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ARCHAEA BIODIVERSITY FROM CHOL BURI MANGROVE FOREST, THAILAND

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Abstract: **Mangrove forests are one of the most typical coastline ecosystems in Thailand having importance for ecology of the country and exhibiting high biodiversity. The aim of the study was to characterize microbial community as well as to estimate methanogenesis in these mangrove sediments. The phylogenetic trees constructed from 16S rDNA of archaeal library revealed the strong dominance of two phylogenetic groups: ammonium oxidizers and methanogens. It was shown that the dominance of the classes *Delta-* and *Gammaproteobacteria* were dominant microbial groups in bacterial community. The former included sulphate-reducers from the genera *Desulfobulbus*, *Desulfobacterium* and *Desulfopila*. Archaeal community was dominated by ammonium oxidizers belonging to Candidatus *Nitrosopumilus* and methanogens from the orders *Methanomicrobiales* and *Methanosarcinales*. Methanogenesis in the samples incubated at 25°C has been strongly stimulated by trimethylamine and methanol, indicating the importance of methylotrophic pathway of CH_4 production. The 16S rRNA gene copy number of *Archaea* was approximatly two times higher than that of *Bacteria* according to qPCR. Statistical analysis revealed much higher diversity of bacteria compared to archaea. The microbial community of Chol Buri mangrove sediments comprises different trophic groups with predominance of sulphate-reducing bacteria as well as ammonia-oxidizing and methanogenic archaea. Thus, the 16S rRNA gene analysis exhibited the high microbial diversity in the sediments of Chol Buri mangrove of Thailand. The coexistence of two functional archaeal groups, namely anaerobic methanogens and aerobic ammonia-oxidizers which are highly abundant in the same sample site can be explained by the specific hydrology of mangrove ecosystems with highly varying red-ox potential resulted from the daily tidal period. The abundance of the above described three main functional groups of microorganisms in the sediment of Chol Buri mangrove in Thailand could be an indication of the primary importance of appropriative metabolic processes: sulphate reduction, ammonia-oxidation and methanogenesis. Methanogens and sulphate-reducers are likely to be the main terminal microbial groups in the anaerobic community of mangrove. This work is a contribution to the study of microbial diversity and microbial processes in mangrove ecosystems.**

Keywords:* *Mangrove, Archaea, ammonium oxidizers, methanogens

I. INTRODUCTION

Mangroves are coastal wetland forests which occupy about 1.8×10^5 km^2 of tropical and subtropical coastlines world-wide [1]. They are located in the intertidal zone that is frequently inundated. The mangroves play an important role in the regulation and optimization of tropical marine environments. It is a complex ecosystem with highly interactive plants, animals and microorganisms. The latter are composed of a combination of microorganisms from terrestrial soil, marine and freshwater ecosystems [2]. The mangrove forests are recognized as one of the most productive ecosystems of the world and are characterized by high turnover rates of organic matter and nutrient recycling between the ocean and terrestrial habitats [3, 4]. It provides the base for a large and complex food web and various ecological niches to different microorganisms playing major roles in degradation processes with

producing detritus that is organic matter in the active process of degradation. Mangroves act as sources of greenhouse gases, methane and nitrous oxide in particular [5-7]. In addition, some mangroves can receive high nutrient inputs due to anthropogenic activities [1, 3, 5], which can further stimulate CH_4 and N_2O emissions. Due to the influence of marine environment, mangroves exhibit salinity similar to sea water. Salinity has been shown to be the primary driver of changes in vegetation type and sediment microbial community [8].

Mangrove sediments are mainly anaerobic with an overlying thin aerobic zone, although the redox status of soils in tidal ecosystems can fluctuate in response to the tidal cycle [9]. In the anaerobic layer decomposition occurs mainly through sulphate-reduction and methanogenesis [10, 11]. These microbial processes are responsible for the terminal electron removal during decomposition of organic matter and also play important role in different saline environments [12-14].

There are reports on various aspects of study of mangroves from different continents, in North America [8], Central America [15], Africa [10] and Australia [5]. Nevertheless, the highest species diversity and area extent is characteristic of tropical mangroves in South Asia. In opposite to mangroves of India [7] and China [16, 17], the Thai mangroves located in the South-East Asia are still remaining poor studied ecosystems regarding microbial processes and diversity. Thailand comprises three different types of mangroves according to classification given by Lugo and Snedaker [18]: fringe, riverine and overwash forests. The fringe mangroves are the most extensive coastal ecosystem in Thailand and have an important ecological, climatic and industrial significance for the country. Up to now, there is a lack of data on the diversity of bacteria inhabiting mangroves. Very limited data are available on archaeal composition in mangrove sediments. They are mostly obtained by cultivation-based analysis that identifies only a small part of native microbial population [19, 20]. There is no information on microbial community and its metabolic potential in Thai mangroves. The aim of the study was to characterize microbial community inhabiting the sediments of Chol Buri Mangrove located close to one of the most populated area in Thailand near to Bangkok. Molecular biological methods based on culture-independent techniques such as 16S rRNA gene cloning and quantitative polymerase chain reaction (qPCR) have been applied to genetically identify the structure of bacterial and archaeal community. The potential and the composition of methanogenic archaea in the mangroves have been also estimated. The mangrove samples were incubated in the presence of selective substrates in order to estimate the metabolic potential of different microbial groups, in particular methanogens.

II. EXPERIMENTAL SECTION

2.1 Study areas

The 38 km^2 Chol Buri mangrove locates in Chonburi province, which lies approximately 80 kilometers southeast of Bangkok in the eastern part of the Gulf of Thailand. The Gulf covers approximately 515 km and includes about 470 km^2 of mangrove area [22]. The annual precipitation is about 2660 mm. The mean temperature is about 29°C, for the relative humidity comprising between 80–85%. The average salinity is 2.9% (w/v). The pH of water is 7.2 [22]. The concentration of the main ions was (in mg l-1): Na^+ (2150), K^+ (92), Ca^{2+} (100), Mg^{2+} (118), Cl^- (2370), NO^{3-} (1.0), SO_4^{2-} (780). Sediment samples were immediately transferred to sterile 1 L glass bottles while flushing with nitrogen to keep the anaerobic conditions. The bottles were tightly closed and transported on ice to the laboratory and stored at +4°C until further analysis.

2.2 Incubation experiments

The mangrove samples were handled anoxically under a N_2 atmosphere. An aliquot (15 ml) was placed into a sterile glass bottle (60 ml), gassed with N_2, closed with a sterile black butyl rubber stopper. The samples then were incubated in triplicates in darkness without shaking. Depending on the aims of the experiments, the samples were supplemented by different substrates (glucose, acetate, methanol, trimethyamine (TMA), formate or gas mixture H_2 and CO_2 in the ratio 4:1) and incubated at 25°C. The final concentration of substrate in the incubated samples was 20 mM for each soluble compound whereas 10 ml of the gas mixture were added into the head space.

2.3 Analytical techniques

Methane and CO_2 were analysed on a gas chromatograph with flame ionization detector (Carlo Erba, Milan, Italy). Carbon dioxide was determined after conversion to CH_4 using a methanizer (Ni-Catalyst at 330°C, Chromopak, Middelburg, Netherlands). Inorganic anions were detected by ionic high performance liquid chromatograph (IC-1000, Biotronik, Germany) using a conductivity detector. Separation column BT II An and suppression column BT S AG were used for the analysis. The flow rate of the eluent (2.6 mM bicarbonate buffer) was 1.2 ml min^{-1}. The volume of the sample was 30 ml. Detection limit was 0.1 mg l^{-1}.

2.4 DNA extraction and PCR amplification

DNA extraction was ca. 10 g of wet sediment samples using DNA Clean Mega Soil Kit (MoBio, USA) according to the manufacturer's instructions. The primer pair 27f and 1492r (Table 1) was used for PCR to amplified the bacterial 16S rRNA gene. The archaeal 16S rRNA gene was amplified by using the primer pair 23f and ARCH915r (Table 1). Each 50 µl of PCR reaction contained 10 ng of template DNA, 10 pmol of each primer, 80 µM dNTP, 1× buffer B and 1.25 U Taq DNA polymerase (EuRx, Poland). Cycling parameters consisted of 94°C of initial denaturation for 2 minutes, followed by 30 cycles of 94°C denaturation for 30s, 30s for annealing and 72°C extension for 1 min, and a final extension step of 72°C for 3min. The annealing temperatures for bacterial primer pair was 52°C and 55°C for the archaeal one.

Table 1 PCR primers used in this study

Primer	Sequence 5'→3'	Reference
23f	TCYGGTTGATCCTGCC	[23]
27f	AGAGTTTGATCMTGGCTCAG	[24]
364af	CGGGGYGCASCAGGCGCGAA	[25]
519f	CAGCMGCCGCGGTAANWC	[24]
907r	CCGTCAATTCMTTTRAGTTT	[24]
ARC915r	GTGCTCCCCCGCCAATTCCT	[26]
1492r	TACGGYTACCTTGTTACGACTT	[24]

2.5 Cloning of PCR products

PCR products were purified using MinElute (QIAGEN, Germany). Cloning was conducted using pGEM-T vector system II (Promega, Germany). PCR products (100 ng) were ligated to pGEM-T vector and transformed into Escherichia coli JM109 competent cells. Transformants were plated on LB agar with Ampicilin/IPTG/X-Gal. Plates were incubated overnight at 37°C. Positive clones were selected and cultured overnight at 37°C in LB broth with 100 mg/ml Ampicilin. Recombinant plasmid was extracted using Montage Plasmid Miniprep96 Kit (Millipore, Germany) according to the manufacturer's instructions.

2.6 Sequence analysis

Recombinant plasmid was sequenced twice using M13 forward and reverse primers (Fermentas, Germany) with Big Dye reagent kit (ABI, Germany). Sequencing was performed on ABI Genetic Analyzer (AB3130XL) (ABI, Germany). Chimeric artifacts were removed using Bellerophon [27] and Mallard [28]. Non-chimeric sequences were subsequently compared to the sequences deposited at Genbank using BLAST [29].

2.7 Computational analysis

Sequences were aligned using MUSCLE 3.7 [30] with closely related strains and environmental clones. Evolutionary models for the data sets were calculated using Modeltest 3.8 [31]. Bayesian inference (BI) was carried out with MrBayes 3.1.2 [32]. Bayesian posterior probabilities (BPP) were obtained by performing two separate runs with four Markov chains (MC). Each run was conducted with 4×10^6 generations and sampled every 100 generations. Convergence was checked by examining the generation plot visualized with TRACER 1.4 [33] and computing the potential scale reduction factor (PSRF) with the sump command in MrBayes 3.1.2. A consensus tree was calculated after discarding the first 25% of the iterations as burn-in. Aligned sequences were clustered into operational taxonomic units (OTUs) using DOTUR program [21]. Jukes-Cantor evolutionary distances were calculated using DNADIST of Phylip 3.68 package [34].

2.8 Conditions of qPCR and data analysis

Amplification of 16S rRNA gene of archaea and bacteria gene was performed with primer sets 364f and ARC915r, 519f and 907r respectively (Table 1). All reactions (25 µl) contained: 12.5 µl SYBR Green Ready Mix (Bio-Rad, Hercules, CA) (1×), 4.0 µl MgCl2 (25 mM), 0.25 µl from 50 µM of each primer, 0.25 µl BSA (Bovine Serum Albumin), (20 µg/µl), 0.25 µl FITC (Fluorescein Isothiocyanate) (1/1000), and 2 ng of template DNA. Cycling conditions consisted of an initial 3 min denaturation step at 94°C, followed by 45 cycles of repeated denaturation at 94°C for 45 sec, annealing at 52°C for 30 sec, and extension step at 72°C for 45 sec. PCR was carried out in iCycler thermal cycler (Bio-Rad, Hercules, CA). qPCR assays with C_T values over 40 were considered negative. For each PCR run, a negative (no-template) control was used to test for false-positive results or contamination. Absence of nonspecific products or primer dimmers was confirmed by observation of a single melting peak in a melting curve analysis. Calibration curves were generated using *Anoxybacillus flavithermus* DSM 2641, *Methanosarcina lacustris* DSM 13486 as templates for quantification of bacteria and archaea respectively. Different 16S rRNA gene copy numbers in a range of 100-107 were amplified. Logarithmic values of different 16S rRNA gene copy numbers were plotted against threshold cycle number from qPCR assays. To select the linear standard curves, r^2 of the slope was chosen for the value at least equal to 0.993. Experiments were carried out in triplicate. Appropriate dilutions of sediment samples were done prior to amplification in order to fit the C_T value in the linear range of

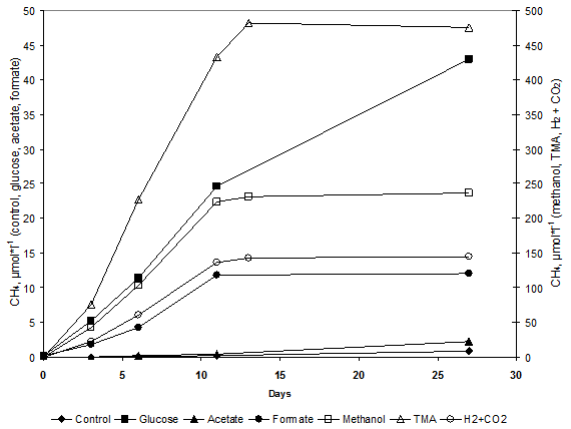
each standard curve. Number of 16S gene copies was determined by comparing the C_T values of each sample to their corresponding standard curves. Data collection and statistical analysis were performed using CFX Manager 1.1 (Bio-Rad, Germany).

III. RESULTS AND DISCUSSION

3.1 Incubation experiments

Methanogenesis has been observed in all native samples incubated at 25°C with the rate of 4.6 nmol $l^{-1}day^{-1}$. It was highly and immediately stimulated by the addition of mostly C1-compounds such as TMA and methanol as well as by H_2+CO_2 gas mixture (Figure 1). Methane production was also slightly stimulated with glucose, formate and acetate as substrates. In the samples supplemented with glucose, the first stage was its fast fermentation with production of CO_2. The second stage was methanogenesis and seemed to be initiated by the excess of carbon dioxide.

Figure 1. Methanogenesis in the Chol Buri mangrove samples supplemented with different substrates at 25°C.



3.2 Analysis of 16S rRNA gene

From the archaeal library, 93 clones were analyzed. Five sequences were found not to have any similarity to known sequences from public database. They were discarded from further analysis. Two distinct phyla were detected in the archaeal library from the sediment of the Chol Buri mangrove: The Candidatus *Thaumarchaeaota*, accounting for 28.1% of the total library and the phylum *Euryarchaeota*, accounting for 79.1% of the total archaeal library (Figure 2 and 3). A total of 25 sequences from 88 archaeal sequences of the clone library showed 93-99% similar to the specie *Nitrosopumilus maritimus*, which was the dominant specie in the archaeal library. The phylum *Euryarchaeaota* included 64 sequences grouped in four lineages of the archaeal domain: Methanogen, *Thermoplasmatales*, Candidatus *Nitrososphaera* and Uncultured euryarchaote. A total of 26 sequences accounting for 40.6% of the euryarchaeotal community were grouped in three methanogenic orders: *Methanobacteriales*, *Methanosarcinales* and *Methanomicrobiales*. Members of the order *Methanobacteriales* represented the most abundant methanogenic community (57.7% of the total methanogenic community). A total of seven clones were affiliated to representatives of the genus *Methanobacterium*, four to the genus *Methanothermobacter*, three to the genus *Methanobrevibacter* and two to the genus *Methanoculleus*. These genera are known to perform the hydrogen-dependent methanogenesis. With 8 clones accounting for 30.8% of the total methanogenic community, the *Methanosarcinales* represented the second important methanogenic group. A total of three clones were affiliated to the genus *Methanolobus*, known to have high affinity to methanol and methylated compounds such as trimethylamine. Two clones were affiliated to acetate-obligate methanogen of the genus *Methanosaeta*. The genus *Methanococcoides* was represented by two clones and the genus *Methanomethylovorans* by one clone. *Methanomicrobiales* accounted for 11.5% of the total methanogenic community. They were all affiliated to the members of the genus *Methanoculleus*. Sequences representing the *Thermoplasmatales* were all related to uncultured *Thermoplasmatales* archaeon. They accounted for 11.2% of the total archaeal community. The uncultured formed the most important group, with 26 sequences of a total of 88, accounting for 29.5% of the total euryarchaeotal community.

From the bacterial clone library, 92 clones were analyzed. The phylogenetic analysis revealed that the bacterial clones represented 8 major lineages of the bacterial domain: *Acidobacteria*, *Bacteroides*, *Chloroflexi*, *Nitrospirales*, *Planctomycetales*, *Proteobacteria*, *Spirochaetes* and *Verrucomicrobia* (Figure 4 and 5). A total of 61 from 90 clones accounting for 67.9% of the bacterial clone library were grouped within the five subdivisions of *Proteobacteria*. The *Deltaproteobacteria* were the most abundant Proteobacterial group (43.5% of the total proteobacterial community) and accounted with the *Gammaproteobacteria* for 80% of the total proteobacterial community, pointing out their important role in the community. Many of the sequences of the *Deltaproteobacteria* were phylogenetically affiliated with members of the sulphate and elemental sulfur reducers belonging to genera *Desulfobulbus*, *Desulfobacterium*, *Desulfopila*, *Synthrophobacter* and iron (III) reducers belonging to genera *Desulfuromonas*, *Geobacter* and *Pelobacter*. The unique sequence representing the subdivision *Betaproteobacteria*

showed 87% similarity to *Burkholderia andropogonis*. Nine clones, accounting for 14.5% of the total proteobacterial community felt phylogenetically in the subdivision of *Alphaproteobacteria*. Two clones branched with the 16S rRNA gene of *Sulfurovum* sp. that represented the subdivision *Epsilonproteobacteria*, accounting for 3.2% of the total proteobacterial community. The *Bacteroides*, with 13 sequences, represented 14.4% of the total bacterial library and found to be the second important bacterial group. Three sequences were phylogenetically associated to *Cytophaga* sp. and two were branched with 16S rRNA gene of *Rhodothermus marinus*. Each of the eight sequences remaining clustered with cultivated organisms showing similarity of 82-95%. Each of other bacterial groups identified had no more than four sequences, representing less than 5% of the total bacterial community.

Figure 2. Phylogenetic tree was constructed based on archaeal 16S rRNA gene using MrBayes. Cenarchaeum symbiosum was used as outgroup. GTR+I+G model was selected for phylogenetic construction. PSRF values were 1.000 (all parameters). Average standard deviation was close to 0.001. Two runs of MC were convergent after TRACER analysis (data not shown). Four million generations of MC were sufficient and the two runs of analysis converged onto stationary distribution.

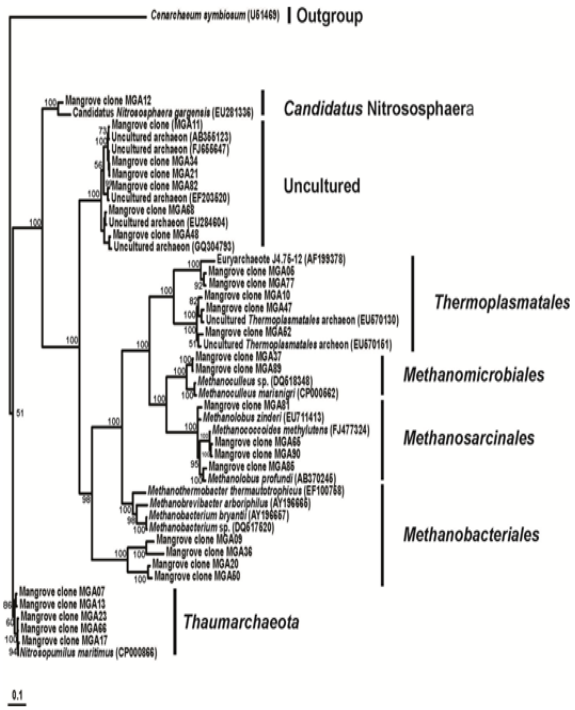


Figure 3. Proportion of different archaeal groups: MB: Methanobacteriales; MM: Methanomicrobiales; MS: Methanosarcinales; TA: Thaumarchaeota; TP: Thermoplasmatales; UA: Uncultured archaeon; CN: Candidatus Nitrososphaera

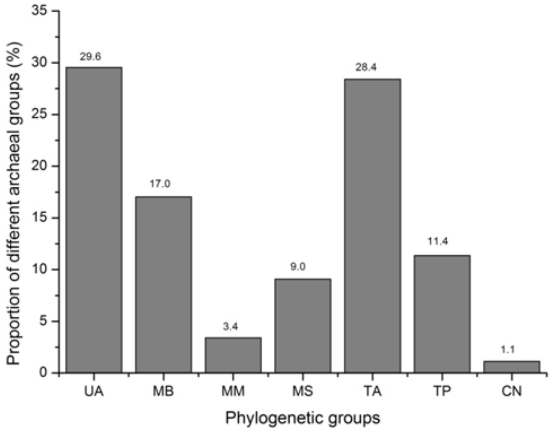


Figure 4. Phylogenetic tree was constructed based on bacterial 16S rRNA gene using MrBayes. *Aquifex pyrophylus* was used as outgroup. GTR+I+G model was selected for phylogenetic construction. PSRF values were 1.000 (all parameters). Average standard deviation was close to 0.001. Two runs of MC were convergent after TRACER analysis (data not shown). Four million generations of MC were sufficient and the two runs of analysis converged onto stationary distribution.

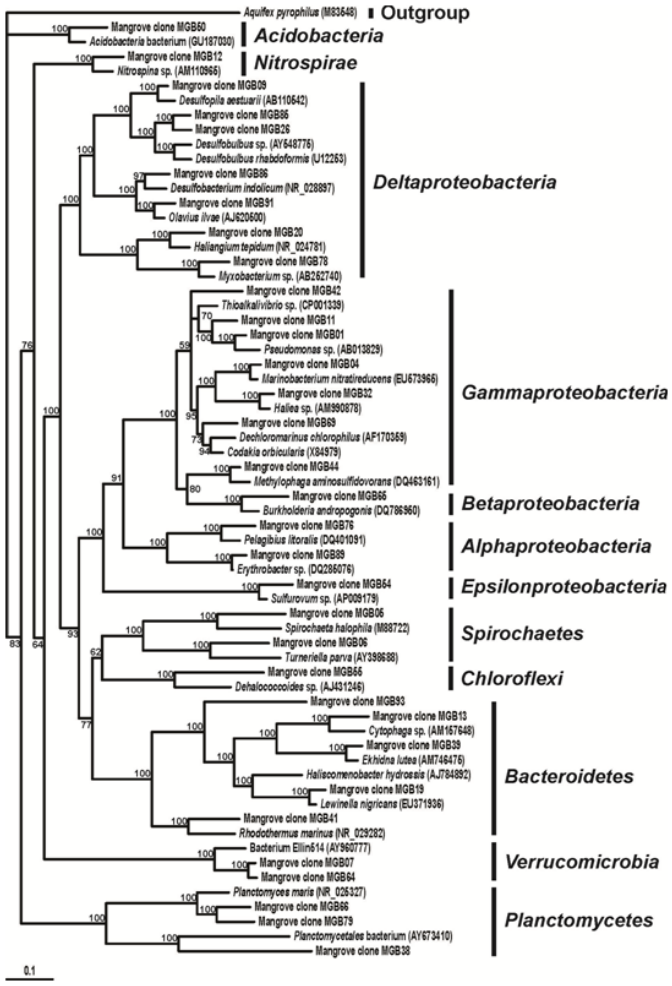
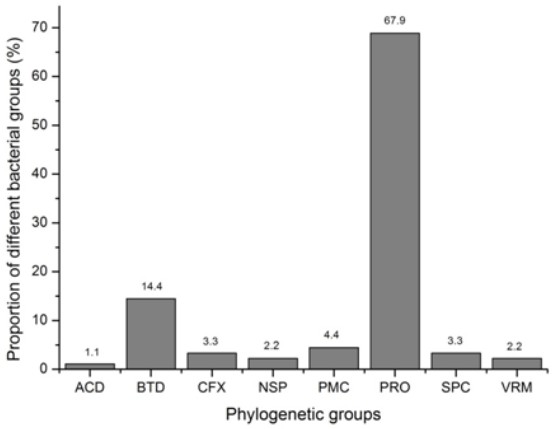


Figure 5. Proportion of different bacterial groups: ACD: *Acidobacteria;* BTD: *Bacteroidetes;* CFX: *Chloroflexi;* NSP: *Nitrospirae;* PMC: *Planctomycetes;* PRO: *Proteobacteria;* SPC: ***Spirochaetes;*** **VRM:** *Verrucomicrobia*



3.3 Analysis of qPCR

Quantification of 16S rRNA gene of *Bacteria* and *Archaea* was performed by using real-time PCR assays. The copy number of 16S rRNA gene of *Archaea* ($2.7\times10^6\pm 7.34\times10^4$) was approximately two times higher than that of *Bacteria* ($1.3\times10^6\pm 1.49\times10^4$).

3.4 Diversity indices and statistical analysis

The diversity indices and the richness estimators were calculated and the randomized rarefaction curve constructed using DOTUR program. Once DOTUR assigns sequences to OTUs, it performs a random sampling without replacement procedure. The probability of drawing a representative from an OTU is the number of times the OTU was observed divided by the total number of sequences in the library [21]. A total of 88 and 90 sequences were obtained from the archaeal and bacterial clone library respectively. Rarefaction curves using an evolutionary distance of 0.03 (equal to three mismatches per 100 bp) were computed using DOTUR (Figure 6). Species richness was estimated using the abundance-based coverage estimator (ACE), bootstrap richness estimators (BRE), Chao1 richness estimator (Chao1), Jackknife richness estimator (Jack) and Shannon weaver index of diversity (SW) (Table 2). This analysis indicated that the expected richness for the bacterial was higher than the archaeal clone library (Table 2).

Figure 6. Rarefaction curves of bacterial and archaeal 16S rRNA gene sequences from the sediment of Chol Buri mangrove, Thailand.

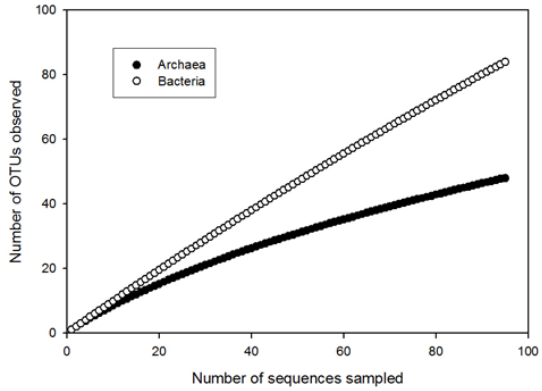


Table 2 Richness estimators for the archaeal and bacterial libraries from the sediment of Chol Buri mangrove

Library	Richness estimators				
	ACE	**BRE**	**Chao1**	**Jack**	**Shannon**
Archaea	111.74	60.67	99.67	111.21	3.46
Bacteria	387.12	112.32	354.10	375.05	4.55

IV. CONCLUSIONS

The Chol Buri mangroves belong to tropic fringe mangrove forests which are a wide-spread coastal ecosystem in Thailand. Due to the relatively open exposure along shoreline, the mangrove is affected by strong winds resulting in causing breakage and the accumulation of debris. The mangrove is under a strong influence of the marine inflows and has the mineral composition that is very similar to marine environments with the concentration of NaCl ranging between 2.2 and 2.9%. The microbial community degrading debris is highly diverse. It contains bacteria and archaea in a ratio 1:2. However, the rarefaction curve analysis as well as richness estimators have shown that the bacterial diversity was higher than that of archaea (Figure 6 and Table 2).

The main bacterial group is *Proteobacteria* accounting for about 70% of clones obtained (Figure 4). Sulphate reducers have been indentified to be the most abundant functional group belonging to *Deltaproteobacteria*. They were affiliated with about 43.5% and 12% of clones among *Proteobacteria* and total bacteria, respectively. The clones were clustered within genera *Desulfobulbus*, *Desulfobacterium* and *Desulfopila*. Sulphate-reducing bacteria have been shown to play a major role in mangrove sediments as the terminal microbial group in the anaerobic trophic chain [11, 15]. Sulphates as the necessary electron acceptor for these microorganisms come with sea water. In marine environments, sulphate reduction is the main terminal process of organic matter decomposition [14]. The same number of clones has been assigned to *Gammaproteobacteria*. This

microbial group is also typical for marine ecosystem. The clones were affiliated to representatives of this bacterial family exhibiting different metabolic functions such as sulfur-oxidation, nitrate- and chlorate-reduction. Other groups of *Proteobacteria* were represented by two (*Alpha*-) or one (*Beta*- and *Epsilon*-) clones. Minor bacterial groups detected in the mangrove sediment were *Acidobacteria*, *Nitrosopirae*, *Spirochaetes* and *Chloroflexi*. The latter was represented by *Dehalococcoides* that obtains energy via the oxidation of hydrogen and subsequent reductive dehalogenation of halogenated organic compounds. The presence of such compounds is an indication of anthropogenic influence on the mangrove ecosystem. Thus, the representatives from bacterial groups involved in the turnover of carbon, sulfur and nitrogen as the most abundant elements in mangroves have been identified. The importance of bacteria participating in the transformation of the above mentioned elements has been also documented for other mangroves [8, 17]. The bacterial structure of the Thai mangrove, Chol Buri appeared to have the closest similarity to that of the Futain mangrove located in China [16]. Both mangroves are located in tropic South-West Asia and hence similar climatic conditions. The phylogenetic trees constructed from 16S rDNA of archaeal library revealed the strong dominance of two phylogenetic groups: ammonium oxidizers and methanogens. The former belongs to a newly-proposed Candidatus *Thaumarchaeota* [35]. The members of this archaeal group are aerobes and play role in nitrogen cycle as autotrophic ammonia-oxidizing microorganisms [36]. They gain energy by oxidizing ammonia to nitrate and are capable of oxidizing ammonia at levels as low as 10 nanomolar, near the limit to sustain its life [36]. The clones identified with this family account for 28% of total archaeal clones. This microbial group can be involved in the processes of nitrogen transformation in the upper sediment layer exposed to oxygen. Methanogenic archaea identified in the mangrove samples account for 30% of archaeal community and belong to genera *Methanolobus*, *Methanococcoides and Methanoculleus*. Two first genera contain methylotrophic methanogens whereas representatives of the last genus are able to use H_2+CO_2 and formate. Four clones were assigned to a separate not identified cluster. The confirmation of the native activity of methanogens was methanogenesis occurred in the mangrove samples incubated at 25°C. The rate of methanogenesis in the native samples was relatively slow 46 $nmol.l^{-1}.day^{-1}$. However, it was highly and immediately stimulated by the addition of some substrates selective for methanogens that indicates their high number and limitation by available substrates. The highest stimulation has been achieved by the addition of C1-compounds such as methanol and TMA indicating the predominance of methylotrophic methanogens. The fact of the prevalence of C1-methanogenic pathway in mangrove sediments have been already documented and new methylotrophic methanogens belonging to genera *Methanococcoides* and *Methanosarcina* have been isolated [11].

H_2+CO_2 and formate to less extent have also stimulated methanogenesis whereas acetate had almost no effect on methanogenic activity. Absence of clones affiliated to acetoclastic methanogens of the genera *Methanosarcina* or *Methanosaeta* [37] points out the minor importance of the appropriative pathway. However, acetate in the mangrove ecosystem can be used by sulphate-reducers which are the most predominant group among bacteria. Addition of glucose had a moderate stimulating effect on methanogenesis. At the degradation of glucose, hydrogen and carbon dioxide could be substrates for methanogens indicating their involvement in the trophic microbial chain functioning in the Chol Buri mangrove.

Thus, the 16S rRNA gene analysis exhibited the high microbial diversity in the sediments of Chol Buri mangrove of Thailand. The coexistence of two functional archaeal groups, namely anaerobic methanogens and aerobic ammonia-oxidizers which are highly abundant in the same sample site can be explained by the specific hydrology of mangrove ecosystems with highly varying red-ox potential resulted from the daily tidal period. The abundance of the above described three main functional groups of microorganisms in the sediment of Chol Buri mangrove in Thailand could be an indication of the primary importance of appropriative metabolic processes: sulphate reduction, ammonia-oxidation and methanogenesis. Methanogens and sulphate-reducers are likely to be the main terminal microbial groups in the anaerobic community of mangrove. The concentration of electron acceptors other than bicarbonate and sulphate is low in the ecosystem and contribution of other terminal anaerobes seems to be negligible. However, iron-reducers (belonging to genera *Geobacter* and *Pelobacter*) have been also found in the samples and can also contribute to the degradation processes in the Chol Buri mangrove. Iron reduction has been shown to be the important terminal process in Thai mangroves [38]. Usually, sulphate reducing bacteria outcompete methanogenic archaea for common substrates. It has been proposed that methanogens can coexist with sulphate reducers by using non-competitive C1-substrates or if the organic input is large enough to support both microbial groups [7]. Methanol can be available for methanogens through bacterial degradation of lignin or pectin [39] whereas methylated amines can be products of degradation of choline and glycine [40]. The latter compounds

are osmolytes found in many marine organisms. The third archaeal group detected in the mangrove sediments was assigned to the order *Thermoplasmatales*. The organisms in this order are extremly acidophilic and their role in mangroves are not known.

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