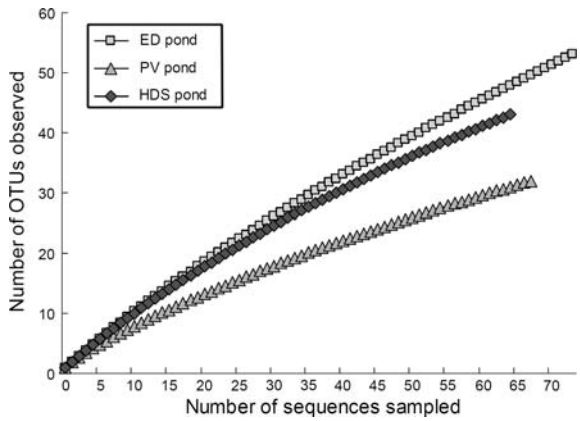


Fig. 2 Rarefaction curves generated for 16S rDNA gene sequences in clone libraries

Thirteen OTUs associated with the *Gammaproteobacteria* fell into seven clusters (Fig. 4B). Gamma-c and -a clusters with OTUs only from the HDS pond contained sequences retrieved from seawater and asphalt, respectively. A cloned sequence in gamma-c cluster was distantly related (91% identity) to the sequence of a clone obtained from gills of *Bankia setacea* (Sipe et al., 2000), whereas gamma-d cluster containing OTUs only from the PV pond included a sequence from soil.

The OTUs belonging to the *Actinobacteria* were grouped into seven clusters in this study (Fig. 4C), with the exception of act-a cluster containing one OTU from the HDS pond and act-b and d containing one OTU from the PV pond, all other clusters consisted of the OTUs shared by the ED and PV pond. OTUs from the PV pond occurred mainly in these clusters. However, none of the clusters consisted of OTUs from all the three ponds. The cluster containing OTUs from only the ED pond was not found. In addition, some clusters included species from a wide range of environments. For example, clusters act-c and act-f, and act-g included sequences from seawater and coastal water; act-e contained a sequence from brackish pond; act-b contained a sequence from sponge tissue, and act-a contained a sequence from grassland soil.

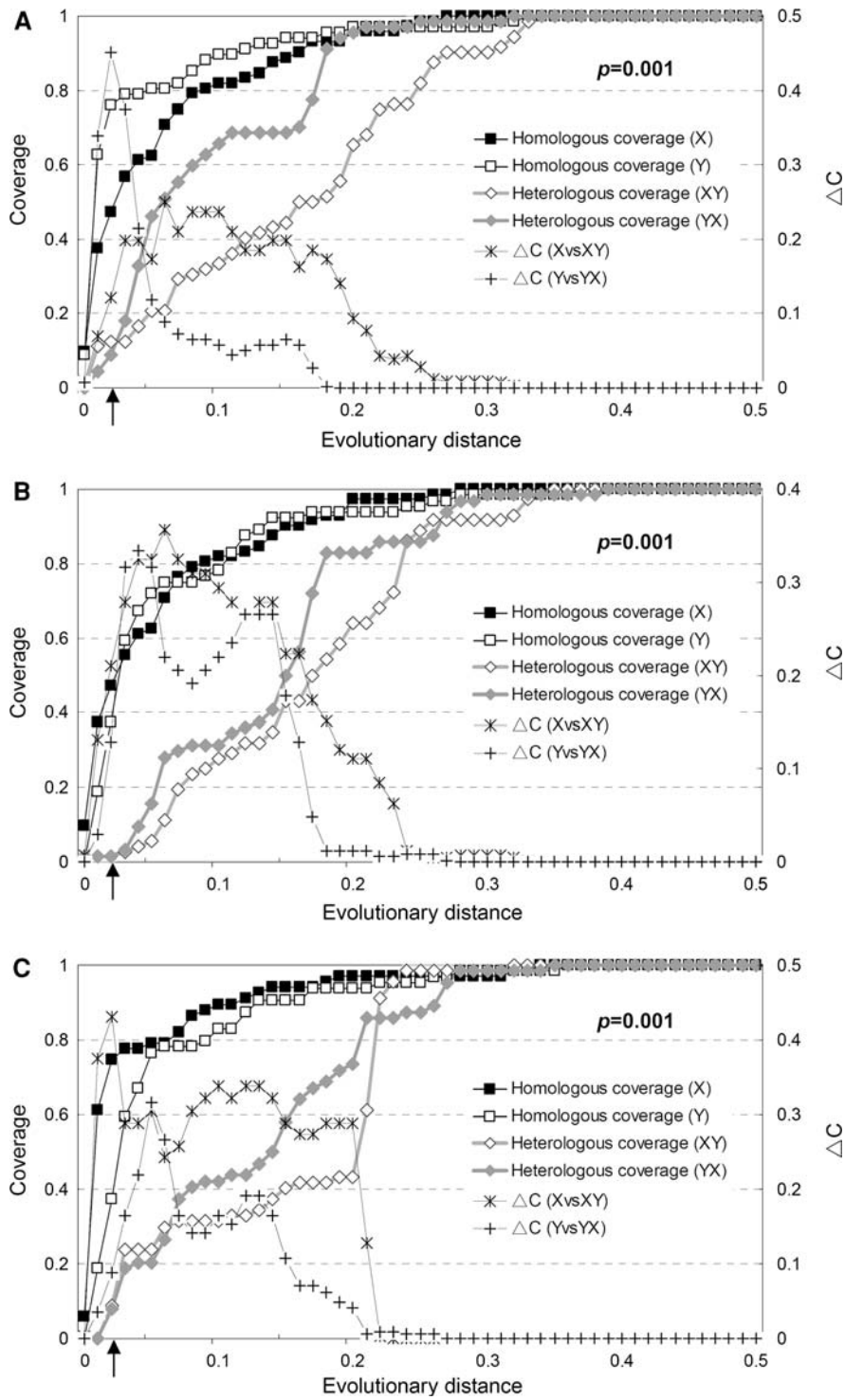


Fig. 3 LIBSHUFF pairwise comparisons of the homologous coverage and the heterologous coverage of the three libraries. The number of sequences from ED, PV, and HDS pond library, which were used for the comparison, were 72, 67, and 64, respectively. Libraries were considered significantly different when $P < 0.025$, whereas the *P*-values in these comparisons were less than 0.001 (Singleton et al., 2001). **A** Coverage for ED pond versus PV pond. **B** Coverage for ED pond versus HDS pond. **C** Coverage for PV pond versus HDS pond. *Arrows* indicate 0.03 sequence distance (i.e., 97% similarity)

A total of 23 OTUs (mainly from the ED pond) affiliated with *Bacteroidetes* were grouped into 18 clusters (Fig. 4D). Fourteen of them (bac-a to e and k to m, bac-h, i, and o) contained the OTUs only from the ED pond. Bac-o cluster was characterized by sequences affiliated with one *Flexibacteraceae*

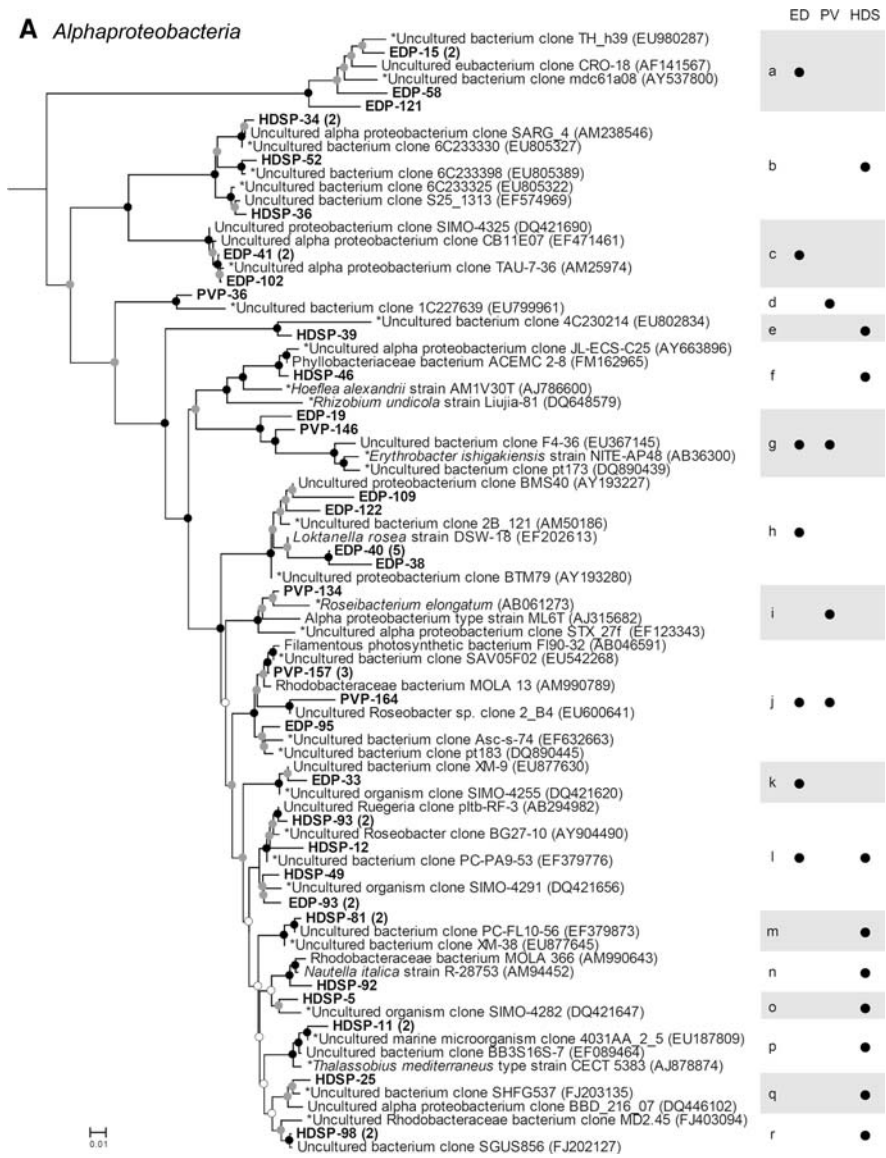
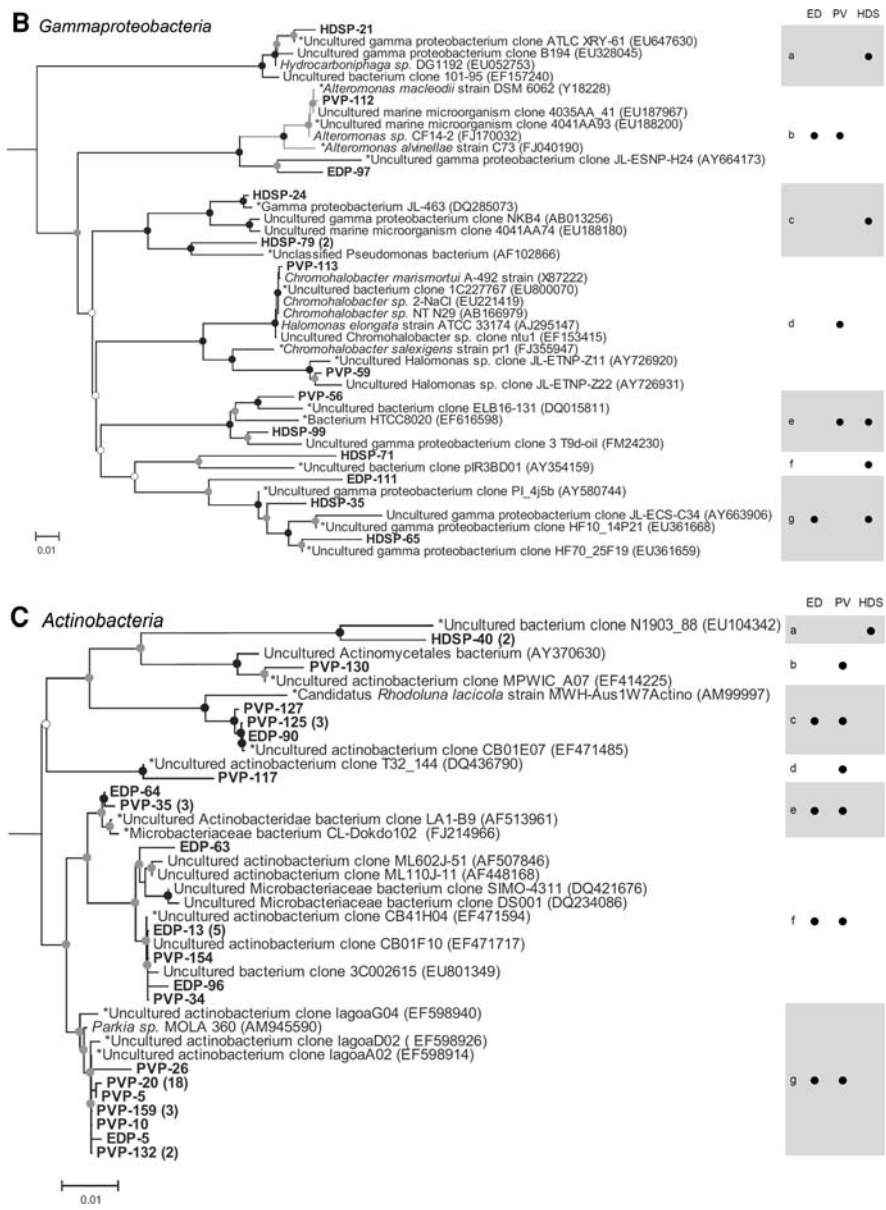


Fig. 4 Phylogenetic trees based on analysis of the partial 16S rDNA sequences obtained from the three clone libraries of ED pond (EDP-*), PV pond (PVP-*) and HDS pond (HDSP-*). Numbers of clones in each OTU are given in *parentheses*. Bootstrap values above 90%, 70%, and below 70% are marked with *black*, *gray*, and *open circles*, respectively. The closest neighbors of each OTU in GenBank are marked with *asterisks*. The *scale bars* indicate the estimated sequence divergence. Listed right of the tree are the cluster numbers and their distribution in ponds. Separate phylogenetic trees are shown for (**A**) *Alphaproteobacteria*; (**B**) *Gammaproteobacteria*; (**C**) *Actinobacteria*; (**D**) *Bacteroidetes*; (**E**) *Cyanobacteria*; (**F**) minor groups, including *Acidobacteria*, *Deltaproteobacteria*, *Firmicutes*, Candidate division OP11, *Planctomycetes*, and *Verrucomicrobia*

bacterium obtained from a marine dinoflagellate (*H. circularisquama*) and from coastal waters. Bac-m cluster was represented by a sequence affiliated with the family *Flavobacteriaceae*, from an isolate identified as UST030701-295, which was first isolated from the surface of a sponge and is able to grow on marine agar (Lau et al., 2005). Bac-o had a sequence from the same family but was isolated from marine sediment (Khan et al., 2007). Bac-h and -i clusters contained sequences closely related to the environmental clones retrieved from mangrove soil, coastal water, and coral tissues. In addition, bac-f and j, and bac-n clusters contained OTUs only from the HDS and PV pond, respectively. They harbored sequences from bacteria of seawaters. The OTUs shared by the ED and HDS pond formed one cluster, bac-g, characterized by sequences found in coastal water bacteria.

Twenty-eight OTUs belonging to *Cyanobacteria* fell into 11 clusters. The OTUs shared by the HDS and PV ponds formed two clusters, cya-a and cya-b. The former contained a sequence clustered with strain RS9916 that belonged to the family *Chroococcales* from seawater; the latter included some sequences of the same family but from lakes and estuaries. Four OTUs from the ED pond and four

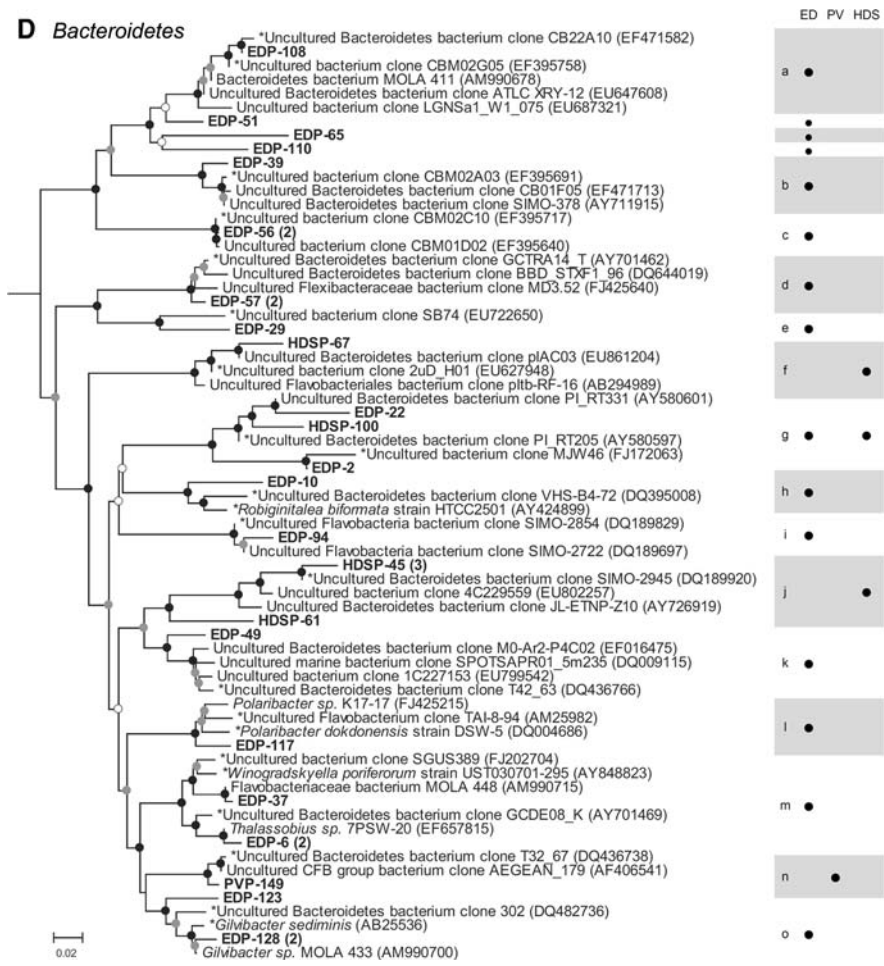
Fig. 4 continued



from the HDS pond formed the cya-k cluster, which included clones closely related to the environmental sequences from salt marsh sediment. The OTUs originating only from the ED pond were grouped into three clusters (cya-e, h, and i) which were characterized by sequences of uncultured organisms from lake water, soils, and salt marsh. The OTUs only from the HDS pond grouped into clusters cya-f, g, and j, which were characterized by the environmental sequences from aquatic systems (Fig. 4E).

Finally, phylogenetic analysis showed that one of the three remaining OTUs only from the ED pond was affiliated with *Deltaproteobactera*; the other two were assigned to *Planctomycetes* whose representative clones matched *Schlesner* 664 from compost heap and *Schlesner* 130 from seawater. Two OTUs that were distantly related (86% to 91% identity) to *Acidobacteria* and OP11, respectively, and one OTU belonging to *Firmicutes* was retrieved only from the HDS pond. However, one OTU that was ascribed to

Fig. 4 continued



Verrucomicrobia was only found in the PV pond (Fig. 4F).

Discussion

Previous studies have demonstrated that different cultured animals in mariculture have significant impact on aquatic environments (Qian et al., 2001; La Rosa et al., 2002; Vezzulli et al., 2002). It is well known that microbial communities may respond to the changes in substrate supply by either physiological adaptation or alterations in the community composition. Our results show that there are large differences in bacterial compositions in the three mariculture ponds, and the differences in the water samples were primarily associated with the distributions of the different phylotypes.

In this study, the clone libraries from the ED, PV, and HDS pond were dominated by the heterotrophic bacterial sequences affiliated with *Bacteroidetes*, *Actinobacteria*, and *Alphaproteobacteria*, respectively (Table 2). The difference in the distribution of dominant phyla might be explained by the differences in animal feed for the three mariculture animals. The leftover feed for ED was mainly comprised of fish and corn powders, which could be decomposed to complex organic polymers and high molecular weight dissolved organic matter (DOM). Some members of *Bacteroidetes* are known to play an important role in the degradation and consumption of this material (Cottrell & Kirchman, 2000; Kirchman, 2002). The phyla *Bacteroidetes* is dominated by anaerobes which are commonly members of gut microflora (Humayoun et al., 2003). In contrast, HDS pond was fed with *Gracilaria*, which

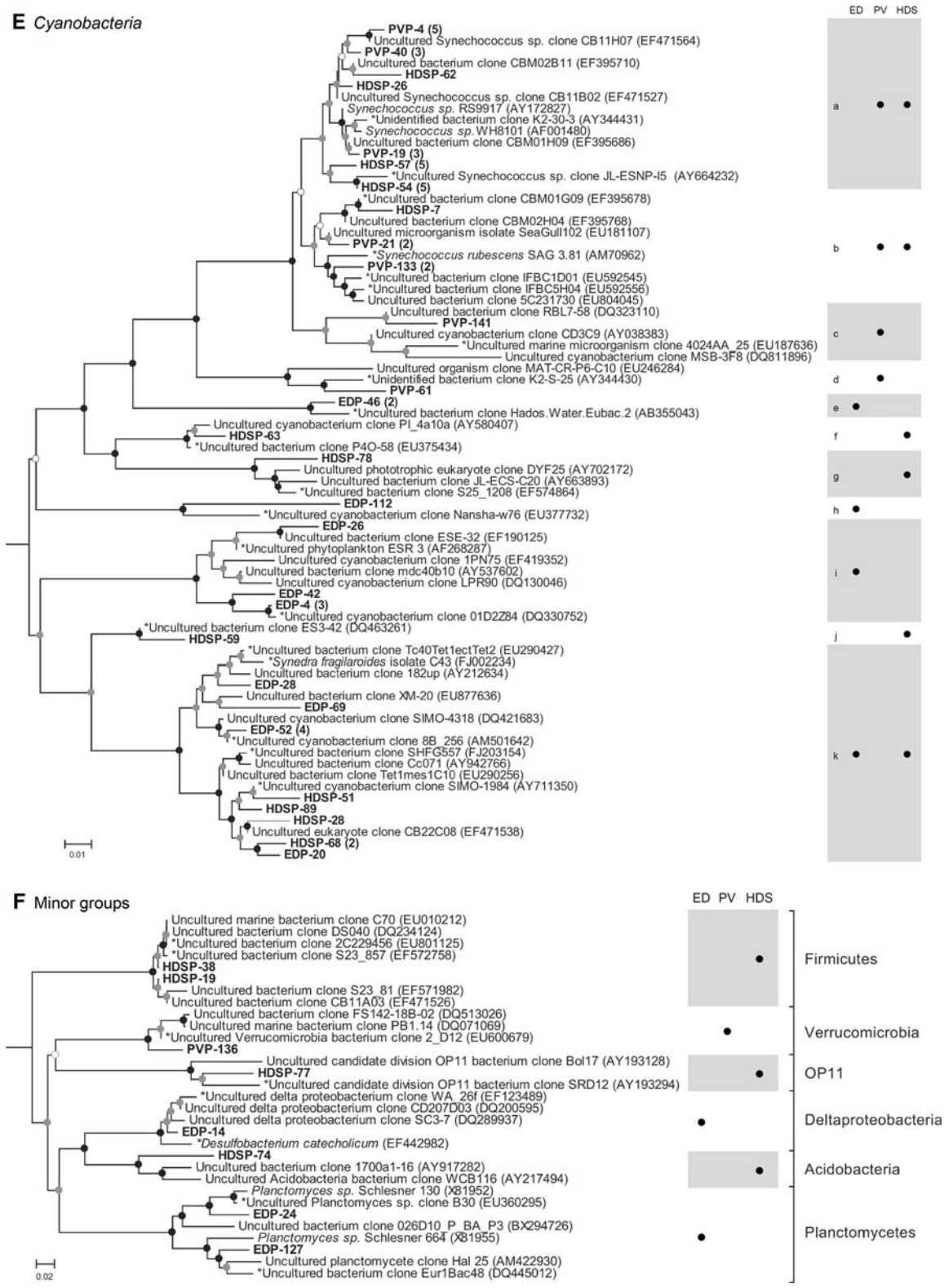


Fig. 4 continued

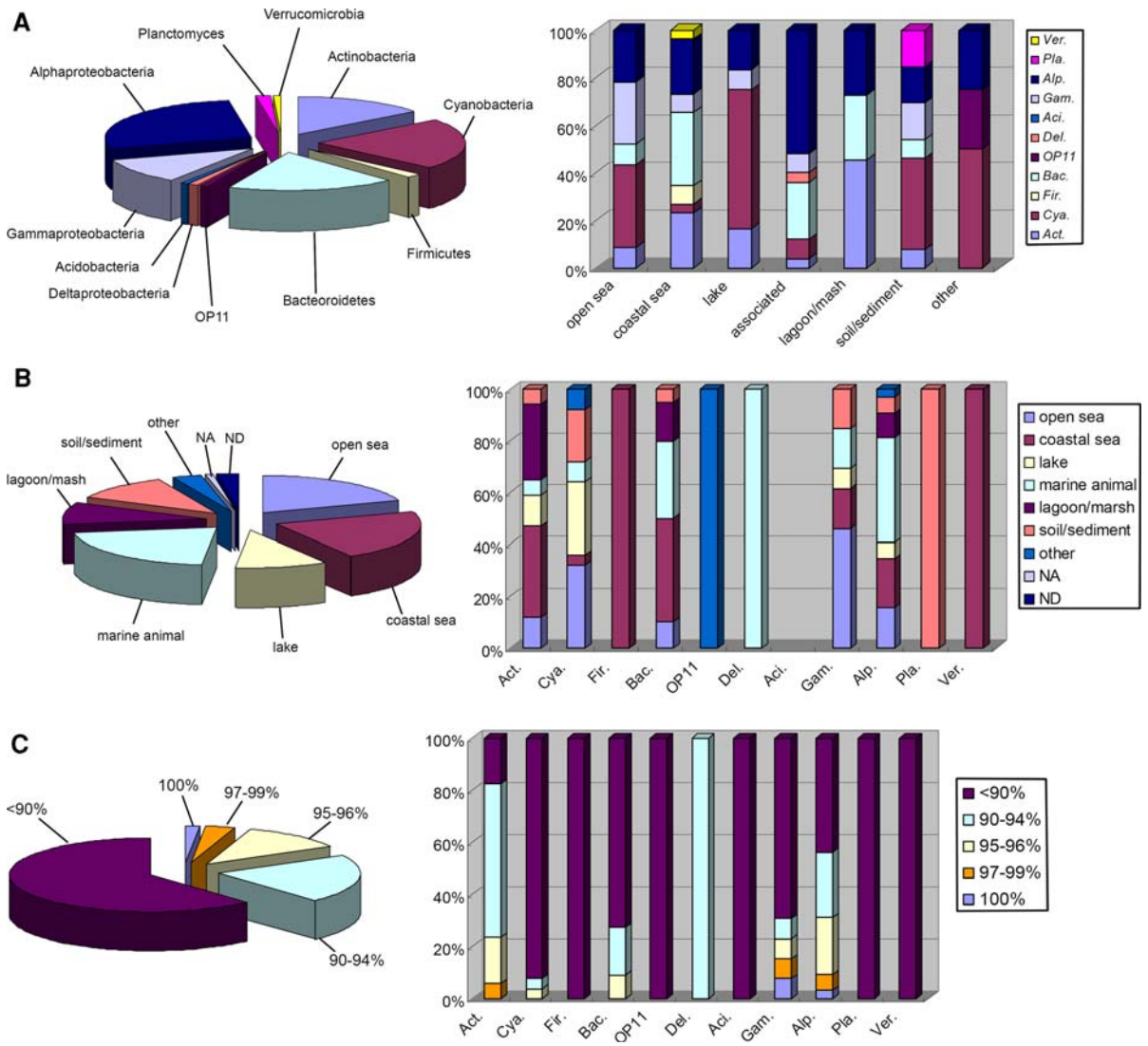


Fig. 5 Statistical analysis of OTUs distribution based on their taxonomic affiliation (**A**), and the source environments (**B**), or similarity values (**C**) of their nearest neighbors in GenBank

was predominantly converted to low molecular weight DOM. As a result, *Alphaproteobacteria* which utilize DOM could more efficiently dominate the bacterial population (Kirchman, 2002). It should be noted that the feed for PV contained antibiotics, which may have a major impact on the microbes and might select against some specific bacteria. It was reported that there are bacteria resistant to antibiotics present in large quantities in maricultures (Smith et al., 1994). When all the 118 OTUs from the three ponds are pooled for analysis, the dominant phyla are *Alphaproteobacteria* (27.1%), *Bacteroidetes* (18.6%), *Actinobacteria* (14.4%), and *Gammaproteobacteria* (11%) (Fig. 5A), indicating that they are likely the most successful bacterial groups in mariculture environments.

It is very interesting to note that certain OTUs were associated with specific animals (see Table 5). In the ED pond library, one OTU (EDP-58) affiliated with the *Alphaproteobacteria* was 94% identical to a clone retrieved from the gut of gnotobiotic zebrafish (Rawls et al., 2004); another OTU (EDP-95) affiliated with *Alphaproteobacteria* shared 96% identity to a clone retrieved from abdominal setal tuft of

Table 5 The closest neighbors of each OTU in GenBank

OTU	Clone	Affiliation	The closest neighbors in GenBank				
			Uncultured		%	Cultured type strain	%
1.	PVP-20, PVP-l4, PVP-58, PVP-114, PVP-39, PVP-11, PVP-158, PVP-15, PVP-38, PVP-155, PVP-37, PVP-140, PVP-110, PVP-142, PVP-148, PVP-152, PVP-161, PVP-31, PVP-1, PVP-147, PVP-6, PVP-9	Act.	Clone LA1-B9 (AF513961)	Hawaiian take water	97	*Yonghaparkia alkaliphila* (AB376086)	90
2.	PVP-132, PVP-50	Act.	Clone lagoaA02 (EF598914)	Araruama Lagoon	99	*Mycetocola saprophilus* (AM410677)	94
3.	PVP-117	Act.	Clone T32_144 (DQ436790)	Mediterranean seawater	99	*Rhodoluna lacicola* (AM999979)	94
4.	PVP-5	Act.	Clone T32_144 (DQ436790)	Mediterranean seawater	99	ND	
5.	PVP-159	Act.	Clone lagoaDO2 (EF598926)	Araruama Lagoon	96	*Yonghaparkia alkaliphila* (AB376086)	93
6.	EDP-63	Act.	Clone lagoaG04 (EF598940)	Araruama Lagoon	97	ND	
7.	**EDP**-64, **PVP**-35, PVP-28, PVP-139	Act.	Clone CL-Dokdo102 (FJ214966)	Seawater	96	*Yonghaparkia alkaliphila* (AB376086)	92
8.	**EDP**-13, EDP-36, EDP-12, EDP-18, EDP-54, **PVP**-34	Act.	Clone CB41H04(EF471594)	Chesapeake bay seawater	98	*Microcella alkaliphila* (AB362249)	96
9.	PVP-154	Act.	Clone CBO1F10 (EF471717)	Chesapeake bay seawater	99	*Microcella alkaliphila* (AB362249)	97
10.	EDP-96	Act.	Clone 5o42 (EU644794)	Soap Lake	98	*Microcella alkaliphila* (AB362249)	94
11.	PVP-10	Act.	Clone CB41H04 (EF471594)	Chesapeake bay seawater	97	*Microcella alkaliphila* (AB362249)	95
12.	PVP-26	Act.	Clone lagoaG04 (EF598940)	Araruama Lagoon	95	*Labedella gwakjiensis* (DQ533552)	91
13.	**EDP**-90, **PVP**-127	Act.	Clone CB01E07 (EF471485)	Chesapeake bay seawater	99	*Aquiluna ruba* (AJ565416)	95
14.	PVP-125	Act.	Clone Pl_4b7e(AY580340)	USA coastal seawater	99	*Aquiluna ruba* (AJ565416)	93
15.	EDP-5	Act.	Clone lagoaG04 (EF598940)	Araruama Lagoon	94	*Labedella kawkjii* (DQ533552)	93
16.	PVP-130	Act.	Clone MPWIC_A07 (EF414225)	Sponge associated	98	*Acidothermus cellulolyticus* (CP000481)	91
17.	HDSP-40, HDSP-88	Act.	Clone N1903_88 (EU104342)	Activated sludge	94	ND	
18.	EDP-4, EDP-1, EDP-89	Cya.	Clone 01D2Z84 (DQ330752)	Microbial mat	99	ND	
19.	EOP-26	Cya.	Chloroplast clone (AF268287)	Lake Esrum	99	ND	
20.	EDP-42	Cya.	Clone 01D2Z84 (DQ330752)	Microbial mat	95	ND	
21.	EDP-52, EDP-107, EDP-60, EDP-11	Cya.	Clone 8B_256 (AM501642)	Lagoon sediment	99	ND	
22.	EDP-28	Cya.	Chloroplast clone (FJ002234)	NA	97	ND	
23.	HDSP-68, HDSP-22	Cya.	Clone SIMO-1984 (AY711350)	Surface sediment	99	ND	
24.	HDSP-51	Cya.	Clone SIMO-1984 (AY711350)	Surface sediment	99	ND	

Table 5 continued

OTU	Clone	Affiliation	The closest neighbors in GenBank				
			Uncultured		%	Cultured type strain	%
25.	**EDP**-20, **HDSP**-89	Cya.	Clone SIMO-1984 (AY711350)	Surface sediment	99	ND	
26.	HDSP-28	Cya.	Clone SHFG557 (FJ203154)	Coral diseased tissue	99	ND	
27.	EDP-69	Cya.	Clone Tc40Tet1 (EU290427)	Sponge associated	98	ND	
28.	HDSP-59	Cya.	Clone ES3-42 (DQ463261)	River sediment	96	ND	
29.	EDP-112	Cya.	Clone Nansha-w76 (EU377732)	SCS seawater	91	ND	
30.	HDSP-78	Cya.	Clone S25_1208(EF574864)	Seawater	95	ND	
31.	HDSP-63	Cya.	Clone P4O-58 (EU375434)	Lake water	98	ND	
32.	EDP-46, EDP-25	Cya.	Clone: Eubac.2.(AB355043)	Lake water	98	ND	
33.	PVP61	Cya.	Clone K2-S-25 (AY344430)	Lake water	95	*Leptolyngbya nodulose* (EF 122600)	92
34.	PVP-4, PVP-123, PVP-163, PVP-138, PVP-120	Cya.	Clone JL-SCS-M24 (AY664031)	SCS seawater	96	ND	
35.	HDSP-57, HDSP-87, HDSP-96, HDSP-60, HDSP-69	Cya.	Clone C88 (EU010221)	Singapore seawater	97	ND	
36.	PVP-40, PVP-135, PVP-25, PVP-26	Cya.	Clone JL-SCS-M24 (AY664031)	SCS seawater	96	ND	
37.	HDSP-62	Cya.	Clone C88(EU010221)	Singapore seawater	94	ND	
38.	PVP-19, PVP-143, PVP-57	Cya.	Clone K2-30-3 (AY344431)	Lake water	97	ND	
39.	HDSP-54, HDSP-75, HDSP-86, HDSP-76, HDSP-58	Cya.	Clone JL-ESNP-15 (AY664232)	Pacific seawater	99	ND	
40.	PVP-133, PVP-121	Cya.	Clone IFBC5H04 (EU592556)	Lake water	97	ND	
41.	PVP-21, PVP-162	Cya.	IFBC1D01 (EU592545)	Lake water	96	ND	
42.	HDSP-7	Cya.	Clone CBM01G09 (EF395678)	Chesapeake bay seawater	99	*Synechococcus rubescens* (AM709629)	96
43.	PVP-141	Cya.	Clone 4024AA_25(EU187636)	ALOHA seawater	95	ND	
44.	HDSP-19, HDSP-90	Fir.	Clone 2C229456 (EU801125)	Delaware bay seawater	97	ND	
45.	HDSP-38, HDSP-91, HDSP-31, HDSP-83	Fir.	Clone S23_857(EF572758)	Coco island seawater	98	ND	
46.	EDP-51	Bac.	Clone CB22A10 (EF471582)	Chesapeake bay seawater	94	ND	
47.	EDP-108	Bac.	Clone CBM02G05 (EF395758)	Chesapeake bay seawater	99	ND	
48.	EDP-110	Bac.	Clone CBM02G05 (EF395758)	Chesapeake bay seawater	91	ND	
49.	EDP-65	Bac.	ND			ND	
50.	EDP-56, EDP-124	Bac.	Clone CBM02C10 (EF395717)	Chesapeake bay seawater	100	ND	

Table 5 continued

OTU	Clone	Affiliation	The closest neighbors in GenBank				
			Uncultured		%	Cultured type strain	%
51.	EDP-39	Bac.	Clone CBM02A03 (EF395691)	Chesapeake bay seawater	96	ND	
52.	**EDP**-2, **HDSP**-100	Bac.	Clone MJW46 (FJ172063)	Tirez lagoon	100	ND	
53.	EDP-22	Bac.	Clone PI_RT205 (AY580597)	USA coastal seawater	98	ND	
54.	HDSP-45, HDSP-41, MOSP-55	Bac.	Clone SIMO-2945 (DQ189920)	Salt mash	99	ND	
55.	HDSP-61	Bac.	ND				
56.	EDP-49	Bac.	Clone T42_63 (DQ436766)	Mediterranean seawater	95	ND	
57.	EDP-6, EDP-85	Bac.	Clone GCDE08_K (AY701469)	Shellfish associated	96	ND	
58.	EDP-37	Bac.	Clone SGUS389 (FJ202704)	Coral associated	95	*Winogradskyella poriferorum* (AY848823)	96
59.	EDP-128, EDP-120	Bac.	Clone 302 (DQ482736)	Dinoflagellate associated	94	*Gilvibacter sediminis* (AB255368)	96
60.	EDP-123	Bac.	Clone 302 (DQ482736)	Dinoflagellate associated	95	*Gilvibacter sediminis* (AB255368)	92
61.	PVP-149	Bac.	Clone T32_67 (DQ436738)	Mediterranean seawater	99	*Formosa agariphila* (AJ893518)	94
62.	EDP-117	Bac.	Clone TAI-8-94 (AM259822)	Sponge associated	95	*Polaribacter dokdonensis* (DQ004686)	93
63.	EDP-94	Bac.	Clone SiMO-2854 (DQ189829)	Salt mash	99	ND	
64.	EDP-10	Bac.	Clone VHS-B4-72 (DQ395008)	Victoria Harbour, HK	92	*Robiginitalea biformata* (AY424899)	91
65.	HDSP-67	Bac.	Clone 2uD_H0l (EU627948)	Water near fish pens	99	ND	
66.	EDP-57, EDP-113	Bac.	Clone GCTRA14_T (AY701462)	Dinoflagellate associated	97	ND	
67.	EDP-29	Bac.	Clone SB74 (EU722650)	Laggon sediment	93	ND	
68.	HDSP-77	OP11	Clone SRD12 (AY193294)	River filaments	98	ND	
69.	EDP-14	Del.	Clone WA_26f (EF123489)	Coral associated	97	*Desulfobacterium catecholicum* (EF442982)	92
70.	HDSP-74	Aci.	ND			ND	
71.	HDSP-35	Gam.	Clone PI_4j5b (AY580744)	USA coastal seawater	98	*Haliea salexigens* (AY576769)	92
72.	HDSP-65	Gam.	Clone HF10 (EU36l668)	ALOHA seawater	98	ND	
73.	EDP-111	Gam.	Clone HF70 (EU361659)	ALOHA seawater	94	ND	
74.	HDSP-71	Gam.	Clone pIR3BD01 (AY354159)	Ocean sediment	92	ND	
75.	HDSP-79, HDSP-82	Gam.	Env. clone (AF102866)	Shipworm associated	91	ND	
76.	HDSP-24	Gam.	Bact. JL-463 (DQ285073)	South China sea	98	ND	
77.	EDP-97	Gam.	Clone JL.ESNP.H24 (AY664173)	Pacific seawater	94	ND	
78.	PVP-112	Gam.	Clone 4041AA93(EU188200)	ALOHA seawater	100	*Alteromonas macleodii* (Y18228)	100
79.	PVP-113	Gam.	Clone 1C227767 (EU800070)	Newport Harbour	99	*Chromohalobacter salexigens* (FJ355947)	99
80.	PVP-59	Gam.	Clone JL.ETNP.Z11 (AY726920)	Pacific seawater	96	*Halomonas meridiana* (EU652041)	96
81.	PVP-56	Gam.	Clone ELB16-131 (DQ015811)	Lake water	94	ND	

Table 5 continued

OTU	Clone	Affiliation	The closest neighbors in GenBank				
			Uncultured		%	Cultured type strain	%
82.	HDSP-99	Gam.	Clone 3T9d-oil (FM242300)	Sediment	95	ND	
83.	HDSP-21	Gam.	Clone ATLC.61 (EU647630)	Algae culture	99	ND	
84.	HDSP-39	Alp.	Clone 4C230214 (EU802834)	Panama coast	94	ND	
85.	EDP-40, EDP-115, EDP-68, EDP-8, EDP-104	Alp.	Clone BTM79 (AY193280)	Marine tidal mat	100	*Loktanella rosea* (EU687492)	100
86.	EDP-109	Alp.	Clone 2B_121 (AM501869)	Lagoon sediment	96	*Loktanella vestfoldensis* (EU687493)	93
87.	EDP-122	Alp.	Bact. plateOTU8 (EU346495)	Sponge associated	98	*Loktanella rosea* (EU687492)	95
88.	EDP-93, EDP-7	Alp.	Clone SIMO-4291 (DQ421656)	Salt marsh	97	*Leisingera aquamarina* (AM900415)	95
89.	HDSP-49	Alp.	Clone DS023 (DO234107)	Mangrove	98	*Citreicella thiooxidans* (EU440958)	94
90.	HDSP-12	Alp.	Clone PC-PA9-53 (EF379776)	Victoria Harbor	98	ND	
91.	HDSP-93, HDSP-42	Alp.	Clone BG27-10 (AY904490)	Mexico coast	98	ND	
92.	HDSP-5	Alp.	Clone SIMO-4282 (DQ421647)	Salt marsh	95	ND	
93.	EDP-33	Alp.	Clone SIMO-4255 (DQ421620)	Salt marsh	99	*Pelagibaca bermudensis* (DQ178660)	94
94.	PVP-157, PVP-128, PVP-13	Alp.	Clone SAV05F02 (EU542268)	Pondweed associated	99	*Thalassobacter oligotrophus* (AJ631302)	95
95.	EDP-95	Alp.	Clone pt183 (DQ890445)	Decapoda associated	96	*Tatevamaria omphalii* (AB193438)	93
96.	HDSP-81, HDSP-53	Alp.	Clone XM-38 (EU877645)	Chinese coastal seawater	100	ND	
97.	HDSP-11, HDSP-95	Alp.	Clone 4031AA (EU187809)	ALOHA seawater	98	*Thalassobius mediterraneus* (AJ878874)	96
98.	HDSP-98, HDSP-47	Alp.	Clone MD2.45 (FJ403094)	Coral associated	98	*Pseudoruegeria aquimaris* (DQ675021)	97
99.	MDSP-92	Alp.	Clone MPW1C (EF414227)	Sponge associated	98	*Phaeobacter inhibens* (AY177712)	94
100.	HDSP-25	Alp.	Clone SHFG537 (FJ203135)	Coral associated	97	*Thalassobius gelatinovours* (DQ915639)	95
101.	PVP-134	Alp.	Clone STX (EF123343)	Coral associated	98	*Roseibacterium elongatum* (AB061273)	96
102.	EDP-38	Alp.	Clone AS-26 (AJ391187)	Adriatic seawater	93	*Loktanella vestfoldensis* (AY771771)	92
103.	EDP-19	Alp.	Clone pt173 (DQ890439)	Decapoda associated	91	ND	
104.	PVP-146	Alp.	Clone pt173 (DQ890439)	Decapoda associated	94	*Altererythrobacter luteolus* (AY739662)	92
105.	PVP-164	Alp.	Clone Asc-s-74 (EF632663)	Sediment	97	*Pseudoruegeria aquimaris* (DQ675021)	95
106.	HDSP-46	Alp.	Clone JL-ECS-C25 (AY663896)	East China Sea	97	*Hoeflea alexandrii* (AJ86600)	94
107.	EDP-41, EDP-35	Alp.	Clone TAU-7-36 (AM259743)	Sponge associated	99	ND	
108.	EDP-102	Alp.	Clone TAU-7-36 (AM259743)	Sponge associated	99	ND	
109.	HDSP-34, HDSP-43	Alp.	Clone 6C233330 (EU805327)	Panama offshore seawater	98	ND	
110.	HDSP-36	Alp.	Clone 6C233325 (EU805322)	Panama offshore seawater	98	ND	
111.	HDSP-52	Alp.	Clone 6C233398 (EU805389)	Panama offshore seawater	97	ND	
112.	PVP-36	Alp.	Clone 1C227639 (EU799961)	Newport Harbour	94	ND	

Table 5 continued

OTU	Clone	Affiliation	The closest neighbors in GenBank				
			Uncultured		%	Cultured type strain	%
113.	EDP-15, EDP-86	Alp.	Clone TH_h39 (EU980287)	Lake water	97	*Rhizobium undicola* (DQ648579)	97
114.	EDP-58	Alp.	Clone mdc61a08 (AY537800)	Zebrafish gut	94	ND	
115.	EDP-121	Alp.	Clone TH_h39 (EU980287)	Lake water	97	ND	
116.	EDP-24	Pla.	Clone B30 (EU360295)	Deep sea sediment	91	ND	
117.	EDP-127	Pla.	Clone Eur1Bac48 (DQ445012)	Permafrost	93	ND	
118.	PVP-136	Ver.	Clone 2_D12 (EU600679)	Spain coast	94	ND	

EDP, *Epinephelus diacanthus* pond; PVP, *Penaeus vannamei* pond; HDSP, *Haliotis diversicolor* supertexta pond; Act., *Actinobacteria*; Cya., *Cyanobacteria*; Fir., *Firmicutes*; Bac., *Bacteroidetes*; OP11, a candidate division; Del., *Deltaproteobacteria*; Aci., *Acidobacteria*; Gam., *Gammaproteobacteria*; Alp., *Alphaproteobacteria*; Pla., *Planctomycetes*; Ver., *Verrucomicrobia*

ND no significant results obtained (similarity <90), *NA* not available

Thalassinidea (described in GenBank, accession No. DQ890445). In the HDS pond library, one OTU (HDSP-79) was distantly related to a *Gammaproteobacteria* that came from the gills of *Bankia setacea* (Sipe et al., 2000); another OTU (HDSP-28) affiliated with *Cyanobacteria* was 99% identical to a clone from coral diseased tissues. The nearest neighbor of both the OTU EDP-19 from the ED pond and the OTU PVP-146 from the PV pond was an uncultured bacterium associated with Decapoda. These findings indicate that some unique bacterial compositions might be created by different mariculture animals but no definite identifications have been made. In total, 21% of the OTUs in this study have closest neighbors from marine animals, slightly less than those from coastal seas (22%) and more than those from open seas (19.5%) (Table 5; Fig. 5B).

Dramatic differences in diversity existed in the three ponds. Relative to other reported aquatic environments, e.g., a shallow hypertrophic freshwater lake in China (Wu et al., 2007), the diversities of species in the ED and HDS pond library were quite high, while the diversity in the PV libraries was much lower, as indicated by the Chao1 estimate (Table 3). Within the three ponds, taxa divergence was extensive. The ED and HDS pond library contained 34 and 28 clusters, respectively; in contrast, the PV pond library contained only 19 clusters. This discrepancy was also reflected in rarefaction analysis. A likely explanation for this discrepancy was that water environments of the ED and HDS pond were more complex than that of the PV pond, and the complexity of environmental conditions may determine the microbial composition (Horner-Devine et al., 2004).

Phylogenetic analysis showed that most clusters from the three pond libraries, except for EDP-51, EDP-65, and EDP-110 which are affiliated with *Bacteroidetes*, have previously been found in other environments, suggesting that the bacterial composition in mariculture ponds of this study has few novel bacterial groups. It also differed significantly from marine coastal waters that had been studied previously (Hollibaugh et al., 2000), which confirms the great impact by mariculture on planktonic bacterial communities. However, in general, bacteria in mariculture ponds are diverse, covering 11 phyla or subphyla (Fig. 5A). OTUs of 61.9% and 94.9% have less than 90% and 97% similarity to their nearest neighbors in public databases, respectively (Fig. 5C),

indicating that most species in these mariculture ponds are novel and remain to be identified.

Conclusion

The comparative analysis of the three mariculture ponds revealed that the structures and diversity of the bacterial communities in these ponds were significantly different. The bacterial population in a mariculture pond might be characterized by abiotic and biotic features of the aquatic ecosystems, which are influenced by stock feed, faecal excretion of the mariculture animals, and a range of other factors. Our study might provide a useful indicator and new information for environmental assessment of mariculture ponds, and help to better understand microbial ecology in them.

Acknowledgments This study was supported by the NSFC projects (40632013, 40521003), and the MOST project (200805068, 2006BAC11B04). We are grateful to Yanlin Zhao and Dechao Yang for sampling and field assistance. We thank Dr. Oliver Yu at the Donald Danforth Plant Science Center, USA, for his help in editing and improving the manuscript. Thanks are also due to the editor for his detailed revisions on the language and contents, and to the reviewers for their critical comments.

References

Carlson, C., S. Giovannoni, D. Hansell, S. Goldberg, M. Parsons, M. Otero, K. Vergin & B. Wheeler, 2002. Effect of nutrient amendments on bacterioplankton production production, community structure, and DOC utilization in the northwestern Sargasso Sea. Aquatic Microbial Ecology 30: 19–36.

Caruso, G. , L. Genovese, M. Mancuso & A. Modica, 2003. Effects of fishing on microbial enzyme activities and densities: comparison between three Mediterranean sites. Letters in Applied Microbiology 37: 324–328.

Chao, A., 1984. Non-parametric estimation of the number of classes in a population. Scan. J. Stat 11: 265–270.

Cotner, J. B. & B. A. Biddanda, 2002. Small players, large role: microbial influence on biogeochemical processes in pelagic aquatic ecosystems. Ecosystems 5: 105–121.

Cottrell, M. T. & D. L. Kirchman, 2000. Natural assemblages of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. Applied and Environmental Microbiology 66: 1692–1697.

Feng, Y. Y., L. C. Hou, N. X. Ping, T. D. Ling & C. I. Kyo, 2004. Development of mariculture and its impact in Chinese coastal waters. Reviews in Fish Biology and Fisheries 14: 1–10.

Fuhrman, J. A., D. E. Comeau, A. Hagstrom & A. M. Chan, 1988. Extraction from natural planktonic microorganism of DNA suitable for molecular biological studies. Applied and Environmental Microbiology 54: 1426–1429.

Gonzalez, J. M., R. Simo, R. Massana, J. S. Covert, E. O. Casamayor, C. Pedros-Alio & M. A. Moran, 2000. Bacterial community structure associated with a dimethylsulfoniopropionate-producing North Atlantic algal bloom. Applied and Environmental Microbiology 66: 4237–4246.

Haukka, K., E. Kolmonen, R. Hyder, J. Hietala, K. Vakkilainen, T. Kairesalo, H. Haario & K. Sivonen, 2006. Effect of nutrient loading on bacterioplankton community composition in lake mesocosms. Microbial Ecology 51: 137–146.

Hill, T. C. J., K. A. Walsh, J. A. Harris & B. F. Moffett, 2003. Using ecological diversity measures with bacterial communities. FEMS Microbiology Ecology 43: 1–11.

Hollibaugh, J. T., P. S. Wong & M. C. Murrell, 2000. Similarity of particle-associated and free-living bacterial communities in northern San Francisco Bay, California. Aquatic Microbial Ecology 21: 103–114.

Horner-Devine, M. C., K. M. Carney & B. J. M. Bohannan, 2004. An ecological perspective on bacterial biodiversity. Proceedings of the Royal Society of London. Series B: Biological Sciences 271: 113–122.

Humayoun, S. B., N. Bano & J. T. Hollibaugh, 2003. Depth distribution of microbial diversity in Mono Lake, a meromictic soda lake in California. Applied and Environmental Microbiology 69: 1030–1042.

Ivanova, E. P., N. V. Zhukova, A. M. Lysenko, N. M. Gorshkova, A. F. Sergeev, V. V. Mikhailov & J. P. Bowman, 2005. Loktanella agnita sp. nov. and Loktanella rosea sp. nov., from the north-west Pacific Ocean. International Journal of Systematic and Evolutionary Microbiology 55: 2203–2207.

Jensen, S., L. Øvreås, B. Øivind & T. Vigdis, 2004. Phylogenetic analysis of bacterial communities associated with larvae of the Atlantic halibut propose succession from a uniform normal flora. Systematic and Applied Microbiology 27: 728–736.

Jespersen, A. M. & K. Christoffersen, 1987. Measurements of chlorophyll a from phytoplankton using ethanol as extraction solvent. Archiv fur Hydrobiologie 109: 445–454.

Khan, S. T., Y. Nakagawa & S. Harayama, 2007. Sediminibacter furfurosus gen. nov., sp. nov. and Gilvibacter sediminis gen. nov., sp. nov., novel members of the family Flavobacteriaceae. International Journal of Systematic and Evolutionary Microbiology 57: 265–269.

Kirchman, D. L., 2002. The ecology of Cytophaga-Flavobacteria in aquatic environments. FEMS Microbiology Ecology 39: 91–100.

Kuhner, M. K. & J. Felsenstein, 1994. A simulation comparison of phylogeny algorithms under equal and unequal evolutionary rates. Molecular Biology and Evolution 11: 459–468.

La Rosa, T., S. Mirto, E. Favaloro, B. Savona, G. Sara, R. Danovaro & A. Mazzola, 2002. Impact on the water column biogeochemistry of a Mediterranean mussel and fish farm. Water Research 36: 713–721.

Lau, W. W., P. A. Jumars & E. V. Armbrust, 2002. Genetic diversity of attached bacteria in the hindgut of the deposit-

feeding shrimp Neotrypaea (formerly Callianassa) californiensis (decapoda: thalassinidae). Microbial Ecology 48: 455–466.

Lau, S. C. K., M. Y. T. Mandy, X.-C. Li, I. Plakhotnikova, S. Dobretsov, K. W. K. Lau, M. Wu, P.-K. Wong, J. R. Pawlik & P.-Y. Qian, 2005. Winogradskyella poriferorum sp. nov., a novel member of the family Flavobacteriaceae isolated from a sponge in the Bahamas. International Journal of Systematic and Evolutionary Microbiology 55: 1589–1592.

Mantzavrakos, E., M. Kornaros, G. Lyberatos & P. Kaspiris, 2007. Impacts of a marine fish farm in Argolikos Gulf (Greece) on the water column and the sediment. Desalination 210: 110–124.

Massana, R., A. Murray, C. M. Preston & E. F. Delong, 1997. Vertical distribution and phylogentic characterization of marine planktonic Archaea in the Santa Barbara Channel. Applied and Environmental Microbiology 63: 50–56.

National Oceanographic Bureau in China, 1999. Water Monitoring and Analysis, Specification of Oceanographic Survey (GB 17378.4-98). Standards Press of China, Beijing.

Odum, E. P., 1971. Principles and concepts pertaining to organization at the community level. In Odum, E. P. (ed.), Fundamentals of Ecology. Saunders College Publishing, Philadelphia: 140–161.

Øvreås, L., D. Bourne, R. Sandaa, E. Casamayor, S. Benlloch, V. Goddard, G. Smerdon, M. Heldal & T. Thingstad, 2003. Response of bacterial and viral communities to nutrient manipulations in seawater mesocosms. Aquatic Microbial Ecology 31: 109–121.

Pitta, P., E. T. Apostolaki, T. Tsagaraki, M. Tsapakis & I. Karakassis, 2006. Fish farming effects on chemical and microbial variables of the water column:a spatio-temporal study along the Mediterranean Sea. Hydrobiologia 563: 99–108.

Qian, P. Y., M. C. S. Wu & I. H. Ni, 2001. Comparison of nutrients release among some maricultured animals. Aquaculture 200: 305–316.

Rawls, J. F., B. S. Samuel & J. I. Gordon, 2004. Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. Proceedings of the National Academy of Sciences of the United States of America 101: 4596–4601.

Sandaa, R. A., T. Magnesen, L. Torkildsen & Ø. Bergh, 2003. Characterisation of the bacterial community associated with early stages of great scallop (*Pecten maximus*), using denaturing gradient gel electrophoresis (DGGE). Systematic and Applied Microbiology 26: 202–311.

Sarà, G., 2007. Aquaculture effects on some physical and chemical properties of the water column: a meta-analysis. Chemistry and Ecology 23: 251–262.

Schäfer, H., L. Bernard, C. Courties, R. Lebaron, P. Servais, R. Pukall, E. Stackebrandt, M. Troussellier, T. Guindulain, J. Vives-Rego & G. Muyzer, 2001. Microbial community dynamics in Mediterranean nutrient-enriched seawater mesocosms: changes in the genetic diversity of bacterial populations. FEMS Microbiology Ecology 34: 243–253.

Schloss, P. D., B. R. Larget & J. Handelsman, 2004. Integration of microbial ecology and statistics: a test to compare gene libraries. Applied and Environmental Microbiology 70: 5485–5492.

Singleton, D. R., M. A. Furlong, S. L. Rathbun & W. B. Whitman, 2001. Quantitative comparisons of 16S rDNA sequence libraries from environmental samples. Applied and Environmental Microbiology 67: 4373–4376.

Sipe, A. R., A. E. Wilbur & S. C. Cary, 2000. Bacterial symbiont transmission in the wood-boring shipworm Bankia setacea (Bivalvia: Teredinidae). Applied and Environmental Microbiology 66: 1685–1691.

Smith, P., M. Hiney & O. Samuelsen, 1994. Bacterial resistance to antimicrobial agents used in fish farming: a critical evaluation of method and meaning. Annual Review Fish Disease 4: 273–313.

Souchu, P., A. Vaquer, Y. Colllos, S. Landrein & J.-M. Deslous-Paoli, 2001. Influence of shellfish farming activities on the biogeochemical composition of the water column in Thau lagoon. Marine Ecology Progress Series 218: 141–152.

Tamura, K., J. Dudley, M. Nei & S. Kumar, 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24: 1596–1599.

Thompson, J. D., G. J. Toby, F. Plewniak, F. Jeanmougin & D. G. Higgins, 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25: 4876–4882.

Vezzulli, L. C. E., G. Riccardi & M. Fabiano, 2002. Bacterial community structure and activity in fish farm sediments of the Ligurian sea (Western Mediterranean). Aquaculture International 10: 1–19.

Wu, X., W. Xi, W. Ye & H. Yang, 2007. Bacterial community composition of a shallow hypertrophic freshwater lake in China, revealed by 16S rRNA gene sequences. FEMS Microbiology Ecology 61: 85–96.