Journal of Marine Science and Technology; Vol. 18, No. 3; 2018: 286–295
DOI: 10.15625/1859-3097/18/3/10642
http://www.vjs.ac.vn/index.php/jmst

BIODIVERSITY ASSESSMENT OF MICROORGANISMS ASSOCIATED WITH TWO MARINE SPONGES (*Haliclona oculata* AND *Amphius huxleyi*) COLLECTED AT THE LANG CO BAY OF VIETNAM

Ton That Huu Dat[1], Pham Viet Cuong[1], Tran Thi Kim Dung[1], Vu Thi Thu Huyen[2], Nguyen Thi Kim Cuc[2,*]

[1]*Mientrung Institute for Scientific Research, VAST, Vietnam*
[2]*Institute of Marine Biochemistry, VAST, Vietnam*
[*]E-mail: kcnguyenthi@gmail.com

Received: 28-8-2017; accepted: 18-11-2017

Abstract. Sponges (Phylum Porifera) are ancient sedentary and filter-feeding animals which harbour very diverse and abundant associated microbial community in their tissues with density up to 40–50% of sponge tissue volume. In this study, the diversity of associated microorganisms with two marine sponges *Haliclona oculata* and *Amphius huxleyi* collected at the Lang Co bay of Vietnam was assessed by analysis of hypervariable V3 and V4 regions of the 16S rRNA gene using Illumina MiSeq system. The taxonomic diversity of sponge-associated microorganisms was classified to different taxonomic levels (kingdom, phylum, class, order, family, and genus). Based on Bayesian classification method and reference sequences derived from Greengenes database, the associated microorganisms in studied sponges were assigned to 17 phyla (*H. oculata*) and 13 phyla (*A. huxleyi*). Many microbial taxa were detected in two sponge species, however, they were distinctive by the abundance. *Proteobacteria* was the most dominant phylum in both sponge species, and all of 4 classes *Epsilonproteobacteria, Gammaproteobacteria, Alphaproteobacteria*, and *Deltaproteobacteria* were found in *H. oculata* and *A. huxleyi*.

Keywords: *Amphius huxleyi*, *Haliclona oculata*, Illumina Miseq system, sponge-associated microorganisms.

INTRODUCTION

Sponges are well known as the most primitive and simplest multicellular metazoans. As the filter feeders, sponges are able to filter thousands of litres of water per day, which makes them shelter significant numbers of diverse microbes on their surface and internal mesohyl matrix. These associated microbes play crucial roles in supplying nutrients, stabilizing sponge skeletons and protecting sponge from bio-fouling or predation [1–3].

Depend on the abundance of their associated microbial communities, sponges can be divided into two main groups. The high microbial abundance (HMA) sponges contain diverse and abundant microbial communities that are distinctive from the microbial communities from the surrounding seawater. Furthermore, HMA sponges are also characterized by lower pumping rates and a higher frequency of hosting photosynthetic symbionts. On the other hand, the low microbial abundance (LMA) sponges contain associated microorganisms with significantly lower abundances that are more similar to the microbial communities from the surrounding seawater. LMA sponges often have higher pumping rates and a higher rate of heterotrophic feeding on particulate organic matter [1, 4]. Although studies have used a variety of fingerprinting techniques for investigation of microbial community associated with sponges such as DGGE, TRFLPs, ARISA, DNA library, NGS [4–6], their genomes as well as actual relationship between them and hosts are poorly understood [1]. Amplicon sequencing, especially small subunit of the 16S rRNA gene is a widely used approach for assessment of composition, structure and spatio-temporal patterns of microbial communities due to its ubiquity across all domains of life [7]. Recent high-throughput sequencing (HTS) technology and the application of barcode indexing have allowed collecting thousands of sequences from a large number of samples simultaneously [8]. These approaches have revealed deeper insights into the diversity of microbial communities [9].

In this study, we assessed the diversity of microorganisms associated with two sponge species: *Haliclona oculata* and *Amphius huxleyi* collected at the Lang Co bay, Thua Thien Hue province by analysis of V3 and V4 regions of 16S rRNA gene, using Illumina sequencing method on MiSeq system.

MATERIALS AND METHODS

Collection of sponges. Sponge specimens (3 samples per species) were collected by SCUBA diving at Lang Co bay, Thua Thien-Hue province in May 2015 at depth of 15 m. Each of specimens was reserved in a separate bottle with 30% glycerol on the ice during transferred to the laboratory of Institute of Marine Biochemistry. In the lab, the sponge specimens were stored at 4°C until used (not longer than 3 weeks). Specimens were identified as *Haliclona oculata* and *Amphius huxleyi* based on the initially morphological characters (at Institute of Marine Environment & Resources, VAST) and 18S rRNA gene sequences (at Mientrung Institute for Scientific Research).

DNA extraction. Sponge associated-microorganisms were extracted according to the protocol described by Ouyang et al., (2009) [10]. Briefly, sponge specimens were washed three times with sterile autoclaved artificial seawater, dried in a drain in 5–10 mins. Ten weighed grams of each specimen was cut into small pieces, homogenized into a cell suspension on ice with TE buffer (10 mM Tris-HCl, 1 mM ethylene diamine tetra acetic acid (EDTA), pH 8.0). First, the cell suspension was filtered by two layers of cheesecloth, and then centrifuged at 250 g for 1 min to remove sponge debris. The supernatant was centrifuged again at 8,000 g for 15 mins to collect pellets containing bacteria. Obtained bacterial pellets were washed with TE50 (10 mM Tris-HCl, 50 mMEDTA, pH 8.0). Total DNA was extracted using Genomic-tips 20/G (Qiagen, Germany) according to manufacturer's instructions.

Amplification of V3 and V4 regions of 16S rRNA gene

First PCR. Amplification of V3 and V4 regions of 16S rRNA gene was performed in 25 µl reaction in 96 well 0.2 ml PCR plate, including 2.5 µl DNA template (total DNA, 5 ng/µl), 1 µl primers with overhang adapters 16S Amplicon Forward Primer (TCGTCGGCAGCGTCAGATGTGTATAAG AGACAGCCTACGGGNGGCWGCAG) and 16S Amplicon Reverse Primer (GTCTCGTGGGCTCGGAGATGTGTATAA GAGACAGGACTACHVGGGTATCTAACC), and 12.5 µl 2x KAPA HiFi HotStart ReadyMix. The primer pair sequences of the V3 and V4 regions created single amplicons of approximately ~ 460 bp. PCR was performed in a thermal cycler using the program of 95°C for 3 mins, 25 cycles of 95°C for 30 secs, 55°C for 30 secs, 72°C for 30 secs, 72°C for 5 mins, then hold at 4°C. PCR product was purified by AMPure XP beads as described in 16S Metagenomic Sequencing Library Preparation protocol (Illumina Inc.) to purify the V3 and V4 amplicons away from free primers and primer dimer species.

Second PCR: This step attaches dual indices and Illumina sequencing adapters using the Nextera XT Index Kit. The PCR reaction (50 µl) contained 5 µl of PCR product from first PCR, 5 µl of Nextra XT Index primer 1 (N7xx), 5 µl of Nextra XT Index primer 2 (S5xx), 25 µl of 2x KAPA HiFi HotStart Ready Mix and 10 µl of PCR grade water. Thermal program of PCR processing was 95°C for 3 mins, 8 cycles of 95°C for 30 secs, 55°C for 30 secs, 72°C for 30 secs, 72°C for 5 mins, hold at 4°C. The final library was purified using AMPure XP beads before quantification.

DNA concentration and purification were determined using Nanodrop 2000 spectrophotometer (Thermo Scientific) at the wave of 260 nm and 280 nm. For metagenomic sequencing, DNA concentration was calculated in nM, based on the size of DNA amplicons, and determined by an Agilent Technologies 2100 Bioanalyzer (Agilent Technologies, Palo Alto, Calif.).

Sequencing by MiSeq System. The purified and quantified PCR products were pooled together at an equal concentration, then denatured and loaded onto the Illumina MiSeq sequencing system following 16S Metagenomics protocol of manufacture. The library was sequenced at DNA Analyzing Centre - Gentis Hanoi, Vietnam.

16S Metagenomic analysis. Sequence data and the taxonomic assignment were analyzed following 16S Metagenomics Workflow using the MiSeq Reporter software v2.4 (Illumina Inc) provided by MiSeq System. Briefly, raw sequenced data was filtered to remove low-quality reads by Illumina chastity filter. The reads that passed quality filter and had Q-score ≥ 30 were then demultiplexed to remove index sequences. Only reads pairing with perfectly matching primers and indexes were subjected to further analysis. High-quality reads were assigned into various taxonomic 6 levels by an RDP Bayesian classifier using the reference GreenGenes 16S rRNA database (http://greengenes.lbl.gov/). In our study, only the taxa with the relative abundance at least 0.1% were further analyzed.

The non-metric multidimensional scaling (nMDS) plot was created by R. v.3.3.0 via the function *metaMDS* (Bray-Curtis distances) of the vegan package.

Statistical analysis. We used t-test analysis to test the difference of the relative abundance of shared taxa in both sponge species using R. v.3.3.0 software with the $\alpha = 0.05$.

RESULTS

An average of 83.5% high-quality reads (in a total of 6,337,151 reads) of species *H. oculata* and 84.7% reads (in a total of 5,716,412 reads) of species *A. huxleyi* passed the quality requirement. The reads were classified into various taxonomic levels according to the described method.

Diversity of microbial communities associated with two sponge speices. According to the above described classification method, the microbial communities associated with two sponge species were identified at different taxonomic levels (table 1). The taxonomic analyses showed that the microbial community in *Haliclona oculata* was more diverse than that in *Amphius huxleyi* at the phylum level, but less diverse at lower taxonomic levels (e.g., order, family and genus levels).

Table 1. Number of microbial taxa identified at different taxonomic levels

No	Taxonomic level	*H. oculata*	*A. huxleyi*	*Number of shared taxa*
1	Kingdom	3	3	3
2	Phylum	17	13	11
3	Class	23	23	19
4	Order	33	36	26
5	Family	41	45	24
6	Genus	39	43	23

Although many taxa were found in both sponge species, they were very different in relative abundance (see next section). The non-metric multidimensional scaling analysis (fig. 1) revealed that the two sponge species hosted the different microbial communities.

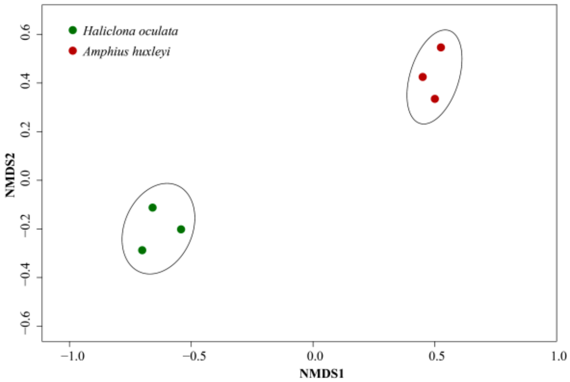

Fig. 1. Non-metric multidimensional scaling (nMDS) plot derived from Bray-Curtis distance analysis of sponge microbial communities

The abundance of main microbial taxa associated with two sponge species. The obtained reads of microorganisms associated with *H. oculata* were assigned to three kingdoms including bacteria (99.07% reads), Archaea (0.27% reads), viruses (0.48%), and only 0.29% reads were not identified. For sponge *A. huxleyi*, associated microorganisms also belonged to three kingdoms, mainly bacteria (98.73% reads), viruses (0.74% reads), Archaea (0.04% reads), and 0.49% reads were not classified at this level. The reads were assigned into 17 different phyla for *H. oculata* and 13 phyla for *A. huxleyi*. The most dominant phylum in both sponge species was *Proteobacteria* (21.66% reads in *H. oculata* and 29.46% reads in *A. huxleyi*). Some other phyla were detected in both sponges but with different abundance. For example, phylum *Cyanobacteria* was more dominant in *A. huxleyi* (23.59% reads) than in *H. oculata* (6.14% reads). Similar results were found in different phyla such as *Actinobacteria* (16.41% reads for *A. huxleyi* versus 5.56% reads for *H. oculata*), *Firmicutes* (5.71% reads for *A. huxleyi* and 3.74% for *H. oculata*), *Chloroflexi* (4.65% reads and 1.99% for *A. huxleyi* and *H. oculata*, respectively). The phylum *Caldithrix* had relatively similar abundance in both sponges (3.60% of reads in *H. oculata* and 3.88% of reads in *A. huxleyi*), whereas the phylum *Nitrospirae* was more abundant in sponge *H. oculata* (6.56% reads) than in sponge *A. huxleyi* (1.84% reads). Statistical analyses indicated that the relative abundance of all shared phyla in both species was significantly different ($\alpha = 0.05$) In addition, 5 phyla detected from sponge *H. oculata* and 2 phyla from sponge *A. huxleyi* were absent in each other (table 2). At phylum level, 45.89% reads from *H. oculata* were unidentified, meanwhile, only 9.54% reads from *A. huxleyi* were unclassified (table 2).

Table 2. Microbial phyla identified from 2 sponge species

H. oculata			***A. huxleyi***		
No	Phylum	% total reads	No	Phylum	% total reads
1	Unclassified	45.89	1	Unclassified	9.54
2	*Proteobacteria*	21.66	2	*Proteobacteria*	29.46
3	*Nitrospirae*	6.56	3	*Cyanobacteria*	23.59
4	*Cyanobacteria*	6.14	4	*Actinobacteria*	16.4
5	*Actinobacteria*	5.56	5	*Firmicutes*	5.71
6	*Firmicutes*	3.74	6	*Chloroflexi*	4.65
7	*Caldithrix*	3.60	7	*Caldithrix*	3.88
8	*Chloroflexi*	1.99	8	*Nitrospirae*	1.84
9	*Synergistetes*	1.45	9	*Acidobacteria*	1.60
10	*Spirochaetes*	0.80	10	*Bacteroidetes*	1.01
11	*Acidobacteria*	0.44	11	*Synergistetes*	0.70
12	*Bacteroidetes*	0.40	12	*Tenericutes*	0.28
13	*Chlorobi*	0.31	13	*Verrucomicrobia*	0.19
14	*Thermotogae*	0.25			
15	*Crenarchaeota*	0.21			
16	*Verrucomicrobia*	0.13			
17	*Deferribacteres*	0.12			

The obtained reads were also assigned into 23 classes for both sponge species with different abundance in each. For sponge *H. oculata*, the phyla *Nitrospira*, *Epsilonproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria* were dominant with 6.55%, 6.14%, 6.12% and 5.58% reads, respectively. Other classes such as *Actinobacteria*, *Synechococcophycideae*, *Caldithrixae*, *Alphaproteobacteria* accounted for 3% to 4.7% reads, whereas three classes *Clostridia*, *Synergistia*, *Anaerolineae* occupied 1.42%, 1.45% and 2.57% reads, respectively. In sponge *A. huxleyi*, most abundant classes were *Synechococcophycideae* (20.65% reads), followed by *Actinobacteria* (15.49% reads) and *Epsilonproteobacteria* (12.75% reads). Four other classes *Deltaproteobacteria*, *Gammaproteobacteria*, *Clostridia*, *Anaerolineae*, and *Caldithrixae* were in a range from 4% to 7% total reads. The classes *Alphaproteobacteria*, *Oscillatoriophycideae*, *Nitrospira*, and *Acidobacteria* had 3.58%, 2.02%, 1.84% and 1.50% total reads, respectively. The rest of classes had less than 1% total reads, comprised 17.26% reads and 11.91% total reads that were unclassified in *H. oculata* and *A. huxleyi*, respectively (fig. 2). Although above mentioned classes were present in both sponges, the relative abundance of most phyla was significantly different (α= 0.05). Class *Synechococcophycideae* in *A. huxleyi* was 5 times higher than in *H. oculata*. Similarly, the relative abundance of classes *Actinobacteria* and *Epsilonproteobacteria* was also 2–3 times higher in *A. huxleyi* than in *H. oculata*.

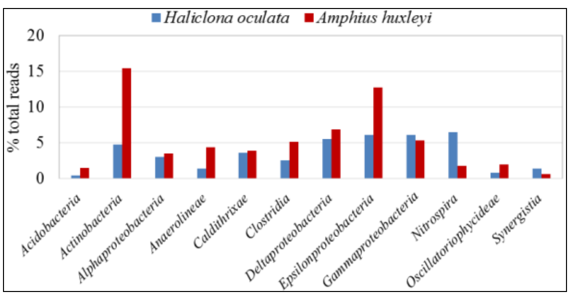

Fig 2. Main classes of sponge-associated bacteria in *H. oculata* and *A. huxleyi*

At order level, the reads were assigned into 33 orders for *H. oculata* and 36 orders for *A. huxleyi*. The main orders such as *Synechococcales*, *Actinomycetales*, *Camphilobacterales*, *Desulfovibrionales*, *Caldithrixales*, and *Rhodospirillales* were detected in both sponges, but with a higher percentage of reads in sponge *A. huxleyi*. The highest read rates identified in *A. huxleyi* were *Synechococcales*, *Actinomycetales* and *Campylobacterales*, with 20.62%, 15.13% and 12.75% total reads, respectively, followed by *Desulfovibrionales*, *Anaerolineales*, *Clostridiales*, and *Caldithrixales* (from 4% to 6% total reads). The order *Nitrospirales* was most abundant in *H. oculata* (6.56% reads), but in *A huxleyi*, it occupied only 1.84% reads (fig. 3).

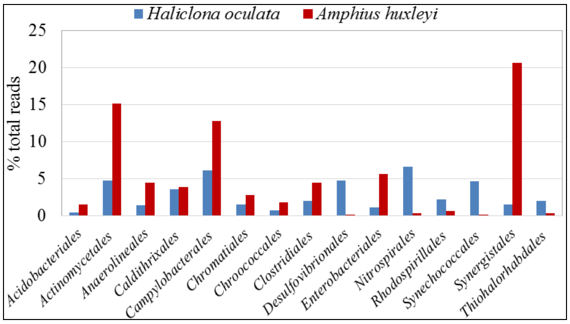

Fig 3. Main orders of sponge-associated bacteria in *H. oculata* and *A. huxleyi*

Based on Bayesian classifier and GreenGenes 16S rRNA database, the qualified reads of *H. oculata* and *A. huxleyi* were further assigned into 41 and 45 different families, respectively. For sponge *H. oculata*, the families *Thermodesulfovibrionaceae*, *Campylobacteraceae*, *Synechococcaceae*, *Caldithrixaceae* were dominant with 3.6% to 6.5% total reads; other 7 families *Thiohalorhabdaceae*, *Rhodospirillaceae*, *Thermomonosporaceae*, *Anaerolinaceae*, *Synergistaceae*, *Chromatiaceae* and *Enterobacteriaceae* occupied from 1% to 2% total reads; the rest accounted for 0.1% to 0.84% reads. In case of sponge *A. huxleyi*, the most important families, *Synechococcaceae* and *Campylobacteraceae*, accounted for 20.62% and 12.74% reads, respectively. The next families, *Pseudonocardiaceae, Desulfovibrionaceae, Streptosporangiaceae, Anaerolinaceae, Caldithrixaceae, Veillonellaceae,* and *Rhodospirillaceae* occupied 2.2% to 5.6% total reads, and the other families *Ectothiorhodospiraceae, Thermodesulfovibrionaceae, Phormidiaceae, Acidobacteriaceae, Thermomonosporaceae* and *Thiohalorhabdaceae* also had more than 1% total reads. The remaining families possessed only from 0.1% to 0.74% total reads. The obtained result showed that main families in both sponges *H. oculata* and *A. huxleyi* were shared, but dissimilar in their abundance. The most abundant families in *A. huxleyi* were *Synechococcaceae* and *Campylobacteraceae* (20.6% and 12.7% reads, respectively), whereas only 1.8% reads were assigned to the family *Thermodesulfovibrionaceae*. In contrast, in sponge *H. oculata*, *Thermodesulfovibrionaceae*, *Campylobacteraceae* were the most abundant families (6.6% and 6.1% reads, respectively), while only 4.6% reads were assigned to family *Synechococcaceae*, much less than in sponge *A. huxleyi*. The difference of relative abundance of the families *Caldithrixaceae* was, *Desulfovibrionaceae*, and *Rhodospirillaceae* in both species was statistically insignificant (α= 0.05) (fig. 4).

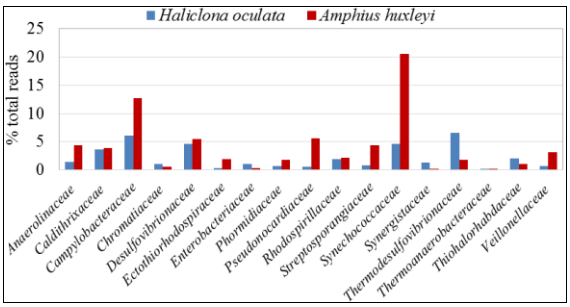

Fig 4. Main families of sponge-associated bacteria in *H. oculata* and *A. huxleyi*

At a lower taxonomic level, representative reads were assigned into 39 genera for *H. oculata* with 2 most dominant genera *Thermodesulfovibio, Campylobacter* (6.55% and 6.11% reads, respectively); and 43 genera for *A. huxleyi*, in which the most dominant genera were *Prochlorococcus* and *Campylobacter* (20.6% and 12.7% reads, respectively). The genera *Saccharopolyspora, Streptosporangium, Longilinea,* and *Selenomonas* were more abundant in sponge *A. huxleyi* (3–5.5%) than in *H. oculata* (0.4–1.2%). The sponge *A. huxleyi* hosted 5 genera (*Candidatus Liberibacter, Slackia, Symploca,* and *Ruegeria)* that were absent in sponge *H. oculata* but only accounted for small abundances in total reads (0.11% to 0.12%) (fig. 5). Among shared genera in both sponge species, only genera *Caldithrix* and *Desulfovibrio* had relatively similar abundance, while the abundance of remaining genera was significantly different (α = 0.05).

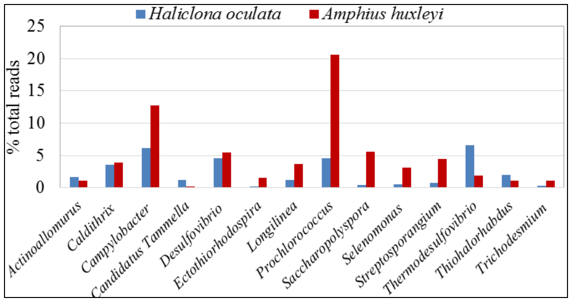

Fig 5. Main genera of sponge-associated bacteria in *H. oculata* and *A. huxleyi*

DISCUSSIONS

Marine sponges are well known to shelter diverse and dense microbes, and this diversity can be explained in a part by the changes of physical, chemical, and biological conditions in sponges that may affect the microbial ecology and evolution [11]. The first studies of sponge-associated microorganisms using the traditional culture-dependent method, or checking sponge tissues under a microscope have shown that microorganisms can contribute up to 50% by volume of the sponges, and this community is specific for sponges [12]. In recent years, many studies have surveyed the diversity of sponge-symbiotic microbes in various marine ecosystems using different culture-independent molecular techniques such as DGGE, TRFLPs, ARISA, clone library sequencing [5, 13]. In our study, we had assessed the microbial diversity of two marine sponge species *A. huxleyi* and *H. oculata* by analyzing V3-V4 regions of the 16S rRNA gene. After the quality filtering step, about 80% reads met the quality requirement, which is similar to the percentage of high-quality reads after filtering in other studies (40–85%) [14–16].

Based on the studies investigating bacterial community by methods such as Denaturing Gradient Gel Electrophoresis (DGGE), 16S rRNA gene sequencing and Fluorescence *In Situ* Hybridization (FISH), it is found that sponge-associated bacterial community comprises more than 25 phyla, including common phyla such as *Proteobacteria, Nitrospira, Cyanobacteria, Bacteriodetes, Actinobacteria, Chloroflexi, Planctomycetes, Acidobacteria, Poribacteria* and *Verrucomicrobia*, and members of Archaea domain. The populations of other organisms living in sponges are fungi and microalgae. Very little is known about the virus in the sponges, although the virus-like particles were detected in the cell nucleus of *Aplysina (Verongia) cavernicola*. Recently, metagenomic approaches have been widely used in assessing the diversity of sponge-associated microbes [17–20]. For example, Alex et al., (2015) [20] used the pyrosequencing for characterization of microbial communities in 12 different co-occurring intertidal marine sponge species sampled from the Atlantic coast. Taxonomic assignment of 16S ribosomal RNA tag sequences estimated altogether 26 microbial groups, represented by bacterial (75.5%) and archaeal (22%) domains. *Proteobacteria* (43.4%) and *Crenarchaeota* (20.6%) were the most dominant microbial groups detected in all the 12 marine sponge species and ambient seawater [20]. In our study, three kingdoms including bacteria, Archaea and viruses were detected in both sponge species, in which bacteria were most abundant (99.07% reads), followed by viruses (0.48% reads) and Archaea (0.21% reads). The common bacterial phyla that were often found from various sponge species in above studies (e.g., *Proteobacteria, Nitrospira, Cyanobacteria, Bacteriodetes, Actinobacteria, Chloroflexi, Planctomycetes, Acidobacteria*, and *Verrucomicrobia*) were also detected in sponge samples in our study.

To date, the microbial communities associated with genus *Haliclona* have been well reported. For example, Jasmin et al., (2015) [21] documented the bacterial diversity associated with the species *Haliclona pigmentifera* cohabiting in coral reef of the Gulf of Mannar by analysis of 16S rRNA gene library and showed that the dominant bacterial phylum in *H. pigmentifera* was *β-Proteobacteria* (33.3%) followed by *Cyanobacteria* (21.5%) [21]. Similarly, Sipkema et al., (2009) [22] after analyzing the 16S rRNA library detected representatives of most bacterial phyla associated with sponge *Haliclona (?gellius)* sp. including α-, β-,γ-, δ- and ε-*Proteobacteria, Cyanobacteria, Planctomycetes, Firmicutes, Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Fusobacteria, Nitrospirae, Verrucomicrobia* [22]. In this study, 17 bacterial phyla were identified in species *H. oculata* and *Proteobacteria* was the most dominant phylum (21.7% reads). At lower taxonomic level, the classes *Alphaproteobacteria* and *Gammaproteobacteria* were less abundant in *H. oculata* in our study (3.04% and 6.12%, respectively) than in Atlantic sponges (12.1% and 10.6% reads, respectively), but the class *Epsilonproteobacteria* was much higher in *H. oculata* (6.1%) than in Atlantic sponges (0.2%) [20]. According to Abe et al., (2012), *Gammaproteobacteria* is the dominant class in *Haliclona simulant* and *Gelliodes carnosa* whereas *Alphaproteobacteria* is the dominant class in some other sponges such as *Halichondria panicea, Rhopaloeides odorabile*, and *Mycale laxissima* [23].

To the best of our knowledge, this is the first report about microbial communities associated with marine sponge *A. huxleyi.* Among 12 phyla found in *Amphius huxley*, the phylum *Proteobacteria* was the most abundant phylum (29.4% reads). Most main bacterial phyla found in *A. huxleyi* were also reported in other marine sponges [20–22].

It is known that marine sponges-associated microbes may have several common taxa, but are very different in the relative abundance, indicating that host-identity plays an important role in structuring their microbial communities.

The similar results are also found in our study. Many microbial taxa are found in both sponge species *H. oculata* and *A. huxleyi*, however, their relative abundance is significantly different. The difference of microbial communities associated with both sponge species in our study is also supported by non-metric multidimensional scaling analysis. This finding is consistent with the previous reports in which samples of same species host the microbiota that is more similar than that of sample from other species [13, 18, 20].

CONCLUSION

Using Next Generation Sequencing on MiSeq system, V3-V4 hypervariable regions of 16S rRNA gene of microbes associated with two sponges *H. oculata* and *A. huxleyi* were sequenced. The sponges *H. oculata* and *A. huxleyi* from Lang Co bay host different bacterial communities, while Archaea are present at very low abundance. Phylum *Proteobacteria* is dominant phylotype in both sponge species, and all 4 classes of *Proteobacteria* (*Epsilonproteobacteria*, *Gammaproteobacteria*, *Alphaproteobacteria*, and *Deltaproteobacteria*) were found in both *H. oculata* and *A. huxleyi*. Both investigated sponges shared many common phyla, but with dissimilar abundance. The genera *Prochlorococcus* and *Campylobacter* are present at high abundance in sponge *A. huxleyi*, up to 20.6% and 12.7%, respectively, much higher than what has been reported for sponges.

Acknowledgment: This research was supported by the Vietnamese Government project in cooperation with the Netherlands, code ĐTĐLCN.17/14 of Ministry of Science and Technology.

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