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Phylogenetically diverse, acetaldehyde-degrading bacterial community in the deep sea water of the West Pacific Ocean

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Abstract

As a major aldehyde pollutant widely existing in industry and our daily life, acetaldehyde is more and more harmful to human health. As characteristic habitat niche, bacteria from deep sea environments are abundant and distinctive in heredity, physiology and ecological functions. Thus, the development of acetaldehyde-degrading bacteria from deep sea provides a new method to harness acetaldehyde pollutant. Firstly, in this study, acetaldehyde-degrading bacteria in the deep sea water of the West Pacific Ocean were enriched *in situ* and in the laboratory respectively, and then the diversity of uncultured bacteria was studied by using 16S rRNA genes. Then acetaldehyde-degrading strains were isolated from two samples, including enrichment *in situ* and enrichment in laboratory samples of deep sea water from the West Pacific Ocean using acetaldehyde as the sole carbon source, and then the ability of acetaldehyde degradation was detected. Our results showed that the main uncultured bacteria of two samples with different enrichment approaches were similar, including Proteobacteria, Actinobacteria, Firmicutes, Cyanobacteria, but the structure of bacterial community were significant different. Four subgroups, α, γ, δ and ε, were found in Proteobacteria group. The γ-Proteobacteria was dominant (63.5% clones in laboratory enriched sample, 75% clones *in situ* enriched sample). The species belonged to γ-Proteobacteria and their proportion was nearly identical between the two enrichment samples, and *Vibrio* was the predominant genus (45% in laboratory enriched sample, 48.5% *in situ* enriched sample), followed by *Halomonas* (9% *in situ* enriched sample) and *Streptococcus* (6% in laboratory enriched sample). A total of 12 acetaldehyde-degrading strains were isolated from the two samples, which belonged to *Vibrio*, *Halomonas*, *Pseudoalteromonas*, *Pseudomonas* and *Bacillus* of γ-Proteobacteria. Strains ACH-L-5, ACH-L-8 and ACH-S-12, belonging to *Vibrio* and *Halomonas*, have strong ability of acetaldehyde degradation, which could tolerate 1.5 g/L acetaldehyde and degrade 350 mg/L acetaldehyde within 24 hours. Our results indicated that bacteria of γ-Proteobacteria may play an important role in carbon cycle of deep sea environments, especial the bacteria belonging to *Vibrio* and *Halomonas* and these strains was suggested for their potentials in government of aldehyde pollutants.

Key words: acetaldehyde-degrading bacteria, ALDH, deep sea, *in situ*

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1 Introduction

Deep sea is the biggest Gene Bank on the earth, which contains abundant marine microorganisms. Previous studies paid great attentions to the marine microorganisms in shallow sea, and many marine microorganisms have been reported (Okami et al., 1976; Okazaki et al, 1975; Ding and Valentine, 2008). Compared with a lot of research attentions on marine microorganisms in shallow sea, the investigation of marine microorganisms in deep sea is relatively lacking. Deep sea has extremely heavy climate and environment, for example strong acid and base, high or low temperature, high pressure. Therefore, marine microorganisms of deep sea are significantly different with microorganisms from land including genetic constitution, metabolism regulation, metabolite, chemical defense, and many microorganisms which are acidophilic, basophilic, haloduric and so on (Horikoshi, 1998; Ista et al., 2004; Koyama and Yoshida, 2016). The refractory organisms which come from upper layer of the ocean and bottom sediments are the main nutrient source of microorganisms in deep sea (Horikoshi, 1998; Gao et al., 2017). Thus the microorganisms in deep sea have potential of environment government, for example apply in industrial wastewater treatment. Meanwhile, the potential application of microorganisms of deep sea in biomedicine, new biomaterial was noticed. As exhaustion of land resources, the marine microorganisms, especially the microorganisms of deep sea will be one of spot for exploitation.

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The pollutant of aldehydes widely exists in the environment, for example methanol, acetaldehyde, acraldehyde (Zhou et al., 2005; Gesser and Fu, 1990; Xi et al., 1999). Previous studies have reported that the superfluous acetaldehyde in furniture materials have been noticed by people, and that is harmful to humans (Homann et al., 2000). While acetaldehyde dangers have not been noticed yet by people. Acetaldehyde is toxic when applied externally for prolonged periods, an irritant, and a probable carcinogen. At present, enzymatic degradation by the acetaldehyde dehydrogenase is the main efficient way to degrade acetaldehyde (Lubin and Westerfield, 1945; Svegliati-Baroni et al., 2001). Unfortunately, the extraction of acetaldehyde dehydrogenase is costly and difficult. Furthermore, as the complex chemical constituents of acetaldehyde industrial wasted water, gas and residue, such as high heavy metal, strong acid and alkali, so the degradation of acetaldehyde by the acetaldehyde dehydrogenase is infeasible (van der Ploeg et al., 1994; Lachenmeier and Sohnius, 2008; Kurkivuori et al., 2007). To address this issue, recent studies have more focused on microbial catalyzing, especially the bacteria in deep sea.

Although technique of microbial pure culture which obtains microbial pure culture by isolation, purification, culture of microorganism has encountered un-overstepped obstacle for studying diversity of microorganism, it is irreplaceable for study of cellular structure, physiology, genetics and application of microorganism. To isolate microorganisms as far as possible, the technique of microbial pure culture was improved. New technique without high concentration of nutrients and manual preparation could provide primitive ecology for microorganisms, and the enrichment of microorganisms *in situ* is one of frequently used techniques. Kaeberlein et al. (2002) have obtained many more stains used this technique than traditional separation methods. Furthermore, molecular ecological techniques have recently been used to study bacterial diversity *in situ* (Kirk et al., 2004). This stems from the fact that many microorganisms are not easily obtainable as cultured strains in laboratory, which would allow for identification and characterization.

In this study, with the aim to know about the acetaldehyde-degrading bacterial phylogenetic diversity and to search strains with the potential application of acetaldehyde degradation in the deep sea water of the West Pacific Ocean, enrichment *in situ* and in the laboratory respectively, the uncultured and cultured bacterial phylogenetic diversity and the ability of acetaldehyde-degrading of bacteria in the deep sea water of the West Pacific Ocean were investigated. Besides, the potential application of acetaldehyde-degrading bacteria in the deep sea water of the West Pacific Ocean was suggested.

2 Materials and methods

2.1 *Sampling*

Enrichment in laboratory: Deep sea water was sampled at the depth of 2 000 m in the West Pacific Ocean (21°03′30″N, 118°23′17″E) in September 2012. A total of 30 mL deep sea water was loaded in 50 mL reagent bottle with 5 g absorbing 40% acetaldehyde of active carbon, and the reagent bottle was stored in a cold storage (8°C) for 6 months.

Enrichment *in situ*: regent bottles were placed at the depth of 2 000 m in the West Pacific Ocean (21°03′30″N, 118°23′17″E) in September 2012. There were active carbon granules which absorbed 40% acetaldehyde in these regent bottles, and some pores were on these regent bottles for internal and external material exchange, and bacteria were enriched *in situ* for six months.

2.2 *Total genomic DNA extraction, PCR amplification of 16S rRNA gene, and clone library structure*

Total genomic DNA was extracted from two kinds of samples, respectively, using PowerSoil DNA Isolation Kit (MOBIO). The quality and concentration of the soil DNA were assessed using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, USA). The overall size of the total DNA was checked by running an aliquot of DNA on a 1.0% agarose gel, and then was stored in –20°C until further processing.

The resulting genomic DNA was used as templates to amplify the bacterial 16S rRNA gene fragment using common primers 27F and 1492R. The PCR mixture (50 μL) contained 2 μL DNA template, 5 μL 10×Taq buffer (Mg^{2+}), 4 μL dNTP mixture (2.5 mmol/L), 1 μL of each primer (10 μmol/L), 0.5 μL EXTaq DNA polymerase (5 U/μL). The PCR program was carried out with 3 min at 95°C, followed by 30 cycles of denaturation at 95°C for 90 s, annealing at 55°C for 90 s and elongation at 72°C for 90 s, and final elongation at 72°C for 10 min. PCR products were purified using Gel Extraction Kit (Omega). PCR products were ligated into pTA-2 Vector and transformed into *E. coli* DH5α. PCR was performed to detect whether the picked clones were positive recombinants. Two different clone libraries were structured. A total of 200 positive clones were selected from each clone library and sequenced using primers T3 and T7 by Shanghai Majorbio Company.

2.3 *16S rRNA genes phylogenetic diversity and bacterial diversity analysis*

After removing the sequences of plasmid vector, 1 400–1 500 bp 16S rRNA genes were obtained in this study, and were checked chimera on the DECIPHER-Find Chimeras. These sequences were aligned on the Rdp Pipeline Tools to identify sequences similarity. Sequences with similarity not less than 97% were classified as one OTUs, while similarity less than 97% were classified different OTUs. The representative sequences were selected, and then phylogenetic trees based on gene sequences of 16S rRNA were constructed using neighbor-joining method in MEGA 5.0 combined with bootstrap analysis setting with 1 000 replications, respectively. Coverage estimator based on Coverage C (Chao and Lee, 1992) and species richness was assessed with Chao1 (Chao, 1984), while evenness and diversity of species were estimated by Evenness index (E') and Shannon diversity index (H') (Chao and Shen, 2003).

2.4 *Isolation and identification of cultivable acetaldehyde-degrading bacteria*

To further screen cultivable acetaldehyde-degrading bacteria, acetaldehyde was used as sole carbon source for the growth of acetaldehyde-degrading bacteria. Two different samples (5 g) were vortex shocked with 5 min, and then serial 10-fold dilutions (each sample in sterile sea water) were plated on the corresponding solid modified 2216E (0.1% yeast extract, 2% agar powder, 720 mg/L acetaldehyde and sea water) and incubated for 48 h at 37°C. Subsequently, individual bacteria were removed from agar plates, placed on new medium and checked for purity and stored in 20% glycerol at –80°C.

The genomic DNA of acetaldehyde-degrading bacteria was extracted using TaKaRa MiniBEST Bacteria Genomic DNA Extraction Kit Ver. 3.0. The resulting genomic DNA was used as templates to amplify the bacterial 16S rRNA gene fragment using common primers 27F and 1492R. PCR products were sequenced in Shanghai Majorbio Company after analysis by electrophoresis on 1.5% agarose gel. A total of 1 400–1 500 bp of Bacterial 16S

rRNA genes sequences were obtained in this study and aligned on the NCBI database. The most appropriate relative sequences were selected and it can be sure genus or family of bacteria until the similarity not less than 99%.

2.5 *Detection of acetaldehyde-degrading ability of cultivable acetaldehyde-degrading bacteria*

To detect the tolerance of cultivable acetaldehyde-degrading bacteria toward acetaldehyde, bacterial cells were grown in 5 mL 2216E with different concentrations of acetaldehyde (500 mg/L, 800 mg/L, 100 mg/L, 1 500 mg/L, and 2 000 mg/L) at 37°C, 200 r/min. The OD_{600} was detected after 48 h to determine whether these bacteria could tolerate the acetaldehyde.

To further investigate the abilities of acetaldehyde-degradation of these bacteria, acetaldehyde was used as sole carbon for the growth of these bacteria (1 g/L NH_4NO_3, 500 mg/L acetaldehyde and sea water). Acetaldehyde-degrading bacteria cells were cultured at 37°C, 200 r/min for 24 h in 2216E medium. The cultures were centrifuged at 10 000 *g* for 10 min, and the bacterial cells were collected. The cells pellets were washed twice with sterile medium (1 g/L NH_4NO_3 and sea water), and then resuspended in medium (1 g/L NH_4NO_3 and sea water). Subsequently, acetaldehyde-degrading bacteria (2% inoculation amount) were cultured in medium (1 g/L NH_4NO_3, 500 mg/L acetaldehyde and sea water) at 37°C, 200 r/min for 24 h and the medium without acetaldehyde-degrading bacteria were used as the blank control. Then, the cultures were centrifuged at 10 000 *g* for 10 min, and the supernatants were collected to analysis acetaldehyde using HPLC. Previous reporters have described the method of detecting acetaldehyde, 200 μL 1 000 mg/L of 2, 4-dinitrophenylhydrazine (DNPH) and 20 μL acetic acid was added to each of 200 μL samples supernatants and incubated for 15 min at 37°C for derivatization. Each of samples were mixed with 1 200 μL extraction agent (ethyl acetate:n-hexane=1:5, v/v) for vortex shocked 5 min, and then sat for 30 min, supernatants were collected and then were vacuum-dried for 6 h using a speed vacuum concentrator (Labconco, Kansas City, MO, USA). The derivative samples were re-dissolved in solution (acetonitrile:water=85:15, v/v) and then analyzed by Agilent E2695 (Agilent Technologies, Wilmington, DE, USA) equipped with Acclaim 120 C18 (4.6 mm×250 mm) and 2998 PDA detector. Samples were analyzed at 40°C using ethyl acetate:n-hexane=1:5 (v/v) as a mobile phase at a flow rate of 1 mL/min. The concentration of the acetaldehyde-DNPH (ACH-DNPH) was determined by the calibration curves of peak areas vs. the standard curve of acetaldehyde-DNPH, which were obtained from HPLC analysis. In addition, the concentration of acetaldehyde in the blank control was detected to calculate the volatilization of acetaldehyde during cultivation.

2.6 *Nucleotide sequence accession number*

Bacterial 16S rRNA sequences obtained in this study were deposited in GenBank under accession numbers KM873053-KM873119 and KM873133–KM873144.

3 Results

3.1 *Uncultivated acetaldehyde-degrading bacterial community composition of the West Pacific Ocean's deep sea in laboratory and in situ*

To analyze acetaldehyde-degrading bacterial community in deep sea water of the West Pacific Ocean, two sample treatments including enrichment in laboratory and *in situ* were used. To further measure the bacterial diversity presented within two different samples, a series of alpha diversity indices were used (Table 1). Coverages were 91.5% and 92.5% for enrichment in laboratory and *in situ*, respectively, which showed these data were authentic. Shannon, Simpson, and Chao1 diversity indices account for evenness and richness as well as the total number of species obtained. The overall diversity of bacterial populations was showed with Shannon diversity index of 2.29 and 2.07, Evenness index of 0.654 9 and 0.597 2, Chao1 richness estimator of 101 and 40.75 in sample of enrichment in laboratory and *in situ*, receptively.

Table 1. Analysis of the acetaldehyde-degrading bacterial diversity in deep-sea water from the South China Sea

	In laboratory	*In situ*
Numbers of sequenced clones	200	200
Numbers of OTUs	33	32
Coverages/%	91.5	92.5
Shannon diversity index (*H'*)	2.29	2.07
Evenness index (*E'*)	0.654 9	0.597 2
Chao1	101	40.75

As showed in Table 2, a total of 33 and 32 OTUs were obtained from 200 clones in sample of enrichment in laboratory and *in situ*, respectively. To identify these OTUs, BLASTn database was used for 16S rRNA gene identification, and then MEGA 5.0 was employed for phylogenetic analysis. In laboratory, three OTUs belonging to *Vibrio* genus were obtained: OTU ACH-14L-309 and ACH-14L-70 exhibited 99% and 97% 16S rRNA gene similarities to *Vibrio harveyi* ATCC BAA-1116, respectively. OTU ACH-14L-307 showed 98% 16S rRNA gene similarity to *Vibrio crassostreae* strain LGP 7, OTU ACH-14L-308 exhibited 99% 16S rRNA gene similarity to *Streptococcus pseudopneumoniae* IS7493, and OTU ACH-14L-306 showed 99% 16S rRNA gene similarity to *Halomonas meridiana* strain DSM 5425, OTU ACH-14L-67 showed 94% 16S rRNA gene similarity to *Thalassobacter stenotrophicus* strain 5SM22, OTU ACH-14L-86 exhibited 99% 16S rRNA gene similarity with *Staphylococcus caprae* strain ATCC 35538, OTU ACH-14L-287 showed 99% 16S rRNA gene similarity with *Marinobacter vinifirmus* strain FB1, and two OTUs which belonged to *Desulfuromusa* genus were obtained, namely OTU ACH-14L-77 and ACH-14L-60 which have showed 97% and 96% 16S rRNA gene similarities to *Desulfuromusa ferrireducens* strain 102 and *Desulforhopalus singaporensis* strain S'pore T1, respectively. *In situ*, two OTUs belonging to *Vibrio* genus were also obtained, namely OUT ACH-14S-98 and OTU ACH-14S-76 which have 99% 16S rRNA gene similarities to *Vibrio harveyi* ATCC BAA-1116 and *Vibrio chagasii* strain R-3712, respectively. OTU ACH-14S-95 showed 99% 16S rRNA gene similarity with *Halomonas meridiana* strain DSM 5425. OTU ACH-14S-85 exhibited 95% 16S rRNA gene similarity with *Pelagicola litoralis* strain CL-ES2. Four OTUs belonging to *marinobacter* genus were obtained: OTU ACH-14S-211, ACH-14S-74, ACH-14S-63 and ACH-14S-68. All of OTUs' information in laboratory or *in situ* was shown in Table 2.

3.2 *Comparing of uncultivated acetaldehyde-degrading bacterial diversity of the West Pacific Ocean's deep sea in laboratory and in situ*

Previous studies have showed that different ways of sample treatments could enrich different bacteria (Rochelle et al., 1994; Smalla et al., 2007). Eight bacterial phyla were detected, while two OTUs were unclassified. As showed in Fig. 1, the most predominant phyla was γ-proteobacteria (63.5%), followed by Firmicutes (14%), α-proteobacteria (6.5%), β-proteobacteria (5%), δ-

Table 2. Summary of the 16S rDNA sequences identified in the deep sea water of theSouth China Sea by enriching *in situ* or in laboratory

OTUs	Number of clones	Accession No.	Nearest relative	Identity/%
In situ				
ACH-14S-98	87	KM873087	*Vibrio harveyi* ATCC BAA-1116 (NR_102976.1)	99%
ACH-14S-76	10	KM873094	*Vibrio chagasii* strain R-3712 (NR_025480.1)	99%
ACH-14S-6	3	KM873102	*Escherichia fergusonii* ATCC 35469 (NR_074902.1)	99%
ACH-14S-233	1	KM873115	*Haemophilus parainfluenzae* ATCC 33392 (NR_042878.1)	99%
ACH-14S-24	1	KM873108	*Shewanella japonica* strain KMM 3299 (NR_025012.1)	99%
ACH-14S-88	9	KM873091	*Pseudoalteromonas donghaensis* strain HJ51 (NR_104537.1)	97%
ACH-14S-65	5	KM873091	*Pseudoalteromonas phenolica* strain O-BC30 (NR_028809.1)	98%
ACH-14S-276	1	KM873114	*Alteromonas hispanica* strain F-32 (NR_043274.1)	97%
ACH-14S-100	2	KM873109	*Alteromonas macleodii* str. 'Balearic Sea AD45 (NR_074797.1)	99%
ACH-14S-97	2	KM873088	*Idiomarina baltica* OS145 (NR_027560.1)	99%
ACH-14S-39	2	KM873105	*Colwellia psychrerythraea* strain ATCC 27364 (NR_037047.1)	98%
ACH-14S-211	1	KM873117	*Marinobacter adhaerens* HP15 (NR_074765.1)	99%
ACH-14S-74	4	KM873095	*Marinobacter vinifirmus* strain FB1 (NR_043666.1)	99%
ACH-14S-63	1	KM873100	*Marinobacter algicola* DG893 (NR_042807.1)	98%
ACH-14S-68	3	KM873098	*Marinobacter algicola* DG893 (NR_042807.1)	99%
ACH-14S-95	18	KM873095	*Halomonas meridiana* strain DSM 5425 (NR_042066.1)	99%
ACH-14S-81	1	KM873093	*Jannaschia* sp. CCS1 (NR_074163.1)	91%
ACH-14S-85	11	KM873092	*Pelagicola litoralis* strain CL-ES2 (NR_044158.1)	95%
ACH-14S-296	1	KM873111	*Sulfurovum lithotrophicum* strain 42BKT (NR_024802.1)	95%
ACH-14S-280	1	KM873112	*Sulfurimonas autotrophica* DSM 16294 (NR_074451.1)	92%
ACH-14S-70	1	KM873097	*Arcobacter nitrofigilis* DSM 7299 (NR_102873.1)	94%
ACH-14S-228	1	KM873116	*Arcobacter nitrofigilis* DSM 7299 (NR_102873.1)	92%
ACH-14S-90	5	KM873090	*Desulforhopalus singaporensis* strain S'pore T1 (NR_028742.1)	96%
ACH-14S-71	1	KM873096	*Micrococcus luteus* NCTC 2665 (NR_075062.1)	99%
ACH-14S-38	1	KM873106	*Corynebacterium tuberculostearicum* strain Medalle X (NR_028975.1)	99%
ACH-14S-60	3	KM873101	*Propionibacterium acnes* (NR_040847.1)	98%
ACH-14S-59	2	KM873103	*Streptococcus mitis* strain NS51 (NR_028664.1)	99%
ACH-14S-51	7	KM873104	*Dolosigranulum pigrum* strain R91/1468 (NR_026098.1)	99%
ACH-14S-201	1	KM873119	*Jeotgalibacillus marinus* strain 581 (NR_025351.1)	96%
ACH-14S-304	8	KM873110	*Bacillus subterraneus* strain COOI3B (NR_104749.1)	99%
ACH-14S-203	4	KM873118	*Bacillus niabensis* strain 4T19 (NR_043334.1)	98%
ACH-14S-279	1	KM873113	*Trichodesmium erythraeum* IMS101 (NR_074275.1)	87%
ACH-14S-32	1	KM873107	Uncultured bacterium gene for 16S ribosomal RNA (AB250585.1)	90%
In laboratory				
ACH-14L-309	68	KM873054	*Vibrio harveyi* ATCC BAA-1116 (NR_102976.1)	99%
ACH-14L-70	1	KM873071	*Vibrio harveyi* ATCC BAA-1116 (NR_102976.1)	97%
ACH-14L-307	21	KM873056	*Vibrio crassostreae* strain LGP 7 (NR_044078.1)	98%
ACH-14L-54	3	KM873076	*Escherichia coli* str. K-12 (NR_102804.1)	99%
ACH-14L-40	1	KM873077	*Haemophilus parainfluenzae* ATCC 33392 (NR_042878.1)	99%
ACH-14L-291	1	KM873060	*Shewanella japonica* strain KMM 3299 (NR_025012.1)	99%
ACH-14L-94	1	KM873064	*Pseudoalteromonas phenolica* strain O-BC30 (NR_028809.1)	97%
ACH-14L-272	4	KM873063	*Alteromonas macleodii* str. 'Balearic Sea AD45 (NR_074797.1)	99%
ACH-14L-283	5	KM873062	*Idiomarina baltica* OS145 (NR_027560.1)	99%
ACH-14L-31	1	KM873078	*Colwellia psychrerythraea* 34H (NR_074565.1)	95%
ACH-14L-74	1	KM873069	*Colwellia psychrerythraea* strain ATCC 27364 (NR_037047.1)	98%
ACH-14L-287	9	KM873061	*Marinobacter vinifirmus* strain FB1 (NR_043666.1)	99%
ACH-14L-306	11	KM873057	*Halomonas meridiana* strain DSM 5425 (NR_042066.1)	99%
ACH-14L-269	10	KM873081	*Neisseria meningitidis* MC58 strain MC58 (NR_103915.1)	98%
ACH-14L-56	1	KM873075	*Aureimonas altamirensis* strain S21B (NR_043764.1)	99%
ACH-14L-67	10	KM873072	*Thalassobacter stenotrophicus* strain 5SM22 (NR_027205.1)	94%
ACH-14L-215	1	KM873084	*Maritalea mobilis* strain E6 (NR_044447.1)	89%
ACH-14L-72	1	KM873070	*Pseudovibrio denitrificans* strain DN34 (NR_029112.1)	91%
ACH-14L-303	3	KM873058	*Sulfurovum* sp. NBC37-1 (NR_074503.1)	95%
ACH-14L-213	1	KM873085	*Arcobacter nitrofigilis* DSM 7299 (NR_102873.1)	95%

to be continued

Continued from Table 2

OTUs	Number of clones	Accession No.	Nearest relative	Identity/%
ACH-14L-207	1	KM873086	*Arcobacter nitrofigilis* DSM 7299 (NR_102873.1)	94%
ACH-14L-77	1	KM873068	*Desulfuromusa ferrireducens* strain 102 (NR_043214.1)	97%
ACH-14L-60	7	KM873074	*Desulforhopalus singaporensis* strain S'pore T1 (NR_028742.1)	96%
ACH-14L-310	1	KM873053	*Kocuria rhizophila* DC2201 (NR_074786.1)	99%
ACH-14L-245	1	KM873083	*Rothia mucilaginosa* DY-18 (NR_074690.1)	99%
ACH-14L-12	1	KM873080	*Veillonella criceti* strain ATCC 17747 (NR_025046.1)	96%
ACH-14L-308	12	KM873055	*Streptococcus pseudopneumoniae* IS7493 (NR_074987.1)	99%
ACH-14L-261	2	KM873082	*Gemella haemolysans* ATCC 10379 (NR_025903.1)	99%
ACH-14L-86	10	KM873066	*Staphylococcus caprae* strain ATCC 35538 (NR_024665.1)	99%
ACH-14L-88	1	KM873065	*Bacillus arsenicus* strain Con a/3 (NR_042217.1)	98%
ACH-14L-78	2	KM873067	*Bacillus subterraneus* strain COOI3B (NR_104749.1)	99%
ACH-14L-298	6	KM873059	*Trichodesmium erythraeum* IMS101 (NR_074275.1)	86%
ACH-14L-30	1	KM873079	Uncultured *Compostimonas* sp. clone 1_145 (KF758667.1)	98%

proteobacteria (4%), Cyanobacteria (3%), ε-proteobacteria (2.5%), Actinobacteria (1%) and 0.5% unclassified in sample of enrichment in laboratory. And γ-proteobacteria (75%) was also the most predominant phyla in sample of enrichment *in situ*, followed by Firmicutes (11%), α-proteobacteria (6%), δ-proteobacteria (2.5%), Actinobacteria (2.5%), ε-proteobacteria (2%), Cyanobacteria (0.5%) and 0.5% unclassified in sample of enrichment *in situ*, while β-proteobacteria was not obtained (Fig. 2). These results showed that bacteria of γ-proteobacteria were the main bacterial population whether sample enrichment performed in laboratory or *in situ*, and also predicted that these bacteria may play key roles in the degradation of acetaldehyde in sea. These results also indicated there was no significant difference between uncultivated acetaldehyde-degrading bacteria from sample of enrichment *in situ* and in laboratory in phyla level.

In genus level, uncultivated acetaldehyde-degrading bacteria community structure was different between enrichments in laboratory and *in situ* (Fig. 3). *In situ*, a total of 24 genus were detected from 200 clones, and *vibrio* was the most abundant genus accounting for 48.5% of all clones, followed by *halomonas* (9%), *pseudoalteromonas* (7%), *bacillus* (6%), *pelagicola* (5.5%), *marinobacter* (4.5%), *dolosigranulum* (3.5%), *desulforhopalus* (2.5%), *escherichia*, *alteromonas* and *propionibacterium* accounting for 1.5%, and the other genus were less than 1%. While in laboratory, a total of 27 genus were detected, and *vibrio* was also the most abundant genus accounting for 45% of all clones, followed by *streptococcus* (6%), *halomonas* (5.5%), *neisseria*, *thalassobacter* and *staphylococcus* (5%), *marinobacter* (4.5%), *desulforhopalus* (3.5%), *trichodesmium* (3%), *idiomarina* (2.5%), *alteromonas* (2%), *escherichia*, *sulfurovum* and *bacillus* accounting for 1.5%, and the other genus were less than 1%. Although uncultivated acetaldehyde-degrading bacteria community structure was different, *vibrio* was the most predominant genus whether enrichments were performed in laboratory or *in situ*, which was in accord with previous studies. These results maybe deduced that bacteria which belonged to *vibrio* genus were the main bacterial population for acetaldehyde-degrading bacteria community in deep sea.

3.3 *Isolation of acetaldehyde-degrading bacteria of the west Pacific Ocean's deep sea in laboratory and in situ*

To further screen and investigate the acetaldehyde-degrading bacteria of the West Pacific Ocean's deep sea, we have isolated cultivable bacteria from deep sea water which were enriched in laboratory and *in situ*. In laboratory, a total of four acetaldehyde-degrading strains were obtained (Table 3). Two strains belonging to *vibrio* genus were obtained, namely, strain ACH-L-1 and strain ACH-L-2 which exhibited 99% 16S rRNA gene similarities with *vibrio harveyi* ATCC BAA-1116 and *vibrio rotiferianus* CAIM 577, respectively. One strain ACH-L-5 showed 99% 16S rRNA gene similarity with *halomonas axialensis* strain Althf1 and another strain ACH-L-8 exhibited 99% 16S rRNA gene similarity with *halomonas meridiana* strain DSM 5425. While *in situ*, a total of eight acetaldehyde-degrading strains were isolated. Strain ACH-S-1 showed 99% 16S rRNA gene similarity to *vibrio rotiferianus* CAIM 577 and it was also obtained in laboratory. Another strain, namely, ACH-S-12 has shown 99% 16S rRNA gene similarity to *vibrio parahaemolyticus* RIMD 2210633. Strain ACH-S-6 which belonged to *halomonas* genus exhibited 16S rRNA gene similarity to *halomonas axialensis* strain Althf1. Furthermore, two strains as well as ACH-S-7 and ACH-S-10 have showed 97% and 99% 16S rRNA gene similarity to *pseudoalteromonas piscicida* strain IAM 12932 and *pseudomonas xanthomarina* strain KMM 1447, respectively. Strains ACH-S-8 and ACH-S-9 which showed 99% 16S rRNA gene similarity to *bacillus aquimaris* strain TF-12 were obtained. Another strain ACH-S-11 exhibited 99% 16S rRNA gene similarity to *pseudomonas xanthomarina* strain KMM 1447. These results showed that bacteria belonging to *vibrio* and *halomonas* were the main bacterial population for acetaldehyde-degrading bacterial community in deep sea. Furthermore, these results also showed that bacteria of *vibrio* and *halomonas* genus are more suitable for cultivation in laboratory's conditions than other bacteria.

3.4 *Ability of acetaldehyde-degrading for cultivable acetaldehyde-degrading bacteria*

To further investigate the ability of acetaldehyde-degrading of these strains, we have detected the tolerance of cultivable acetaldehyde-degrading bacteria suffering acetaldehyde and the degradation of acetaldehyde by using HPLC analysis. In Table 3, our results have showed that all of strains can grow in medium which contain high concentration of acetaldehyde (0.8–1.5 mg/mL). There were three strains, namely, ACH-L-5, ACH-L-8 and ACH-S-12 can tolerate 1.5 g/L acetaldehyde. Follow eight strains, ACH-L-2, ACH-S-1, ACH-S-6, ACH-S-7, ACH-S-10, ACH-S-11, ACH-S-8 and ACH-S-9 can suffer 1 g/L acetaldehyde. Strain ACH-L-1 can tolerate 0.8 g/L acetaldehyde. Then we have detected the ability of acetaldehyde-degrading of these strains by using acetaldehyde as sole carbon for growth. We have found that

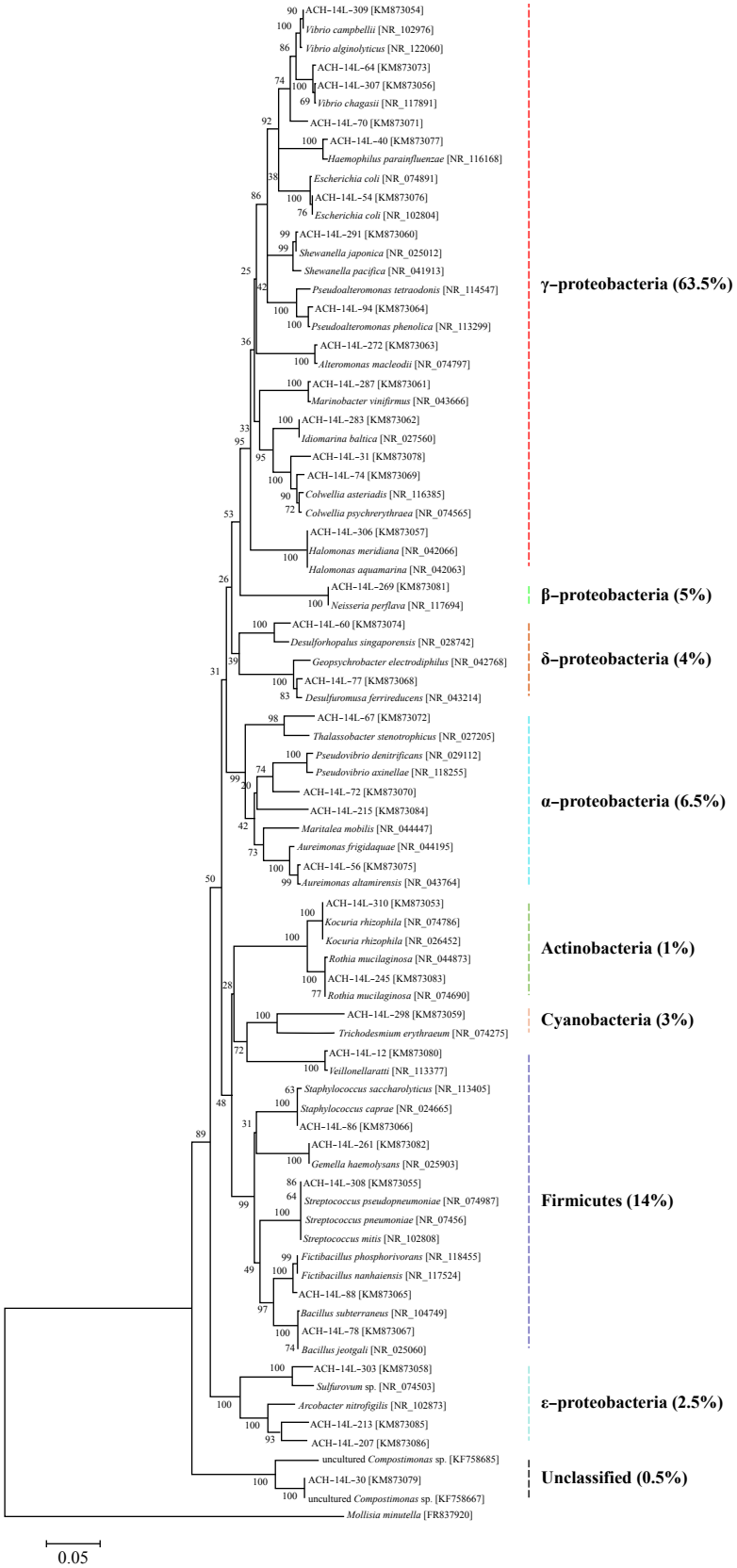

Fig. 1. Phylogenetic analysis of uncultured bacteria which enrichment in laboratory based on 16S rRNA genes sequences. All of bacterial 16S rRNA genes sequences are identified by accession and their ID number or their genus on the tree. The tree was constructed by neighbor-joining method. Bootstrap values (expressed as percentages of 1 000 replications) are shown at branching points.

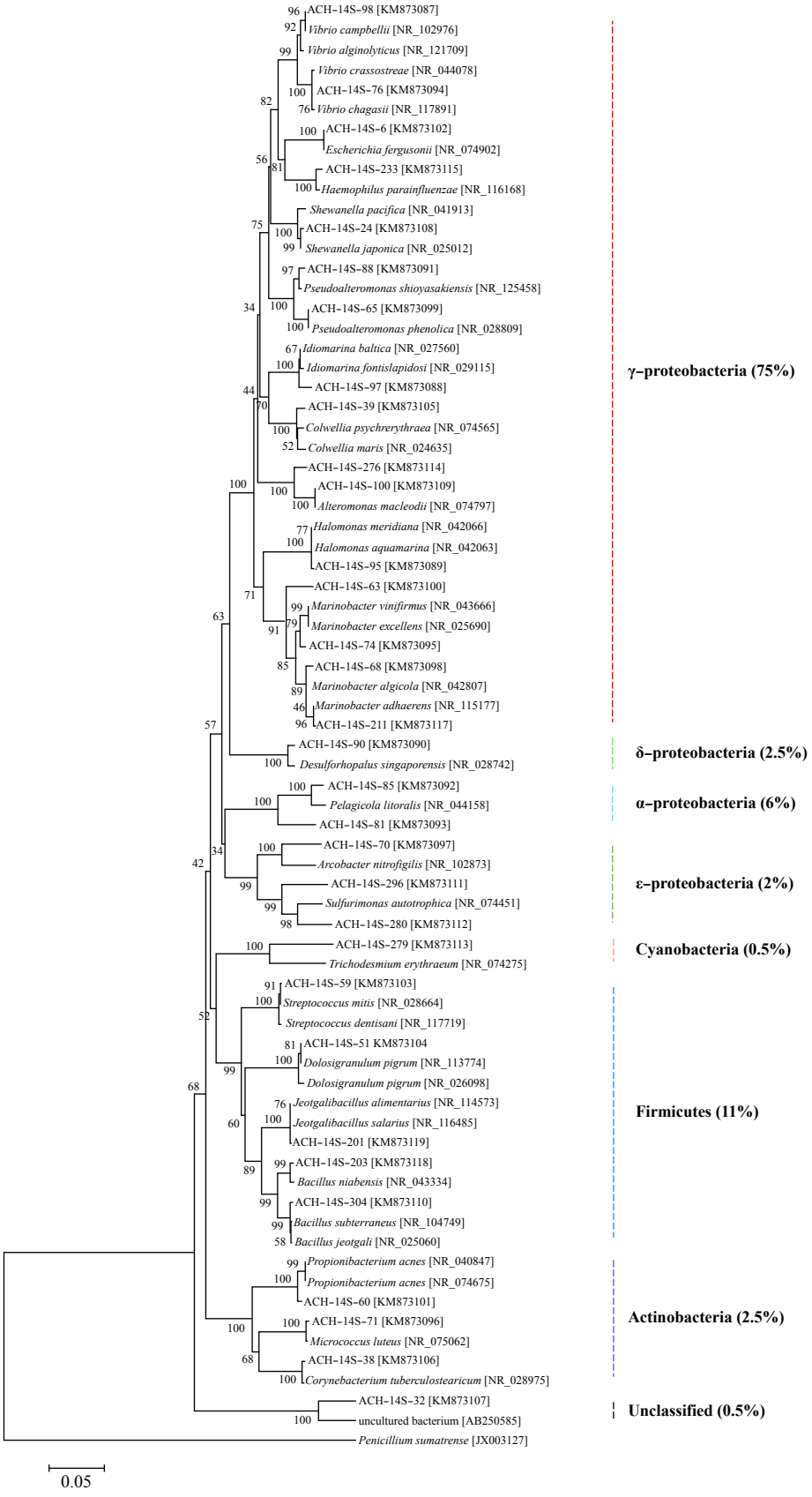

Fig. 2. Phylogenetic analysis of uncultured bacteria which enrichment *in situ* based on 16S rRNA genes sequences. All of bacterial 16S rRNA genes sequences are identified by accession and their ID number or their genus on the tree. The tree was constructed by neighbor-joining method. Bootstrap values (expressed as percentages of 1 000 replications) are shown at branching points.

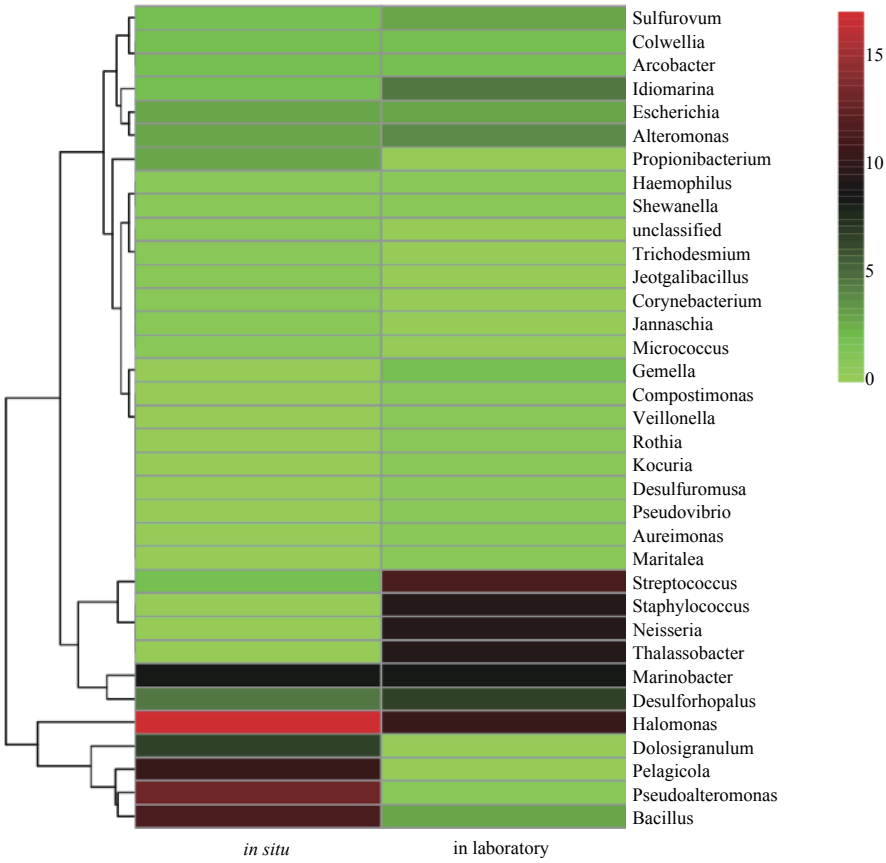

Fig. 3. Heatmap analysis of uncultured bacterial community structure at genus level (except genus *vibrio*) in enrichment in laboratory and *in situ*. The bacterial names are presented. Red indicates high abundance and green low abundance.

most of strains can degrade acetaldehyde. In particular, there were four strains, namely ACH-L-5, ACH-L-8, ACH-S-12 and ACH-S-6, could degrade all of acetaldehyde in medium (500 mg/L) after 24 h. Followed by strain ACH-S-11 which could degrade 82.94% acetaldehyde, strain ACH-S-9 was 73.92%, strain ACH-S-8 have degraded 55.44%, and strains ACH-L-2, ACH-L-1, ACH-S-7, ACH-S-1, ACH-S-10 have degraded 53.90%, 50.40%, 39.50%, 27.00% and 17.10% acetaldehyde, respectively. As shown in Table 4, these results showed that bacteria belonging to the genus *Halomonas* have better ability of acetaldehyde degradation than other genus' bacteria.

4 Discussion

Although, a large number of marine microorganism species have been reported, the vast majority was still unknown (Ding and Valentine, 2015; Arrigo, 2005). Meanwhile, marine microorganisms were more and more used in many fields, including biomedicine, industrial production, environmental management,

Table 3. Analysis of the acetaldehyde-degrading ability of cultured bacterial strains from the deep sea water of the South China Sea by enriching *in situ* or in laboratory

Strain	Accession No.	Nearest relative	Identity/%	MCTA	DA
In laboratory					
ACH-L-1	KM873133	*Vibrio harveyi* ATCC BAA-1116 (NR_102976.1)	99	0.8	50.40
ACH-L-2	KM873134	*Vibrio rotiferianus* CAIM 577 (NR_042081.1)	99	1	53.90
ACH-L-5	KM873135	*Halomonas axialensis* strain Althf1 (NR_027219.1)	99	1.5	100
ACH-L-8	KM873140	*Halomonas meridiana* strain DSM 5425 (NR_042066.1)	99	1.5	100
In situ					
ACH-S-1	KM873138	*Vibrio rotiferianus* CAIM 577 (NR_042081.1)	99	1	27.00
ACH-S-12	KM873144	*Vibrio parahaemolyticus* RIMD 2210633 (NR_074196.1)	99	1.5	100
ACH-S-6	KM873138	*Halomonas axialensis* strain Althf1 (NR_027219.1)	99	1	100
ACH-S-7	KM873139	*Pseudoalteromonas donghaensis* strain HJ51 (NR_104537.1)	97	1	39.50
ACH-S-10	KM873142	*Pseudoalteromonas piscicida* strain IAM 12932 (NR_040946.1)	99	1	17.10
ACH-S-11	KM873143	*Pseudomonas xanthomarina* strain KMM 1447 (NR_041044.1)	99	1	82.94
ACH-S-8	KM873140	*Bacillus aquimaris* strain TF-12 (NR_025241.1)	99	1	55.44
ACH-S-9	KM873141	*Bacillus aquimaris* strain TF-12 (NR_025241.1)	99	1	73.92

Note: MCTA represents the max tolerable concentration of acetaldehyde (g/L) and DA the degradation rate of acetaldehyde (%).

Table 4. The ability of acetaldehyde-degrading in bacterial strains

Strain	Degradation of acetaldehyde every 24 h/mg·g^{-1}	Reference
Metschnikowia pulcherrima C6	662.4	Li and de Orduña (2011)
Zygosaccharomyces bailii C23	547.2	Li and de Orduña (2011)
Candida stellata C35	720.0	Li and de Orduña (2011)
Hansenula anomala C4	345.6	Li and de Orduña (2011)
Candida vini C2	360.0	Li and de Orduña (2011)
Hanseniaspora uvarum C1	604.8	Li and de Orduña (2011)
Schizo- saccharomyces pombe C7	57.6	Li and de Orduña (2011)
ACH-L-5	833.3	this study
ACH-L-8	684.9	this study
ACH-S-12	609.8	this study
ACH-S-6	675.7	this study

and the study of marine microorganisms always was one of the hot spots over the last decades (Gao et al., 2017; Bernan et al., 1997; Kelecom, 2002). Deep sea water could also harbor many microorganisms, especially some typical microorganisms with bioactive functions, such as acetaldehyde-degrading bacteria and polysaccharides-degrading bacteria (Horikoshi, 1998; Koyama and Yoshida, 2016). In previous studies, bacterial diversity studies were analyzed by using streak plate method, sequencing of 16S rRNA (Caporaso et al., 2011). While most of these studies were performed in laboratory. To further investigate the diversity of acetaldehyde-degrading bacteria in the West Pacific Ocean's deep sea. We have analyzed the diversity of cultured and uncultured acetaldehyde-degrading bacteria of deep sea water which were enriched in laboratory and *in situ* by using streak plate method and sequencing of 16S rRNA. Meanwhile, we also have detected the ability of acetaldehyde-degradation of these cultured bacteria. Our studies have showed that α, γ, δ, ε-Proteobacteria, Actinobacteria, Firmicutes, Cyanobacteria dominated the bacterial community by sequencing of 16S rRNA in deep sea water with enrichments in laboratory or *in situ*. But there were only some strains which belonged to γ-Proteobacteria and Firmicutes were obtained by using streak plate method. Meanwhile, these results indicated that most of acetaldehyde-degrading bacteria in deep sea water were not suitable for growth in laboratory's conditions. On the other hand, as shown in Table 1, the uncultured bacterial diversity richness which enrichment in laboratory is better than enrichment *in situ*. Although the bacterial diversity richness in laboratory is better than *in situ*, but the main bacterial community structures are similar. Thus, our study has showed that bacteria of *Vibrio* and *Halomonas* composed the dominant population. Conversely, we have obtained more acetaldehyde-degrading bacterial strains *in situ* than in laboratory. It seems that more uncultured bacteria were enriched in laboratory conditions than *in situ* conditions, while bacterial strains were suitable grown *in situ* environments. We have also demonstrated that these bacteria belonging to genus *Vibrio* and *Halomona* played a key role in degradation of aldehydes material of marine.

In phylum level, previous studies have demonstrated that bacteria of Proteobacteria dominated bacterial community in environments, contain α, γ, δ, and ε-Proteobacteria (O'Sullivan et al., 2002; Franzmann, 1996; Borneman and Triplett, 1997). Previous studies of marine bacterial diversity have showed that bacteria belonging to α-Proteobacteria were the main uncultured bacterial species, while the main cultured bacterial species were the γ-Proteobacteria (Borneman and Triplett, 1997). Interestingly, in this study, γ-Proteobacteria were the dominating bacteria whether cultured or uncultured bacteria. In genus level, *Vibrio* and *Halomonas* were the dominating genus of uncultured bacteria, and we have obtained some strains belonging to *Vibrio* and *Halomonas*, especially only strains of *Vibrio* and *Halomona* were found from enrichment in laboratory. The results of detecting ability of acetaldehyde-degrading have showed these strains belonging to *Vibrio* and *Halomona* were better than the other strains for the ability of acetaldehyde-degrading. It seems that bacteria of *Vibrio* and *Halomona* play a key role in the degradation of acetaldehyde in deep sea water. Similarly, previous studies have reported that bacteria of *Vibrio* and *Halomona* could grow in the high aldehydes concentration (Vedadi and Meighen, 1997; Sripo et al., 2002), but further and systemic investigation should be carried out. In our study, we investigated the diversity of bacteria of acetaldehyde-degrading in deep sea water of the West Pacific Ocean, and compared the different diversity of acetaldehyde-degrading bacteria between with two samples which enrichment *in situ* and in laboratory. Some acetaldehyde-degrading bacterial strains were obtained, especially four strains belonging to *Vibrio* and three strains of *Halomonas*, which have potentials in degradation of aldehydes material.

Previous studies have reported a strain, namely *Acetobacter pasteurianus* belonging to Rhodobacterales, α-Proteobacteria, which have utilized acetaldehyde or ethanol in medium as carbon source for growth (Kanchanarach et al., 2010; Takemura et al., 1993). In this study, 16S rRNA genes of Rhodobacterales and Rhizobiales were also found, it indicates that these bacteria belonging to α-Proteobacteria play key roles in carbon or nitrogen cycles in marine environments. Unfortunately, bacterial strains of α-Proteobacteria were not isolated because of unsuitable culture condition. Previous reports have investigated typical marine bacterial diversity and found that these bacteria belonging to β-Proteobacteria mainly survive in estuary or nearshore area and almost not distribute in deep sea (Sekiguchi et al., 2002; Wu et al., 2004). Similarly, our studies do not find bacteria belonging to β-Proteobacteria, except one 16S rRNA gene from enrichment *in situ*. The bacteria of δ, ε-Proteobacteria which have ability of sulfate reducing or sulfur-oxidizing were frequently found in hydrothermal area and cold seep of deep sea. Meanwhile, in this study, we also have obtained 16S rRNA genes of δ, ε-Proteobacteria from the deep sea water whether enrichment *in situ* or in laboratory. These results may suggest that these bacteria may play important roles in marine carbon cycling and nitrogen cycling.

Bacteria of Firmicutes were frequently isolated from offshore and sediment in shallow sea, and there were debatable about it was marine aborigines or originate from mainland (Ravenschlag et al., 1999). Meanwhile, the bacteria belonging to *Enterococcus*,

Planomicrobium, *Bacillus*, have showed that the ability of degrading acetaldehyde. In this study, not only 16S rRNA genes of Firmicutes were detected, but also cultured bacteria of *Enterococcus*, *Planomicrobium*, *Bacillus* (belong to Firmicutes) were isolated. As far as we know, this was the first reportthat the cultured bacteria of Firmicutes from deep sea could degrade acetaldehyde.

As we all know that there are a larger number of microorganism species in ocean, and many of marine microorganisms have excellent bioactivities for application (Kobayashi and Ishibashi, 1993; Okami et al., 1976; Manivasagan et al., 2016). Unfortunately, most of marine microorganisms are still unknown. In our study, we have screened 16S rRNA genes of uncultured bacteria and isolated cultured bacteria from the deep sea water of the West Pacific Ocean by using enrichment *in situ* and in the laboratory, and then detected the ability of acetaldehyde-degradation of cultured bacteria, respectively. Our study showed that the γ-Proteobacteria (occupied 80% clones) was dominant in the deep sea water of the West Pacific Ocean, especially these bacteria belonging to *Vibrio* and *Halomonas* were widely distributed in deep sea environments and composed the main acetaldehyde-degrading bacterial community in deep sea water whether enrichment *in situ* or laboratory. As far as we known, this is the first systematic report of acetaldehyde-degrading bacteria in the deep sea water of the West Pacific Ocean. Our study will provide a reference for investigation of acetaldehyde-degrading bacteria in the West Pacific Ocean. Based on above investigations, the potential applications of acetaldehyde-degrading bacterial stains from the deep sea water of the West Pacific Ocean was suggested.

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