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Evaluation of micro-biodiversity in three Spratly islands by metagenomics

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Abstract. The biodiversity of micro community of the soil in three islands of Spratly Island, Vietnam has been analyzed by metagenomic. A total of 77,689 reads were obtained from the sample of Spratly Island (Truong Sa Lon - TSL), in the meanwhile there are only 62,447 reads from the sample of Sand Cay Island (Son Ca - SC) and 70,150 reads from that of Southwest Cay Island (Song Tu Tay - STT). The validated reads of islands are 35,253, 10,279, and 10,380, respectively. The reads from TSL were divided into 31 phyla, 80 classes, 185 orders, 291 families, 458 genera and 397 species. Those from SC included 24 phyla, 45 classes, 98 orders, 133 families, 176 genera and 100 species. STT has 20 phyla, 39 classes, 95 orders, 129 families, 169 genera and 110 species. Although the component and proportion of each taxonomic level is unique and different from each other and from those collected in other island research, the results have shown the diversity and characteristics of micro-biomes of TSL, SC and STT islands. These characteristics reflect the natural and human conditions affecting and determine the dominance of bacteria population of each island.

Keywords: metagenomic, microbe, micro-biodiversity, Spratly islands, sea.

Classification numbers: 2.4.2, 2.4.4, 5.2.1.

1. INTRODUCTION

Spratly Islands, a disputed archipelago located at South-Eastern of the South China Sea consist of more than 100 islands, islets, cays, from 6°30’ to 12°00’ North and 111°30’ to 117°20' East. The islands are 3-5 meters high above the sea level. Total area of the islands is only about 3 square kilometers, however, it spread in a large sea region of 160,000 km². The natural conditions and climate in this region are very harsh: sunny and windy, frequent thunderstorms, lack of fresh water, many islands without trees. Some weather phenomena also occur differently than inland. The climate in Truong Sa archipelago can be divided into two

seasons: dry season and rainy season. The dry season is from January to May, the rainy season from May to January of the following year, the average annual rainfall is very high at more than 2,500 mm. The phenomenon of thunderstorms on the waters of this archipelago is very common, it can be said that all year round, there are thunderstorms every month and there are often big storms passing through, focusing on the rainy season months. The soil of most of the islands is mostly biogenic carbonate generated from coral deposit and bird feces and plant deposit. The soil thickness is from 5-10 centimeters. Besides, due to low attitude, the soil is always salty which is not favorable for microbes. Some of the islands has groundwater such as Southwest Cay, Northeast Cay and Spratly islands. The flora and fauna of the islands of Spratly, as well as microflora, have not been investigated. Therefore, in the present study, we analyze the micro-biodiversity in three islands of different island groups of the Spratly Islands: Spratly Island (Truong Sa Lon - TSL), Southwest Cay Island (Song Tu Tay - STT) and Sand Cay Island (Son Ca - SC).

Several methods have been deployed to investigate the microflora of soil, such as the culture-based assay, denaturing gradient gel electrophoresis (DGGE) and next generation sequencing (NGS)-based metagenomics, [1-6]. The culture-based assay, in many cases, could not fully provide information of the microflora because many unculturable microbes occupy a certain rate of ones from soil samples. DGGE method, on the other hand, is able to identify unculturable microbes, however, it could not deal with massive 16S rDNA sequences. Metagenomics has been developed based on NGS technology, has ability to identify both culturable and unculturable microorganisms with massive sequence data [1, 4, 7, 8]. In the present study, metagenomic analysis was performed using the GS-FLX pyrosequencing platform.

2. MATERIALS AND METHODS

2.1. Sample collection

Soil, water, waste samples close to husbandry waste treatment areas of 3 islands: Spratly Island (Truong Sa Lon - TSL) (8°38'46.56" North 111°55'21.07" East), Southwest Cay Island (Song Tu Tay - STT) (8°38'46.55" North 111°55'21.08" East) and Sand Cay Island (Son Ca - SC) (10°22'33.53" North - 114°28'43.50" East, which belong to Spratly islands, Truong Sa district, Khanh Hoa province, Viet Nam, were collected according to Abraham *et al.* [9], in sterilized bottles and conserved at 4C until use.

2.2. DNA extraction for metagenomic analysis

Total DNA was extracted from collected soil samples by DNA Extraction Kit (Quiagen) according to manufacturer's manuals. DNA quality was checked by measurement by Nanodrop (Calibri). Qualified samples were then further tested by electrophoresis in agarose 1 %.

2.3. V3-V4 domain amplification for micro-biodiversity evaluation

16S rRNA gene was amplified by PCR using primer designed for V3 and V4 domains of F515 (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGCCAGCMGCCGCGGTAA-3') and R806 (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACH VGGGWTCT AAT-3'). Total PCR mixture volume is 25 µL including 5 µL each primer with concentration of 10 µM and 2.5 µL DNA template. PCR was carried out on Alpha Cycler 1 PCR

Max with initial denaturation of 3 minutes at 95 °C, following with 35 cycles of 30 seconds at 95 °C, 30 seconds at 55 °C and 30 seconds at 72 °C, and kept at 72 °C in 5 minutes at the end of the run. PCR products were thereafter purified by Kapa purifier with ratio of 0.7 [10].

V3-V4 amplicons were sequenced on Illumina MiSeq 300bp paired-end platform. Raw read assessment was done using fastqc, Cutadapt 3.5 was used to remove primers and adaptors. The whole metagenomics analysis was done using qimme2 (version: qiime2-2022.8). Raw sequence reads were imported to qiime2 with PairedEndFastqManifestPhred33V2 option. We used dada2 with denoise-paired pipeline to clean the data. In this process, first 10 bases of all reads were removed, all length of each read was trimmed at 220 bases, reads less than 220 bases also were discarded, and others thresholds were set as default. We used Silva version 138 99 % OTUs full-length sequences for taxonomy classification. After classification, all Mitochondria, Chloroplast classification were removed for further steps. Alpha and beta diversity indexes were estimated with default option.

3. RESULTS AND DISCUSSION

3.1. Microorganism composition obtained from the sample of TSL

A total of 77,689 reads were obtained from the sample of TSL, in the meanwhile there are only 62,447 reads from the sample of SC and 70,150 reads from that of STT (Table 1). The validated reads of islands are 35,253, 10,279, and 10,380, respectively (Table 1). The reads from TSL were divided into 31 phyla, 80 classes, 185 orders, 291 families, 458 genera and 397 species. Those from SC included 24 phyla, 45 classes, 98 orders, 133 families, 176 genera and 100 species. STT has 20 phyla, 39 classes, 95 orders, 129 families, 169 genera and 110 species. In general, these samples are less diverse in compare to those mentioned in previous researches [11, 12].

Table 1. Pyrosequencing results and number of microbial taxa observed in Spratly Islands.

	TSL		SC		STT	
Number of total reads	77,689		62,447		70,150	
Number of validated reads	35,253		10,279		10,380	
Operational Taxonomic Units	796		281		271	
Number of microbial taxa observed	c[a]	uc[b]	c[a]	uc[b]	c[a]	uc[b]
Phylum	35,155	98	10,258	21	10,371	9
Class	35,108	145	10,232	47	10,347	33
Order	34,350	903	10,160	119	10,134	246
Family	33,730	1,523	9,807	472	9,971	409
Genus	29,688	5,565	9,007	1,272	8,860	1,520
Species	9,471	25,782	3,018	7,261	4,392	5,988

[a] Number of sequencing reads classified to scientific name for taxon. [b] Number of sequencing reads unclassified into sublevel.

3.2. Discussion on microorganism taxonomy diversity

Analyzed data are presented in tables and figures with taking care to avoid unnecessary repetition of tabular data. Information presented in tables should not be repeated in figures, or vice versa. Standard deviations/errors help the reader to follow the trend of results and should be supplied whenever appropriate.

In all three islands, phyla of *Proteobacteria, Actinobacteria*, and *Chloroflexi* all appears with high proportions. Besides, *Bacteroidota* and *Acidobacteriota* are present in TSL samples with high proportions. In SC samples, *Firmicutes* and *Planctomycetota* occupy high proportion, in the meanwhile *Patescibacteria* has 9.14 % of reads of STT. Proportion of unclassified reads in all three islands are low (Table 2). The proportion of unclassified reads of the present study is similar to those from Dongdo island with 7 unclassified reads and from Seodo island with 12 read [13].

Table 2. Taxonomic composition of phyla from Spratly Islands.

Taxonomy	Reads			Proportion (%)		
Phylum	TSL	SC	STT	TSL	SC	STT
d__Bacteria;p__Bacteroidota	5,014	2	75	14.22	0.02	0.72
d__Bacteria;p__Firmicutes	1,531	2,522	482	4.34	24.54	4.64
d__Bacteria;p__Proteobacteria	13,310	3,322	5,398	37.76	32.32	52.00
d__Bacteria;p__Acidobacteriota	3,883	410	69	11.01	3.99	0.66
d__Bacteria;p__Chloroflexi	3,010	847	1,194	8.54	8.24	11.50
d__Bacteria;p__Verrucomicrobiota	526	88	70	1.49	0.86	0.67
d__Bacteria;p__Actinobacteriota	3,737	1,354	1,289	10.60	13.17	12.42
d__Bacteria;p__Gemmatimonadota	951	123	19	2.70	1.20	0.18
d__Bacteria;p__Synergistota	-	42	263	0.00	0.41	2.53
d__Bacteria;p__Myxococcota	1,361	10	-	3.86	0.10	0.00
d__Bacteria;p__Patescibacteria	616	354	949	1.75	3.44	9.14
d__Bacteria;p__Planctomycetota	39	1,006	289	0.11	9.79	2.78
Others	1,177	178	274	3.34	1.73	2.64
Unclassified	98	21	9	0.28	0.20	0.09
Total	35,253	10,279	10380	100	100	100

The list of microbial phyla is only shown for phyla with a read number of more than 1 % in at least one of the three islets of Spratly. Taxon names are concatenated using a semicolon (;).

The main phyla in Dongdo and Seodo are Actinobacteria, Acidobacteria, Chloroflexi, Bacteroidetes, Armatimonadetes, Chlorobi, Cyanobacteria, Deinococcus-Thermus, Elusimicrobia, Fibrobacteres, Firmicutes, Gemmatiomonadetes, Nitrospiae, Planctomycetes, Proteobacteria [13]. In the three islands in the present study, the dominant phyla are less abundant than those of Dokdo islands. Moreover, proportion of each phylum is various in different islets. Proteobacteria is the phylum with highest proportion in all three islets with 37.76 % at TSL; 32.32 % at SC and 52.00 % at STT. TSL has four other dominant phyla with quite similar proportion: Actinobacteria (10.60 %), Acidobacteria (11.01 %), Chloroflexi (8.54 %) and Bacteroidetes (14.22 %); other phyla were less than 5 %. Differently, SC has distribution of 4 dominant phyla with various proportion: Actinobacteria (13.17 %), Firmicutes (24.54 %), Chloroflexi (8.24 %), Planctomycetota (9.79 %). In STT, the second dominant phylum was Actinobacteria with 12.42 %. Followings were, Chloroflexi (11.50 %), Patescibacteria (9.14 %). Bacteroidota and Acidobacteriota has high proportion in TSL, however not in SC and STT. Planctomycetota occupies 9.79 % in SC and 2.78 % at STT, however, only 0.11 % at TSL.

Table 3. Taxonomic composition of classes from Spratly Islands.

Taxonomy	Read			Proportion (%)		
Class	TSL	SC	STT	TSL	SC	STT
d__Bacteria;p__Bacteroidota;c__Bacteroidia	4,596	2	75	13.04	0.02	0.72
d__Bacteria;p__Firmicutes;c__Clostridia	1,392	2,512	461	3.95	24.44	4.44
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria	12,852	3,242	5,213	36.46	31.54	50.22
d__Bacteria;p__Acidobacteriota;c__Acidobacteriae	779	177	38	2.21	1.72	0.37
d__Bacteria;p__Chloroflexi;c__Anaerolineae	1,366	457	993	3.87	4.45	9.57
d__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae	526	88	70	1.49	0.86	0.67
d__Bacteria;p__Actinobacteriota;c__Actinobacteria	2,165	814	970	6.14	7.92	9.34
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria	458	70	172	1.30	0.68	1.66
d__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes	949	123	19	2.69	1.20	0.18
d__Bacteria;p__Synergistota;c__Synergistia	-	42	263	0.00	0.41	2.53
d__Bacteria;p__Myxococcota;c__Polyangia	1,053	8	-	2.99	0.08	0.00
d__Bacteria;p__Patescibacteria;c__Saccharimonadia	533	309	406	1.51	3.01	3.91
d__Bacteria;p__Acidobacteriota;c__Blastocatellia	2,643	202	31	7.50	1.97	0.30
d__Bacteria;p__Chloroflexi;c__AD3	387	-	-	1.10	0.00	0.00
d__Bacteria;p__Planctomycetota;c__Planctomycetes	16	859	260	0.05	8.36	2.50
d__Bacteria;p__Actinobacteriota;c__Acidimicrobiia	1,185	444	241	3.36	4.32	2.32
d__Bacteria;p__Planctomycetota;c__Phycisphaerae	23	147	29	0.07	1.43	0.28
d__Bacteria;p__Patescibacteria;c__Dojkabacteria	18	-	264	0.05	0.00	2.54
d__Bacteria;p__Acidobacteriota;c__Vicinamibacteria	408	3	-	1.16	0.03	0.00
d__Bacteria;p__Chloroflexi;c__Chloroflexia	369	127	148	1.05	1.24	1.43
d__Bacteria;p__Chloroflexi;c__Gitt-GS-136	74	148	-	0.21	1.44	0.00
Others	3,316	458	694	9.41	4.46	6.69
Unclassified	145	47	33	0.41	0.46	0.32
Total	35,253	10,279	10,380	100	100	100

The list of microbial classes is only shown for classes with a read number of more than 1 % in at least one of the three islets of Spratly. Taxon names are concatenated using a semicolon (;).

At class level (Table 3), TSL has 145 unclassified reads account for 0.41 %. Similarly, SC has only 47 unclassified reads account 0.46 %, STT has 33 unclassified reads account for 0.32 %. In TSL, there are 2 dominant classes *Alphaproteobacteria* of *Proteobacteria* phylum with 36.46 %, and *Bacteroidia* of *Bacteroidota* phylum with 13.04 %. Other dominant classes have lower proportion: *Clostridia*, *Anaerolineae*, *Actinobacteria*, *Blastocatellia*, *Acidimicrobiia*. In SC, the first dominant class is also *Alphaproteobacteria* (31.54 %), following by *Clostridia* (24.44 %). Other dominant classes are *Actinobacteria* and *Blastocatellia.* In STT, similarity to TSL occurs with dominance of *Alphaproteobacteria* (50.22 %), along with another dominant class of *Actinobacteria* (9.34 %), *Anaerolineae* (9.57 %).

At order level (Table 4), TSL has 903 unclassified reads (2.56 %) besides 185 orders including *Sphingomonadales* (14.78 %), *Rhizobiales* (8.46 %), *Blastocatellales* (6.61 %), *Cytophagales* (5.68 %), *Chitinophagales* (4.30 %), *Micrococcales* (3.13 %), *Rhodobacterales* (2.77 %), *Gemmatimonadales* (2.69 %), *Oscillospirales* (2.21 %), *Anaerolineales* (2.05 %),

Actinomarinales (2.03 %). There are 11 orders occupying 1 - 2 %, the others have small proportion (less than 1 %). SC has 119 unclassified reads (0.34 %). The most dominant order of SC is *Clostridiales* with 15.90 %. Other 15 orders occupy approximately 2 - 7 %, such as *Rhodobacterales* (7.73 %), *Rhizobiales* (7.52 %), *Peptostreptococcales-Tissierellales* (7.28 %). There are 10 other orders with proportion of about 1 - 2 %. Beside 246 unclassified reads (2.37 %), STT has dominant orders of *Rhodobacterales* (14.78 %), *Defluviicoccales* (12.17 %), *Anaerolineales* (8.05 %), *Corynebacteriales* (6.74 %), *Rhizobiales* (4.27 %), *Saccharimonadales* (3.91 %), *Caulobacterales* (2.93 %), *Parvibaculales* (2.55 %), *Dojkabacteria* (2.54 %), *Synergistales* (2.53 %), *Clostridiales* ((2.18 %), and *Kiloniellales* (2.10 %). The other 13 orders have proportion of 1-2 %. Distributions of orders in all three islands are different from each other.

At family level (Table 5), TSL, SC and STT have similar percentage of unclassified reads. With TSL, the other reads belong to different 291 families, in which *Sphingomonadaceae* has the highest proportion of 14.78 % following with *Blastocatellaceae* (6.61 %), *Gemmatimonadaceae* (2.69 %), Chitinophagaceae (2.33 %), *Beijerinckiaceae* (2.20 %) and *Anaerolineaceae* (2.05 %). The family *Clostridiaceae* has the highest proportion in SC (15.81 %), meanwhile those in STT and TSL occupy only 2.18 % and 0.16 %, respectively. The family of *Rhodobacteraceae* oocupies a proportion of 7.73 %. The family *Sphingomonadaceae*, the third dominant proportion in SC with 2.96 %, has similar proportion in STT (1.61 %), however, has highest proportion of 14.78 % in TSL. *Gemmataceae*, a dominant family in SC with 2.94 %, occupies only 0.20 % in STT and 0.01 % in TSL. On the other hand, *Defluviicoccaceae*, a dominant family in STT with 12.17 %, occupies only 0.75 % SC and 1.41 % in TSL. *Mycobacteriaceae*, the third dominant family in STT with 3.88 %, does not exist in SC and has a very low proportion of 0.22 % in TSL. These results show that in spite of similarities in natural conditions, micro-communities of islets of Spratly Islands are much different.

At genus level (Table 6), there is an increase in proportion of unclassified reads in all three islands (12.37 - 15.79 %). Distribution of genera with highest proportions of all three islands is in accordance with that of families: *Sphingomonas* with 11.16 % at TSL, *Clostridium sensu stricto 1* with 15.39 % at SC, and *Defluviicoccus* with 12.17 % at STT. Several other genera with higher proportions also have similar distribution to those at family level. Among the genera in STT, remarkably many ones are typical for sea micro-community: *Halomonas* is halophilic (0.10 %) and *Marinobacter* (0.20 %) is the genus which survive only at sea; *Marispirillum* (0.62 %) is a genus isolated in deep ocean regions. However, micro-communities of the three islets in the present study are somehow different from those isolated from other oceanic island regions. Genus *Bacteroidetes* exists in Black Sea samples with proportion of 1.8 - 2 % (Suominen 2021), however it occupies a lower proportion in SC and TSL (0.02 % and 0.06 %, respectively), or *Clociamonetes,* a genus account for up to 13 - 14 % in Black Sea regions, has no appearance only at the three Spratly islands. The appearance of *Bacillus, Dietzia,* and *Marinobacter* was reported in seawater samples and oil reservoirs [11]. The three genera also appear in almost 3 islands in the present study with low proportion. Genus *Syntrophus*, usually found in reservoirs and groundwater, on the other hand, does not appear in any of the 3 Spratly islands. *Halomonas*, an exclusive genus reported in the seawater environment [11], also appear in STT with low proportion.

Table 4. Taxonomic composition of orders from Spratly Islands.

Taxonomy	**Read**			**Proportion (%)**		
Order	**TSL**	**SC**	**STT**	**TSL**	**SC**	**STT**
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales	499	-	2	1.42	0.00	0.02
d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales	58	1,625	226	0.16	**15.81**	**2.18**
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales	976	795	1,534	**2.77**	**7.73**	**14.78**
d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales	319	112	-	0.90	1.09	0.00
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales	60	17	108	0.17	0.17	1.04
d__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Propionibacteriales	345	212	54	0.98	**2.06**	0.52
d__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales	949	123	19	**2.69**	1.20	0.18
d__Bacteria;p__Acidobacteriota;c__Acidobacteriae;o__Bryobacterales	148	150	-	0.42	1.46	0.00
d__Bacteria;p__Synergistota;c__Synergistia;o__Synergistales	-	42	263	0.00	0.41	**2.53**
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales	524	308	304	1.49	**3.00**	**2.93**
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Defluviicoccales	496	77	1,263	1.41	0.75	**12.17**
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales	2,983	773	443	**8.46**	**7.52**	**4.27**
d__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales	1,102	223	163	**3.13**	**2.17**	1.57
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales	1,516	-	-	**4.30**	0.00	0.00
d__Bacteria;p__Myxococcota;c__Polyangia;o__Polyangiales	534	-	-	1.51	0.00	0.00
d__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales	206	258	700	0.58	**2.51**	6.74
d__Bacteria;p__Patescibacteria;c__Saccharimonadia;o__Saccharimonadales	533	309	406	1.51	**3.01**	3.91
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__uncultured	361	228	15	1.02	**2.22**	0.14
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Tistrellales	238	213	82	0.68	2.07	0.79
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales	5,211	304	167	**14.78**	**2.96**	1.61
d__Bacteria;p__Acidobacteriota;c__Blastocatellia;o__Blastocatellales	2,329	202	31	**6.61**	1.97	0.30
d__Bacteria;p__Chloroflexi;c__AD3;o__AD3	387	-	-	1.10	0.00	0.00
d__Bacteria;p__Planctomycetota;c__Planctomycetes;o__Gemmatales	2	302	21	0.01	**2.94**	0.20
d__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Anaerolineales	721	28	836	**2.05**	0.27	8.05
d__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Caldilineales	148	231	10	0.42	**2.25**	0.10
d__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales	271	303	110	0.77	**2.95**	1.06
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Kiloniellales	37	95	218	0.10	0.92	**2.10**
d__Bacteria;p__Patescibacteria;c__Dojkabacteria;o__Dojkabacteria	18	-	264	0.05	0.00	**2.54**
d__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales	779	7	-	**2.21**	0.07	0.00
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Parvibaculales	-	-	265	0.00	0.00	**2.55**
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales	430	-	73	1.22	0.00	0.70
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Micavibrionales	40	10	135	0.11	0.10	1.30

d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Cytophagales	2,001	2	-	**5.68**	0.02	0.00
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Acetobacterales	110	64	119	0.31	0.62	1.15
d__Bacteria;p__Planctomycetota;c__Planctomycetes;o__Planctomycetales	-	471	172	0.00	**4.58**	1.66
d__Bacteria;p__Acidobacteriota;c__Vicinamibacteria;o__Vicinamibacterales	408	3	-	1.16	0.03	0.00
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Zavarziniales	-	-	168	0.00	0.00	1.62
d__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Actinomarinales	716	130	48	**2.03**	1.26	0.46
d__Bacteria;p__Chloroflexi;c__Anaerolineae;o__SBR1031	297	115	108	0.84	1.12	1.04
d__Bacteria;p__Chloroflexi;c__Chloroflexia;o__Thermomicrobiales	160	126	148	0.45	1.23	1.43
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Dongiales	397	109	-	1.13	1.06	0.00
d__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales	83	748	170	0.24	7.28	1.64
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Reyranellales	312	124	122	0.89	1.21	1.18
d__Bacteria;p__Chloroflexi;c__Gitt-GS-136;o__Gitt-GS-136	74	148	-	0.21	1.44	0.00
Others	7,572	1,173	1,367	21.48	11.41	13.17
Unclassified	903	119	246	2.56	1.16	2.37
Total	**35,253**	**10,279**	**10,380**	**100**	**100**	**100**

The list of microbial orders is only shown for orders with a read number of more than 1 % in at least one of the three islets of Spratly. Taxon names are concatenated using a semicolon (;).

Table 5. Taxonomic composition of families from Spratly Islands.

Taxonomy	**Read**			**Proportion (%)**		
Family	**TSL**	**SC**	**STT**	**TSL**	**SC**	**STT**
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae	976	795	1,534	**2.77**	**7.73**	**14.78**
d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae	316	112	-	0.90	1.09	0.00
d__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Propionibacteriales;f__Nocardioidaceae	262	212	54	0.74	**2.06**	0.52
d__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae	949	123	19	**2.69**	1.20	0.18
d__Bacteria;p__Acidobacteriota;c__Acidobacteriae;o__Bryobacterales;f__Bryobacteraceae	148	150	-	0.42	1.46	0.00
d__Bacteria;p__Synergistota;c__Synergistia;o__Synergistales;f__Synergistaceae	-	42	263	0.00	0.41	**2.53**
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Parvularculaceae	24	95	167	0.07	0.92	1.61
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Defluviicoccales;f__Defluviicoccaceae	496	77	1,263	1.41	0.75	**12.17**
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae	443	152	55	1.26	1.48	0.53
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Chitinophagaceae	823	-	-	**2.33**	0.00	0.00
d__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Nocardiaceae	44	146	173	0.12	1.42	1.67
d__Bacteria;p__Patescibacteria;c__Saccharimonadia;o__Saccharimonadales;f__Saccharimonadales	329	109	113	0.93	1.06	1.09
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__uncultured;f__uncultured	361	228	15	1.02	**2.22**	0.14
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Tistrellales;f__Geminicoccaceae	238	213	82	0.68	**2.07**	0.79
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae	5,211	304	167	**14.78**	**2.96**	**1.61**

d__Bacteria;p__Acidobacteriota;c__Blastocatellia;o__Blastocatellales;f__Blastocatellaceae	2,329	202	31	**6.61**	1.97	0.30
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Hyphomonadaceae	334	147	135	0.95	1.43	1.30
d__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Mycobacteriaceae	79	-	403	0.22	0.00	**3.88**
d__Bacteria;p__Chloroflexi;c__AD3;o__AD3;f__AD3	387	-	-	1.10	0.00	0.00
d__Bacteria;p__Planctomycetota;c__Planctomycetes;o__Gemmatales;f__Gemmataceae	2	302	21	0.01	**2.94**	0.20
d__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Anaerolineales;f__Anaerolineaceae	721	28	836	**2.05**	0.27	8.05
d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae	58	1,625	226	0.16	**15.81**	**2.18**
d__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Caldilineales;f__Caldilineaceae	148	231	10	0.42	2.25	0.10
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Saprospiraceae	609	-	-	1.73	0.00	0.00
d__Bacteria;p__Patescibacteria;c__Saccharimonadia;o__Saccharimonadales;f__Saccharimonadaceae	137	76	239	0.39	0.74	2.30
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Kiloniellales;f__Fodinicurvataceae	-	26	154	0.00	0.25	1.48
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Beijerinckiaceae	776	80	28	**2.20**	0.78	0.27
d__Bacteria;p__Patescibacteria;c__Dojkabacteria;o__Dojkabacteria;f__Dojkabacteria	18	-	264	0.05	0.00	2.54
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Xanthobacteraceae	506	171	92	1.44	1.66	0.89
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Parvibaculales;f__Parvibaculaceae	-	-	265	0.00	0.00	2.55
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae	468	155	59	1.33	1.51	0.57
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Micavibrionales;f__Micavibrionaceae	4	-	113	0.01	0.00	1.09
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Cytophagales;f__Microscillaceae	1,807	2	-	5.13	0.02	0.00
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Acetobacterales;f__Acetobacteraceae	110	64	119	0.31	0.62	1.15
d__Bacteria;p__Planctomycetota;c__Planctomycetes;o__Planctomycetales;f__Rubinisphaeraceae	-	368	160	0.00	3.58	1.54
d__Bacteria;p__Acidobacteriota;c__Vicinamibacteria;o__Vicinamibacterales;f__Vicinamibacteraceae	404	3	-	1.15	0.03	0.00
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Zavarziniales;f__Zavarziniaceae	-	-	168	0.00	0.00	1.62
d__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales;f__Ilumatobacteraceae	95	148	87	0.27	1.44	0.84
d__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Actinomarinales;f__uncultured	716	130	48	**2.03**	1.26	0.46
d__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae	466	7	-	1.32	0.07	0.00
d__Bacteria;p__Chloroflexi;c__Chloroflexia;o__Thermomicrobiales;f__JG30-KF-CM45	113	78	148	0.32	0.76	1.43
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Dongiales;f__Dongiaceae	397	109	-	1.13	1.06	0.00
d__Bacteria;p__Chloroflexi;c__Anaerolineae;o__SBR1031;f__SBR1031	56	86	108	0.16	0.84	1.04
d__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcaceae	47	679	38	0.13	6.61	0.37
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Reyranellales;f__Reyranellaceae	312	124	122	0.89	1.21	1.18
d__Bacteria;p__Chloroflexi;c__Gitt-GS-136;o__Gitt-GS-136;f__Gitt-GS-136	74	148	-	0.21	1.44	0.00
Others	11,937	2,060	2,192	33.86	20.04	21.12
Unclassified	1,523	472	409	4.32	4.59	3.94
Total	**35,253**	**10,279**	**10,380**	**100**	**100**	**100**

The list of microbial families is only shown for families with a read number of more than 1 % in at least one of the three islets of Spratly. Taxon names are concatenated using a semicolon (;).

Table 6. Taxonomic composition of genera from Spratly Islands.

Taxonomy	Read			Proportion (%)		
Genus	**TSL**	**SC**	**STT**	**TSL**	**SC**	**STT**
d__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Propionibacteriales;f__Nocardioidaceae;g__Nocardioides	229	181	-	0.65	1.76	0.00
d__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__uncultured	886	123	19	**2.51**	1.20	0.18
d__Bacteria;p__Acidobacteriota;c__Acidobacteriae;o__Bryobacterales;f__Bryobacteraceae;g__Bryobacter	148	150	-	0.42	1.46	0.00
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Roseovarius	19	-	366	0.05	0.00	3.53
d__Bacteria;p__Synergistota;c__Synergistia;o__Synergistales;f__Synergistaceae;g__Thermovirga	-	-	165	0.00	0.00	1.59
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Defluviicoccales;f__Defluviicoccaceae;g__Defluviicoccus	496	77	1,263	1.41	0.75	**12.17**
d__Bacteria;p__Patescibacteria;c__Saccharimonadia;o__Saccharimonadales;f__Saccharimonadales;g__Saccharimonadales	329	109	113	0.93	1.06	1.09
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__uncultured;f__uncultured;g__uncultured	361	228	15	1.02	**2.22**	0.14
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Tistrellales;f__Geminicoccaceae;g__Candidatus_Alysiosphaera	157	210	-	0.45	**2.04**	0.00
d__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Mycobacteriaceae;g__Mycobacterium	66	-	403	0.19	0.00	**3.88**
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Amaricoccus	86	330	-	0.24	**3.21**	0.00
d__Bacteria;p__Chloroflexi;c__AD3;o__AD3;f__AD3;g__AD3	387	-	-	1.10	0.00	0.00
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Paracoccus	194	102	216	0.55	0.99	**2.08**
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas	3,933	126	-	**11.16**	1.23	0.00
d__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Anaerolineales;f__Anaerolineaceae;g__uncultured	391	28	271	1.11	0.27	**2.61**
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Saprospiraceae;g__uncultured	552	-	-	1.57	0.00	0.00
d__Bacteria;p__Patescibacteria;c__Saccharimonadia;o__Saccharimonadales;f__Saccharimonadaceae;g__TM7a	129	48	217	0.37	0.47	**2.09**
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Hyphomonadaceae;g__SWB02	248	111	-	0.70	1.08	0.00
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Kiloniellales;f__Fodinicurvataceae;g__uncultured	-	26	154	0.00	0.25	1.48
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Beijerinckiaceae;g__Microvirga	772	80	-	**2.19**	0.78	0.00
d__Bacteria;p__Patescibacteria;c__Dojkabacteria;o__Dojkabacteria;f__Dojkabacteria;g__Dojkabacteria	18	-	264	0.05	0.00	**2.54**
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Hyphomonadaceae;g__Hyphomonas	10	-	122	0.03	0.00	1.18
d__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Nocardiaceae;g__Rhodococcus	44	139	-	0.12	1.35	0.00
d__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Caldilineales;f__Caldilineaceae;g__uncultured	142	231	10	0.40	**2.25**	0.10
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Parvibaculales;f__Parvibaculaceae;g__Parvibaculum	-	-	252	0.00	0.00	**2.43**
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Pedomicrobium	331	137	37	0.94	1.33	0.36
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Cytophagales;f__Microscillaceae;g__uncultured	954	-	-	**2.71**	0.00	0.00
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Acetobacterales;f__Acetobacteraceae;g__Roseomonas	58	57	119	0.16	0.55	1.15
d__Bacteria;p__Acidobacteriota;c__Vicinamibacteria;o__Vicinamibacterales;f__Vicinamibacteraceae;g__Vicinamibacteraceae	404	3	-	1.15	0.03	0.00
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Zavarziniales;f__Zavarziniaceae;g__Zavarzinia	-	-	168	0.00	0.00	1.62
d__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Actinomarinales;f__uncultured;g__uncultured	716	130	48	**2.03**	1.26	0.46
d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_1	58	1,582	181	0.16	**15.39**	1.74
d__Bacteria;p__Chloroflexi;c__Chloroflexia;o__Thermomicrobiales;f__JG30-KF-CM45;g__JG30-KF-CM45	113	78	148	0.32	0.76	1.43
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Dongiales;f__Dongiaceae;g__Dongia	397	109	-	1.13	1.06	0.00

d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Defluviimonas	47	86	210	0.13	0.84	**2.02**
d__Bacteria;p__Chloroflexi;c__Anaerolineae;o__SBR1031;f__SBR1031;g__SBR1031	56	86	108	0.16	0.84	1.04
d__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Anaerolineales;f__Anaerolineaceae;g__Pelolinea	-	-	121	0.00	0.00	1.17
d__Bacteria;p__Planctomycetota;c__Planctomycetes;o__Gemmatales;f__Gemmataceae;g__uncultured	2	131	3	0.01	1.27	0.03
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Reyranellales;f__Reyranellaceae;g__uncultured	283	123	95	0.80	1.20	0.92
d__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcaceae;g__Romboutsia	16	355	37	0.05	3.45	0.36
d__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcaceae;g__Terrisporobacter	9	311	-	0.03	3.03	0.00
d__Bacteria;p__Chloroflexi;c__Gitt-GS-136;o__Gitt-GS-136;f__Gitt-GS-136;g__Gitt-GS-136	74	148	-	0.21	1.44	0.00
d__Bacteria;p__Planctomycetota;c__Planctomycetes;o__Planctomycetales;f__Rubinisphaeraceae;g__SH-PL14	-	240	16	0.00	2.33	0.15
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Cytophagales;f__Microscillaceae;g__Chryseolinea	373	-	-	1.06	0.00	0.00
d__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Anaerolineales;f__Anaerolineaceae;g__Ornatilinea	-	-	139	0.00	0.00	1.34
d__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Anaerolineales;f__Anaerolineaceae;g__ADurb.Bin120	-	-	172	0.00	0.00	1.66
Others	16,200	3,132	3,408	45.95	30.47	32.83
Unclassified	5,565	1,272	1,520	15.79	12.37	14.64
Total	**35,253**	**10,279**	**10,380**	**100**	**100**	**100**

The list of microbial genera is only shown for genera with a read number of more than 1 % in at least one of the three islets of Spratly. Taxon names are concatenated using a semicolon (;).

Table 7. Taxonomic composition of species from Spratly Islands.

Taxonomy	**Read**			**Proportion (%)**		
Species	**TSL**	**SC**	**STT**	**TSL**	**SC**	**STT**
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Amaricoccus;s__uncultured_bacterium	70	287	-	0.20	2.79	0.00
d__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Anaerolineales;f__Anaerolineaceae;g__uncultured;s__uncultured_Chloroflexi	17	-	133	0.05	0.00	1.28
d__Bacteria;p__Patescibacteria;c__Saccharimonadia;o__Saccharimonadales;f__Saccharimonadaceae;g__TM7a;s__uncultured_bacterium	112	48	217	0.32	0.47	2.09
d__Bacteria;p__Patescibacteria;c__Dojkabacteria;o__Dojkabacteria;f__Dojkabacteria;g__Dojkabacteria;s__uncultured_bacterium	8	-	133	0.02	0.00	1.28
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Defluviicoccales;f__Defluviicoccaceae;g__Defluviicoccus;s__uncultured_bacterium	128	-	1,064	0.36	0.00	10.25
d__Bacteria;p__Planctomycetota;c__Planctomycetes;o__Planctomycetales;f__Rubinisphaeraceae;g__SH-PL14;s__uncultured_bacterium	-	137	-	0.00	1.33	0.00
d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_1;s__Clostridium_butyricum	-	153	20	0.00	1.49	0.19
d__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Anaerolineales;f__Anaerolineaceae;g__Ornatilinea;s__uncultured_Chloroflexi	-	-	139	0.00	0.00	1.34
d__Bacteria;p__Planctomycetota;c__Planctomycetes;o__Planctomycetales;f__Rubinisphaeraceae;g__SH-PL14;s__uncultured_Planctomycetaceae	-	103	16	0.00	1.00	0.15
d__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcaceae;g__Terrisporobacter;s__uncultured_bacterium	-	177	-	0.00	1.72	0.00
Others	9,136	2,113	2,670	25.92	20.56	25.72
Unclassified	25,782	7,261	5,988	73.13	70.64	57.69
Total	**35,253**	**10,279**	**10,380**	**100**	**100**	**100**

The list of microbial species is only shown for species with a read number of more than 1 % in at least one of the three islets of Spratly. Taxon names are concatenated using a semicolon (;).

At species level (Table 7), the proportion of unclassified reads increases dramatically to 57.69 - 73.13 %. TSL has many species which has the ability of organic matter decomposition and salt-tolerance such as *Lactococus lactis, Bacillus anthracis*. Besides, there are other dominant species such as *Shewanella, Pseudomonas, Lactococcus, Faecalibacterium, Microbacterium, Streptomyces, E. coli, Acinetobacter*. STT has species typical for oceanic micro-community such as *Halomonas* sp. X28, *Galbibacter marinus*, *Bacillus funiculus*, *Marinicella litoralis*. SC has *Lactobacillus fermentum*, *Lysobacter arseniciresistens* ZS79, *Burkholderiales bacterium* X4, *Desulfomicrobium* sp 63 and many uncultured species.

4. CONCLUSIONS

In the present study, micro-biodiversity of three islets of Spratly Islands has been evaluated by metagenomics. Although the component and proportion of each taxonomic level is unique and different from each other and from those collected in other island research, the results have shown the characteristics of micro-biomes of TSL, SC and STT islands. These characteristics reflect the natural and human conditions affecting and determine the dominance of bacteria population of each island. Further investigation should be carried out to deeper understanding the micro-community of Spratly islands and their changes in global warming.

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Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. Handelsman J. - Metagenomics: application of genomics to uncultured microorganisms. Microbiol. Mol. Biol. Rev., **68**(4) (2004) 669–685. https://doi.org/10.1128/MMBR.68.4.669-685.2004.
2. Hong S. W., Lee J. S., Chung K. S. - Polymerase chain reaction-denaturing gradient gel electrophoresis analysis of bacterial community structure in the food, intestines, and feces of earthworms. J. Microbiol., **49** (2011) 544–550. https://doi.org/10.1007/s12275-011-0423-8.
3. Islam E., Sar P. - Culture-dependent and -independent molecular analysis of the bacterial community within uranium ore. J. Basic Microbiol., **51** (2011) 372–384. https://doi.org/10.1002/jobm.201000327.
4. Shokralla S., Spall J. L., Gibson J. F., Hajibabaei M. - Next-generation sequencing technologies for environmental DNA research. Mol. Ecol., **21** (2012) 1794–1805. https://doi.org/10.1111/j.1365-294X.2012.05538.x.
5. Streit W. R., Schmitz R. A. - Metagenomics--the key to the uncultured microbes. Curr. Opin. Microbiol., **7** (2004) 492–498. https://doi.org/10.1016/j.mib.2004.08.002.

6. Zhalnina K., de Quadros P. D., Camargo F. A., Triplett E. W. - Drivers of archaeal ammonia-oxidizing communities in soil. Front. Microbiol., **3** (2012) 210. https://doi.org/10.3389/fmicb.2012.00210.
7. Gilles A., Meglecz E., Pech N., Ferreira S., Malausa T., Martin J. F. - Accuracy and quality assessment of 454 GS-FLX Titanium pyrosequencing. BMC Genomics, **12** (2011) 245. https://doi.org/10.1186/1471-2164-12-245.
8. Li A., Chu Y., Wang X., Ren L., Yu J., Liu X., Yan J., Zhang L., Wu S., Li S. - A pyrosequencing-based metagenomic study of methane-producing microbial community in solid-state biogas reactor. Biotechnol. Biofuels, **6** (2013) 3. https://doi.org/10.1186/1754-6834-6-3.
9. Abraham B. S., Caglayan D., Carrillo N. V., Chapman M. C., Hagan C. T., Hansen S. T., Jeanty R. O., Klimczak A. A., Klingler M. J., Kutcher T. P., Levy S. H., Millard-Bruzos A. A., Moore T. B., Prentice D. J., Prescott M. E., Roehm R., Rose J. A., Yin M., Hyodo A., Lail K., Daum C., Clum A., Copeland A., Seshadri R., Del Rio T. G., Eloe-Fadrosh E. A., Benskin J. B. - Shotgun metagenomic analysis of microbial communities from the Loxahatchee nature preserve in the Florida Everglades. Environ. Microbiome, **15** (2020) 2. https://doi.org/10.1186/s40793-019-0352-4.
10. Yusof N. S. M., Latiff M. A. B. A., Ridwan A. R. M., Badrulisham A. S., Yaakop S., Nor S. M., Zain B. M. M. - First report on metagenomic analysis of gut microbiome in Island Flying Fox (*Pteropus hypomelanus*) revealing latitudinal correlation as opposed to host phylogeny in island populations of Malaysia. Authorea Preprints, (2020) https://doi.org/10.22541/au.159121433.35769721.
11. Hidalgo K. J., Sierra-Garcia I. N., Dellagnezze B. M., de Oliveira V. M. - Metagenomic Insights Into the Mechanisms for Biodegradation of Polycyclic Aromatic Hydrocarbons in the Oil Supply Chain. Front. Microbiol., **11** (2020) 561506. https://doi.org/10.3389/fmicb.2020.561506.
12. Eze M. O. - Metagenome analysis of hydrocarbon-degrading bacterial consortium reveals the specific roles of BTEX biodegraders. Genes, **12**(1) (2021) https://doi.org/10.3390/genes12010098.
13. Kim Y. E., Yoon H., Kim M., Nam Y. J., Kim H., Seo Y., Lee G. M., Kim Y. J., Kong W. S., Kim J. G., Seu Y. B. - Metagenomic analysis of bacterial communities on Dokdo Island. J. Gen. Appl. Microbiol., **60** (2014) 65–74. https://doi.org/10.2323/jgam.60.65.