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Vertical profile of culturable bacteria from the Makassar Strait, Indonesia

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Abstract. *Siallagan ZL, Kristianti T, Dwivany FM, Nugrahapraja H, Fretes CED, Fadli M, Trinugroho JP, Radjasa OK, Susanto RD. 2023. Vertical profile of culturable bacteria from the Makassar Strait, Indonesia. Biodiversitas 24: 1356-1365.* Indonesia's extensive maritime background is a rich biological and chemical diversity source. This diversity has become as source of unique chemical compounds with the potential for industrial development as enzymes, molecular probes, chemicals, pharmaceuticals, cosmetics, nutritional supplements, and agrochemicals. The present study aimed to analyze the community composition of culturable bacteria from three different layers of depth from Makassar Strait using high throughput DNA Illumina sequencing of 16S rRNA. Bioinformatic analysis has rendered 140336 high-quality sequences with an average of 124127 sequences per sample and a mean read length of 428 bp. Results showed that Firmicutes and Proteobacteria were the two most abundant phyla. Taxonomic analysis showed that Firmicutes dominated all samples. Genus *Streptomycetes* and *Psychrobacter* occurred mainly in the culture from the surface layer. Species richness and diversity for the bacterial communities at 100 m were higher than those at 5 m and 200 m. PCA plot, NMDS plot, and UPGMA clustering demonstrated that the culturable bacterial community compositions of 100 and 200 ms were highly similar and distinct from those in 5 m. This research discovered microbes with potential as sources of marine natural products, including *Streptomyces, Paenibacillus, Bacillus, Psychrobacter, Arthrobacter, Bradyrhizobium, Gemmatimonas*, and *Acidobacteria.*

Keywords: 16S rRNA gene, biodiversity, culturable bacteria, Firmicutes, Proteobacteria

INTRODUCTION

Microorganisms in seawater are diverse and abundant and play an essential role in ecosystems, including the marine environment. Marine microorganisms can remineralize particles in the water column and provide nutrients for microorganisms and seafloor organisms (Seymour 2014). Microorganisms such as marine bacteria have diverse metabolisms and participate in important biogeochemical metabolism and recycling of nutrients, including carbon, nitrogen, sulfur, phosphorus, iron, and other elements (Kapellos et al. 2022). Furthermore, by mediating chemical transformations, marine bacteria influence the nutrient composition and energy flow in the water and sediment (Ren et al. 2019).

The diversity of marine bacteria is undoubtedly related to the physical and chemical conditions of the environment. Wang et al. 2016 reported that the driving factors that shape the composition and pattern of the vertical distribution of bacteria in the eastern Indian Ocean are closely related to physical, chemical, and biological properties. Previous research has shown that proteobacteria were predominant in the Atlantic Ocean (Schauer et al. 2010), Arctic Ocean (Han et al. 2015), and Pacific Ocean (Suh et al. 2014), especially during the summer season. In contrast, Bacteroidetes become more abundant than Proteobacteria in the Pacific Ocean during winter (Suh et al. 2014). Bacteria can grow in various environments associated with marine organisms, such as algae, sponges, seagrass, and soft corals. The advantage for these organisms is that bacteria contribute to hosting defense by producing secondary metabolites in bioactive compounds. The identification of the metabolic potential of microbial communities from marine habitats has been chiefly investigated using various approaches. That includes metagenomic (Kennedy et al. 2008; Kodzius and Gojobori 2015) and enhancing the cultivability of microorganisms. That is to study many things about the bioprospecting of marine microorganisms (Dionisi et al. 2012).

The diversity of bacteria from the South Atlantic Ocean was studied by phylogenetic analysis of the 16S rRNA sequences finding eight classes, namely g-Proteobacteria, α-Proteobacteria, Actinobacteria, Actinomycetales, Bacilli, Flavobacteria, Opitutae and Sphingobacteria (Kai et al. 2017). Metagenomics through sequence-based and function-based screening has led to the discovery and synthesis of many biologically significant compounds. For example, such as polyketide synthase, non-ribosomal

peptide synthetase, antibiotics, and biocatalysts (Mahapatra et al. 2019). Until now, a bacterial culture has been the main source in the search for raw materials for drugs, including antibiotics. Several groups of bacteria from marine ecosystems have been known as a source of antibiotic compounds, including the Firmicutes phylum (genera: *Bacillus, Virgibacillus, Brevibacillus, Aneurinibacillus, Staphylococcus*, *Paenibacillus*), Proteobacteria (genera: *Microbulbifer, Salinivibrio, Pseudoalteromonas, Pseudomonas, Stenotrophomeroides* and *Pontibacter*) (Al-Amoudi et al. 2016).

Makassar strait is the main pathway of the Indonesian throughflow (ITF) from the Pacific to the Indian Ocean (Susanto et al. 2012; Gordon and Fine 1996). The presence of ITF affects the diversity of marine organisms in this strait. In addition, seasonal run-off from rivers, such as the Mahakam River in Kalimantan, into the Makassar strait can affect surface water salinity and nutrient levels in this strait (Rachman et al. 2021). These conditions can affect the community dynamics of marine organisms in the Makassar strait water column, including marine microorganisms. The aim of this paper was to investigate the diversity structure of cultivable marine bacteria in three different layers of depth from Makassar strait using high throughput DNA sequencing of the 16S rRNA gene. This research can provide new information about the diversity of bacteria and their vertical distribution in the Makassar Strait.

MATERIALS AND METHODS

Study area

The research was carried out in the Makassar strait during the TRIUMPH expedition, using the research vessel Baruna Jaya VIII in December 2019 (North East Monsoon / NEM). Seawater samples were collected from Station Balikpapan, with the coordinate of 1^0 44.439' South and 117^0 24.991' East (Figure 1). In addition, samples collected from three layers of depth (5 m, 100 m, and 200 m) and physical vertical profiles of seawater for oceanography analysis were also recorded from surface to 500 m. Station Balikpapan is located on the western side of Makassar Strait, on the edge of the North Makassar Basin, and on the northwestern side of Labani Channel, which has a maximum depth of 504 m.

Procedures

Sample collection and CTD measurement

Seawater samples were collected using a Carousel Water Sampler type SBE 32 equipped with a CTD (Conductivity Temperature Depth) type SBE 911+ to measure the physical vertical profile of seawater (pressure, temperature, salinity, fluorescence, and dissolved oxygen (DO) concentration). CTD casts were conducted from the surface to 500 m depth. The recording interval was set to 32 measurements/second, and the casting speed was kept at a maximum of 50 m/minute to minimize the noise during measurement. Onboard, 2-2.5 L of seawater from the water sample were filtered on 0.22 µm pore-size cellulose nitrate membrane filters. The filtrates were stored in sterile falcon tubes at -20°C.

DNA extraction

DNA extraction was carried out in the laboratory, starting with cutting the filter membrane into small pieces ($1cm^2$), then put into a liquid Zobell medium and incubated in a shaker at 200 rpm for 3-4 days at 37°C. The total cultured bacteria were collected using a centrifuge at 12,000 rpm for 2 minutes to obtain pellets. After that, total genomic DNAs were extracted from pellets, following the instruction of the Genomic Zymo BIOMICS DNA Miniprep Kit. DNA concentration and purity were monitored on 1% agarose gel. According to the concentration, DNA was diluted to 1 ng/µL using sterile water.

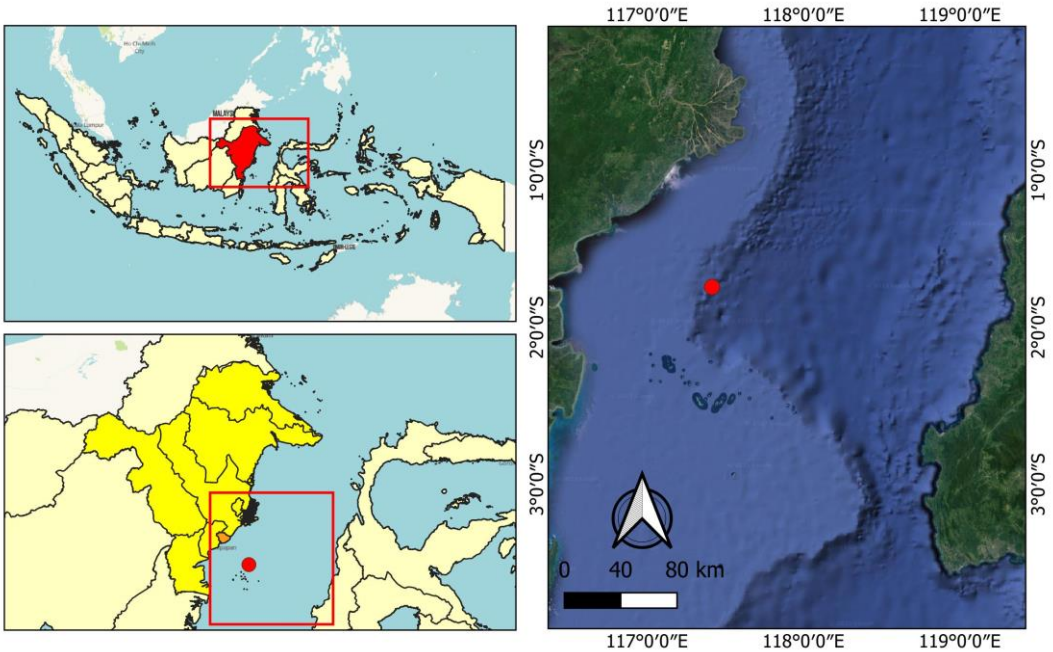


Figure 1. Location of sampling site in the Makassar Strait, Indonesia

PCR amplification and sequencing

In the process from DNA samples to final data, each step, such as sample test, PCR, Purification, library preparation, and sequencing, affected the quality and quantity of data. DNAs were amplified more than three times with universal bacterial primers 16S-341F (5'-CCTAYGGGRBGCASCAG-3') and 16S-806R (5'-GGACTACNNGGGTACTAAT-3') flanking the V3 and V4 regions of the 16S gene. The PCR reaction was carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs). PCR was performed using a Peltier Thermal Cycler (Bio-Rad) with the following conditions: initial denaturation at 95°C for 2 mins; 35 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 40 sec; final extension at 72°C for 7 mins. PCR products were then mixed with 1x loading buffer and subsequently subjected to gel electrophoresis on 2% agarose. Samples with bright main strips between 400bp-450bp were selected for further experiments. PCR products were mixed at equal density ratios. The composite PCR products were purified with Qiagen Gel Extraction Kit (Qiagen, Germany). The libraries generated with NEBNext® UltraTM DNA Library Prep Kit for Illumina and quantified via Qubit and qPCR were analyzed by the Illumina platform.

Data analysis

Paired-end reads (Raw PE) were merged using FLASH (V1.2.7) (Magoč and Salzberg 2011). Quality filtering on the raw tags was performed according to the Qiime (V1.7.0) quality-controlled process (Bokulich et al. 2013; Caporaso et al. 2010). The tags were compared with the reference database (SILVA database) using the UCHIME algorithm. First, the chimera sequences were removed then the Effective Tags were obtained (Haas et al. 2011). Then, Uparse v7.0.1090 software was used to analyze the effective tags of the sequences (Edgar 2013). Sequences with 97% similarity were grouped in the same OTUs. Representative sequences of each OTUs are further annotated. For each representative sequence, Qiime Version 1.7.0 (Altschul et al. 1990) in the Mothur method was performed against the SSUrRNA database of SILVA Database(Q. Wang et al. 2007) for species annotation at each taxonomic rank (Threshold:0.8~1) (Quast et al. 2013). Alpha diversity was calculated with QIIME (Version 1.7.0) and displayed with R software (Version 2.15.3). Beta diversity on both weighted and unweighted unifrac was calculated by QIIME software (Version 1.7.0). Data from CTD measurement will followed quality control (QC) methods adopted and modified from (Valcheva and Palazov 2010; Sea-Bird Scientific 2017) by using SBE Data Processing software provided by Sea Bird Electronic and depicted and analysis by using Ocean Data View Software.

RESULTS AND DISCUSSION

Sequencing statistics and diversity estimation

Amplicon (16S rRNA) sequencing at three different depths obtained a total sequencing of 413,900 (Raw PE), with an average value of 137,967 (Table 1). Meanwhile, raw, clean, and effective tags had mean values of 124,127, 118,355, and 106,071, respectively. The average length (nt) obtained at the three depths was the same as 428. The total number of OTUs (OTU = Operational Taxonomic Unit) was 2265 with an average value of 755, while the total number of OTUs was 11130 and unique OTUs 3710. The number of OTUs, abundance, and diversity at 100 m depths was relatively higher than the two other depths. In addition, coverage for all samples at three depths was not differed (99%). The vertical profile showed that the value of richness (Chao1) and diversity (Shannon Index H 'and Simpson) at 100 m was higher than the depths of 5 m and 200 m.

The OTUs number of samples showed that samples from 100 m had a higher tag unique than 5 m and 200 m (Figure 2). The 5 m level had the highest total and taxon tags compared to 100 m and 200 m. However, 5 m had the lowest unique tags. Based on the average relative abundance analysis at the phylum level, most sequences in the three groups samples were related to 10 phyla: Firmicutes, Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Verrucomicrobia, Rokubacteria, Nitrospirae, and Bacteriodetes. The top three phyla in all samples were Firmicutes, Proteobacteria, and Acidobacteria. The Firmicutes phyla was the most abundant in all depths. The heatmap was drawn to check whether the samples with similar processing were clustered. Heatmaps can visualize the microbial composition of samples belonging to different water samples. According to the Taxonomic abundance cluster heatmap (Figure 3A), there were 35 genera of all samples. *Streptomycetes* and *Psychrobacterium* were the most abundant genera found at 5 m; meanwhile, *Paenibacillus, Bacillus, Candidatus Solibacter, Alphaproteobacteria, Acidobacter,* and *Haliangium* were least abundant in the same depth. On the other hand, *Gammaproteobacteria, Sphingomonas, Dongia, Steroidobacter, Pedomicrobium, Athrobacter, Mizugakiibacter, Reyranella,* and *Bryobacter* were most abundant in water samples of 100 m. *Acidobacteriia, Paenibacillus, Candidatus Udaeobacter, Anaerimyxobacter* and *Chloroflexi* were most abundant in 200 m.

The rarefaction curve and the rank abundance curve are shown in Figures 5A and 5B. When the rarefaction curve approaches a plateau, it indicates that the number of OTUs was sufficient to reveal the original bacterial community in each sample. Meanwhile, the rank abundance curve intuitively reflects the species richness in the sample and the uniformity distribution. The higher the species richness, the greater the range of the curve on the horizontal axis.

Table 1. Diversity estimation for water samples collected from three different depths in Station Balikpapan.

Dept (m)	*Raw PE	No. of sequences	Average length (nt)	OTUs		Observed species	Diversity		Richness	Coverage
				Total	Unique		Shannon index	Simpson	Chao1	Chao (%)
5	140,336	112400	428	676	2046	619	1.842	0.49	656.133	0.999
100	134,790	103120	428	824	5748	824	3.494	0.813	1062.467	0.998
200	138,774	102693	428	765	3336	720	2.083	0.538	774.101	0.999

Note: *Raw PE means the original PE reads off the computer

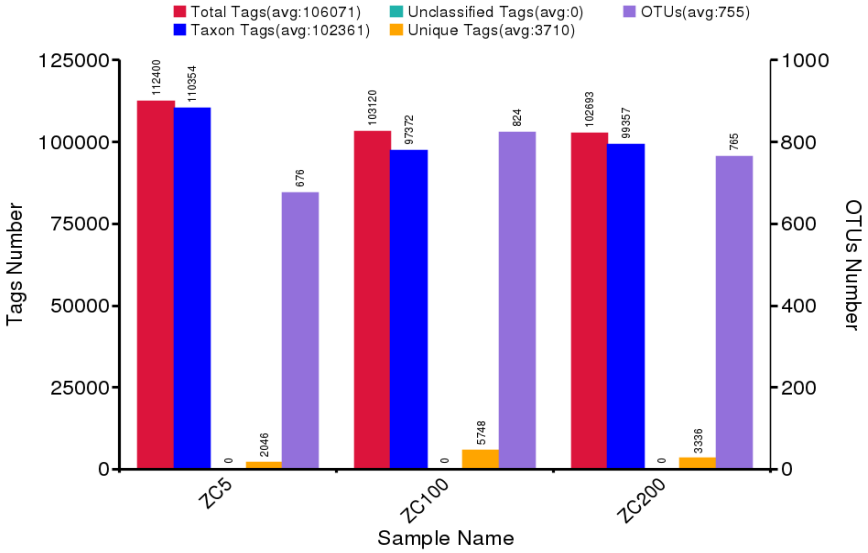
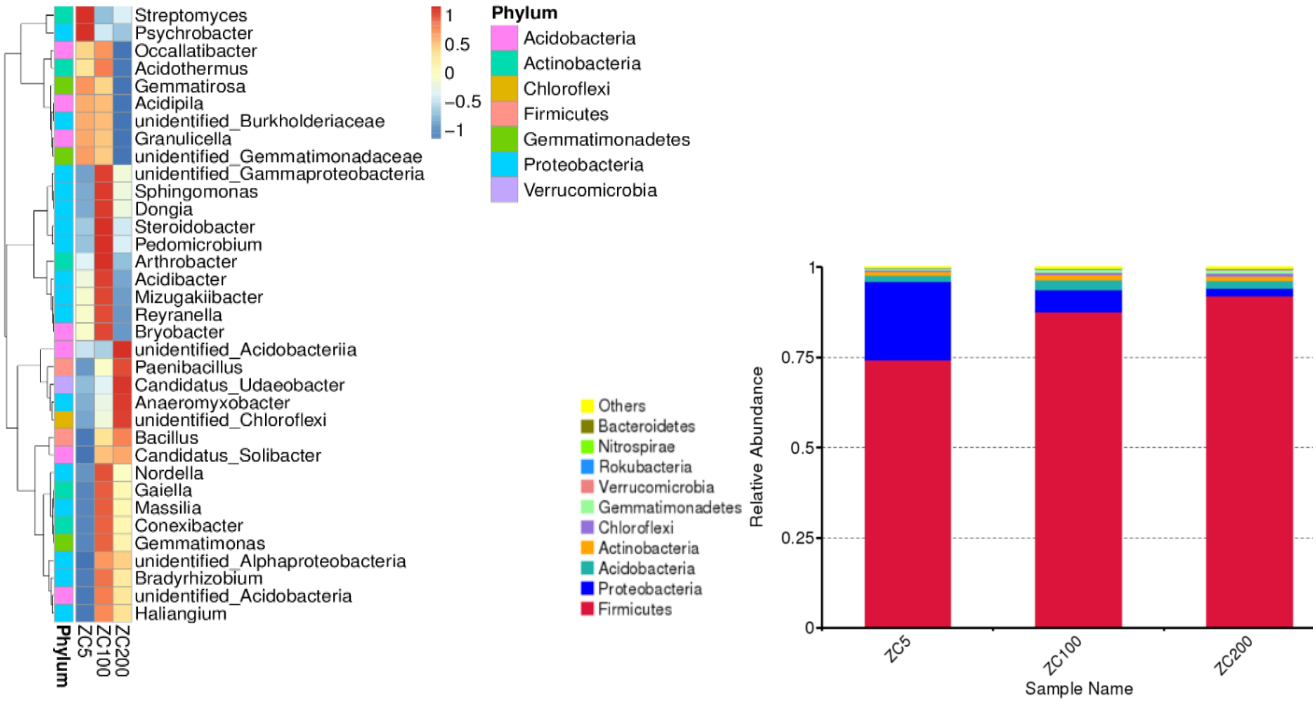


Figure 2. Tags and OTUs number of each sample from three different depths in Station Balikpapan, Makassar Strait, Indonesia



A **B**

Figure 3. A. Taxonomic abundance cluster heatmap; B. Taxa relative abundance in the phylum. X-axis represents "samples name," and Y-axis represents "relative abundance."

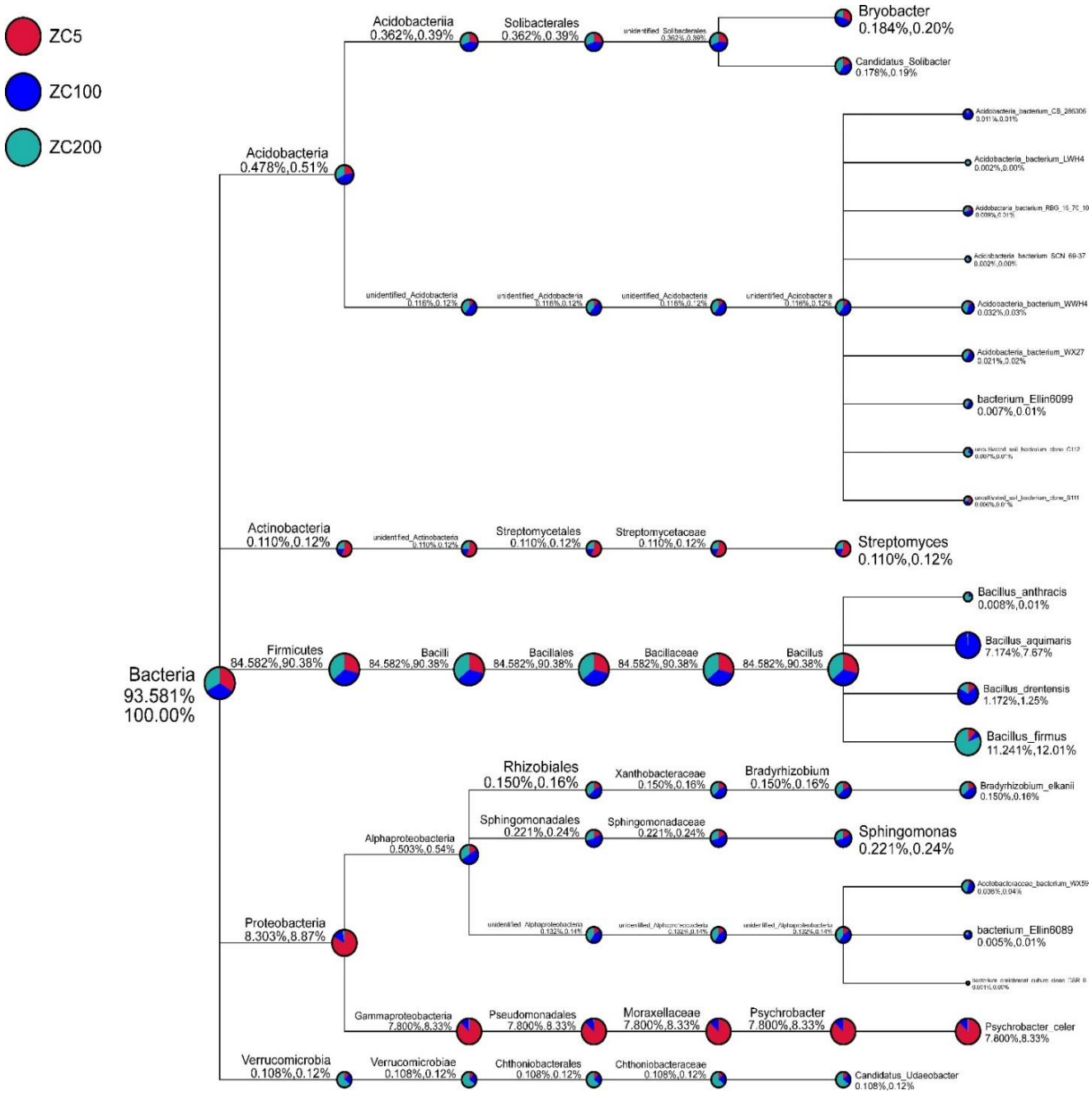


Figure 4. Taxonomy tree of all samples. Different colors represent different taxonomic ranks. The size of the circles represents the relative abundance of species

Microbial community structure

Relative abundance (Figure 3B) was the percent composition of an organism of a particular kind close to the total number of organisms in the area. According to the taxonomic annotation results, the top 10 taxa of each sample or group at each taxonomic rank were selected to form the distribution histogram of the relative abundance of taxa. That is to visualize taxa with a higher relative abundance and proportion in different classification levels of each sample. Furthermore, it was observed that a total of ten phyla were obtained from the samples tested. Firmicutes was the most dominant phylum at all depths, followed by Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Verrucomicrobia, Rokubacteria, Nitrospirae, Bacteroidetes. The phylum Firmicutes at each depth had an increased relative abundance value in contrast with the phylum Proteobacteria, Acidobacteria, which had a decreasing value, while the other phyla had almost the same abundance. The visualization shown by the UPGMA Cluster Tree also shows the same results (Figure 7C). *Bryobacter*, *Candidatus*, *Basillus*, *Psychrobacter*, *Streptomyces*, and *Acidobacteria* were the dominant genera appearing on all samples' taxonomic trees (Figure 4).

Venn diagrams were plotted to show the similarities among different microbial communities at different depths in the same strait region regarding the overlapping of OTUs. From the Venn diagram (Figure 6A), all three water depths had the same 440 OTUs. 5 m and 200 m depths had the same 32 OTUs, 100 m and 200 m depths had the same 141 OTUs, while 5 m and 100 m had the same 106 OTUs. 41 unique OTUs at 5 m depth, 137 unique OTUs at 100 m depth, and 107 unique OTUs at 200 m depth, which means the number of specific OTUs found at 100 m was higher than the others. The profile of microbial community structure between 100 m and 200 m depth had a similarity of about 60%, and the same values were found between 5 m and 100 m depths. The similarity of bacterial community structure at 5 m and 200 m was about 54%. Although the bacterial community structure at the three water depths was similar, the results of the beta diversity heatmap showed that the bacterial community at the three water depths still had differences, but the value was not significantly different (Figure 6B).

The taxonomic abundance cluster heatmap result is presented in Figure 3(A). The graph shows that the most common genus was Proteobacteria (16 genera), followed by Acidobacteria (7 genera), Actinobacteria (5 genera), Firmicutes (2 genera), Chloroflexi (1 genus), Gemmatimonadetes (3 genera), Verrucomicrobia (1 genus). Although Firmicutes was the most dominant phylum in all depths, the number of appearing genus levels was relatively less.

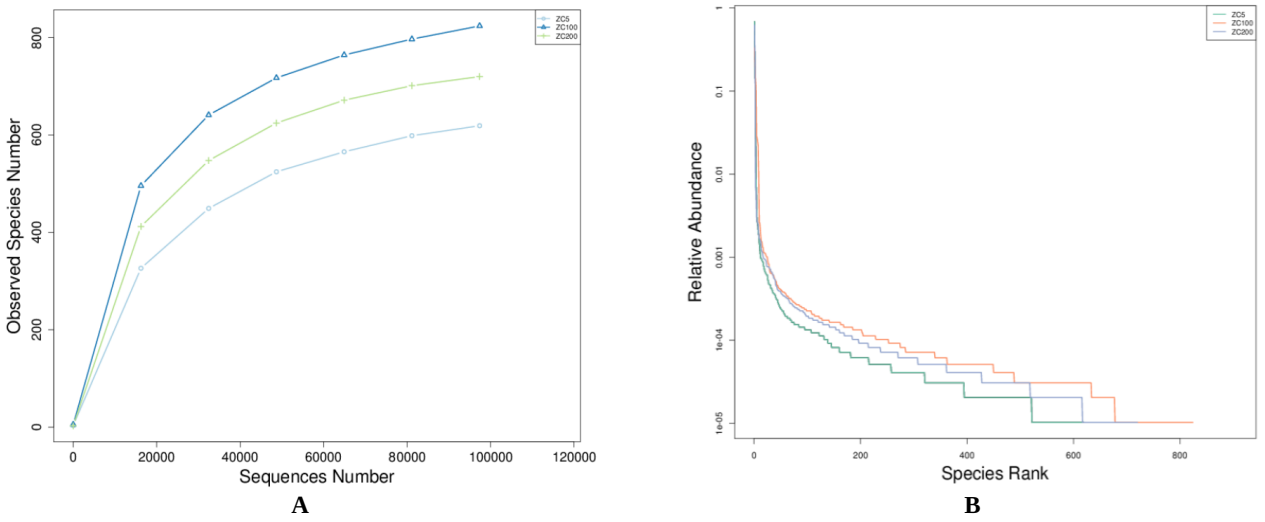


Figure 5. (A) Species rarefaction curve (B) Rank abundance curve

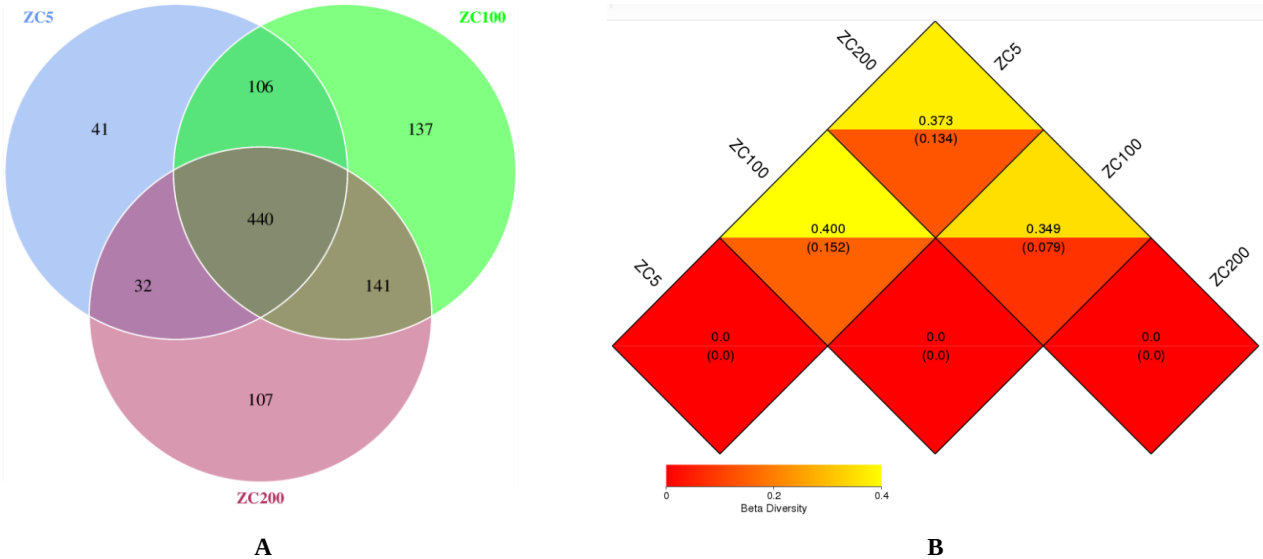


Figure 6. A. Venn diagram (each circle represents one sample or group); B. Beta diversity heatmap (each grid represents pairwise dissimilarity coefficient between pairwise samples)

PCA plot scores were based on the relative abundance of OTUs (Figure 7A). The more similar the community composition was among samples, the closer the distance of their corresponding data points on the PCA graph. PCA results showed that the composition of bacterial communities at depths of 5 m, 100 m, and 200 m differed. It's a non-linear model designed to represent the non-linear biological data structure better, aiming to overcome the flaws in methods based on linear models. The result of the NMDS analysis was based on OTUs. Each data point in the graph represents a sample (Figure 7B). Therefore, the distance between data points reflects the extent of variation. Samples belonging to the same group are in the same color. When the stress factor value is less than 0.2, it's considered that NMDS is reliable to some extent.

Physical oceanographic condition

The water mass dynamics at Station Balikpapan were influenced by the ITF water mass, which underwent a slight change due to the bathymetric structure and the interaction with the water mass of the Mahakam River. This bathymetry configuration makes the mass flow of ITF water on the west side deflected to the southeast to pass through the Labani Channel. Physical oceanography conditions (temperature, salinity, fluorescence, and dissolved oxygen concentration) at a depth of 5 m, 100 m, and 200 m can be observed in Figures 8A and 8B). In contrast, the water mass type can be observed in Figure 8C. Based on temperature data (Figure 8A blue line), the vertical layer can be divided into three layers, i.e., mixed layer (down to 43 m), thermocline layer (43 - 250 m), and deep layer (250 - seabed). The thickness of the mixed layer (based on temperature) is also supported by the vertical profile of dissolved oxygen concentration (DO) (Figure 8B black line). The DO concentration in the mixed layer was about 6.2 mg/L and decreased with depth, except at 120 - 220 m. Decreasing DO concentration in the upper of 120 m was caused by the consumption of plankton (Fluor-max, 0.79 mg/m^3 at 50.73 m, Figure 8B green line) and fishes during night time (CTD measured at night in this station). While the increase of DO concentration in the water column was caused by the intrusion of North Pacific upper water that brings oxygen from the Pacific Ocean. Based on the vertical salinity profile (Figure 8A red line), low salinity (33.42 PSU) was found at the upper layer, where salinity maximum (S-Max, 34.64 PSU) was found at 137 m, and deep salinity minimum (S-Min, 34.45 PSU) was found at 293 m.

Based on the temperature and salinity (TS) diagram (Figure 8C), the surface layer is filled with fresh (33.5-33.75 PSU) and warm (29-30.5°C) water. North Pacific Upper Water (NPU or NP, salinity maximum at 137 m) and North Pacific Intermediate Water (NPIW, salinity minimum at 293 m) were identified in the deeper layer. The core of NPU water (34.64 PSU, 19.48°C, and 24.61 kg/m^3) at 137 ms, with a key characteristic of salinity maximum representing a layer of ITF core. Furthermore, the core of NPIW (34.53 PSU, 10.65°C, and 24.41 kg/m^3) at 293 m, with a key characteristic of salinity minimum at the deeper layer.

Sample 1 was collected at 5 m depth. Freshwater filled this layer with a temperature of 29.17°C, a salinity of 33.47 PSU, chlorophyll-a concentration of 0.36 mg/m^3, and dissolved oxygen concentration of 6.31 mg/L. Sample 2 was collected in the middle of the thermocline layer (100 m). NP or NPU water affected this layer, with a temperature of 23.60°C, a salinity of 34.45 PSU, chlorophyll-a concentration of 0.32 mg/m^3, and dissolved oxygen concentration of 4.92 mg/L. Sample 3 was collected at the bottom of the thermocline layer (200 m). NP or NPU water also affected this layer, with a temperature of 13.84°C and a salinity of 34.51 PSU, respectively chlorophyll-a concentration of 0.45 mg/m^3, and dissolved oxygen concentration of 4.36 mg/L.

Discussion

The abundance of bacterial groups in the environment cannot be separated from the influence of the environment that supports it. Seawater bacteria can live as free-living or by association with certain particles. Bacterial life is influenced by current and wave, sunlight, nutrient conditions or the fertility of the waters, dissolved oxygen levels, temperature, pH, and salinity. The pressure factor underwater also affects the deeper water as the pressure increases. Results of the present study revealed that Firmicute was one of the most dominant phyla found at the three layers' depth. Firmicutes are gram-positive bacteria found at great depths in the water column and marine sediments (da Silva et al. 2013; Ludwig et al. 2009). *Bacillus* was the dominant genus from the Firmicutes group that appeared at all depths. The marine *Bacillus* strain group has been studied taxonomically and can produce biologically active compounds. That include; lipopeptides, polypeptides, macrolactones, fatty acids, polyketides, and isocoumarins (da Silva et al. 2013). Firmicutes were found to be more abundant at three different depths. That indicates it had a wider range of adaptations in the surface layer of water to the thermocline layer. One plausible reason is that members of the Firmicutes group are known to produce spores to survive in low-temperature and oligotrophic areas (Moreno-Letelier et al. 2011).

Proteobacteria are the second most abundant phylum after Firmicutes. In contrast to Firmicutes, Proteobacteria were found to be low at three different depths. This is a cosmopolitan group with enormous diversity in the environment. Proteobacteria is abundant in seabed sediments (Cui et al. 2019; Yu et al. 2018) and ocean surface layers (Wang et al. 2016; Yin et al. 2013). Based on the result, *Psychrobacter* was the dominant genus in the surface layer, while *Gammaproteobacteria, Sphingomonas, Dongia, Steriodobacter, Pedomicrobium, Acidobacter, Mizugakiibacter, Reyranella* were present in the middle layer, and *Anaeromyxobacter* was present in the deepest layer. *Psychrobacter* is a genus of bacteria from the psychrotrophic group, which can live at a temperature of 4°C to 20°C, and has also been found in the Makassar strait region (Radjasa et al. 2007).

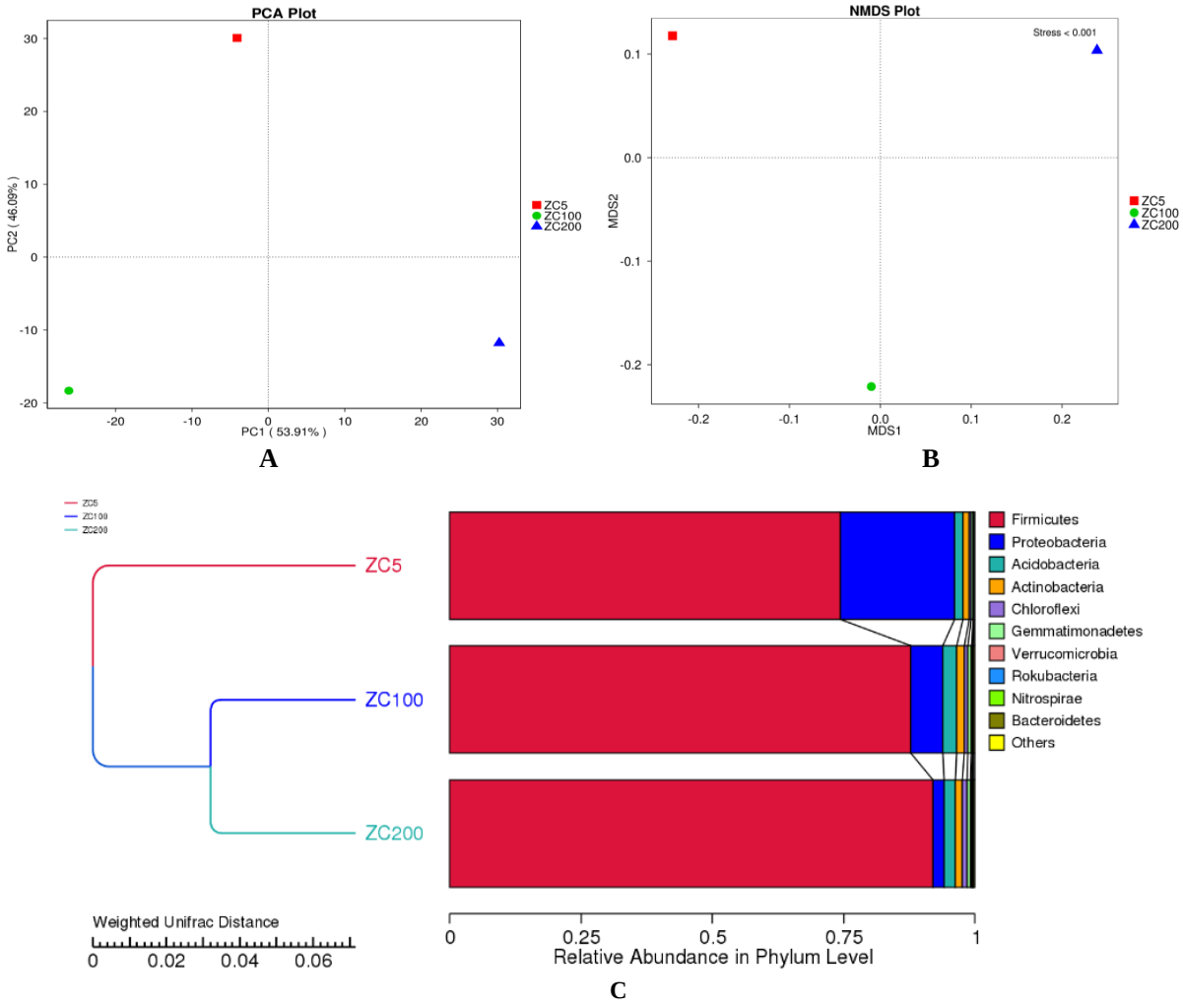


Figure 7. A. PCA based on Weighted Unifrac distance, B. NMDS plot, C. UPGMA cluster tree

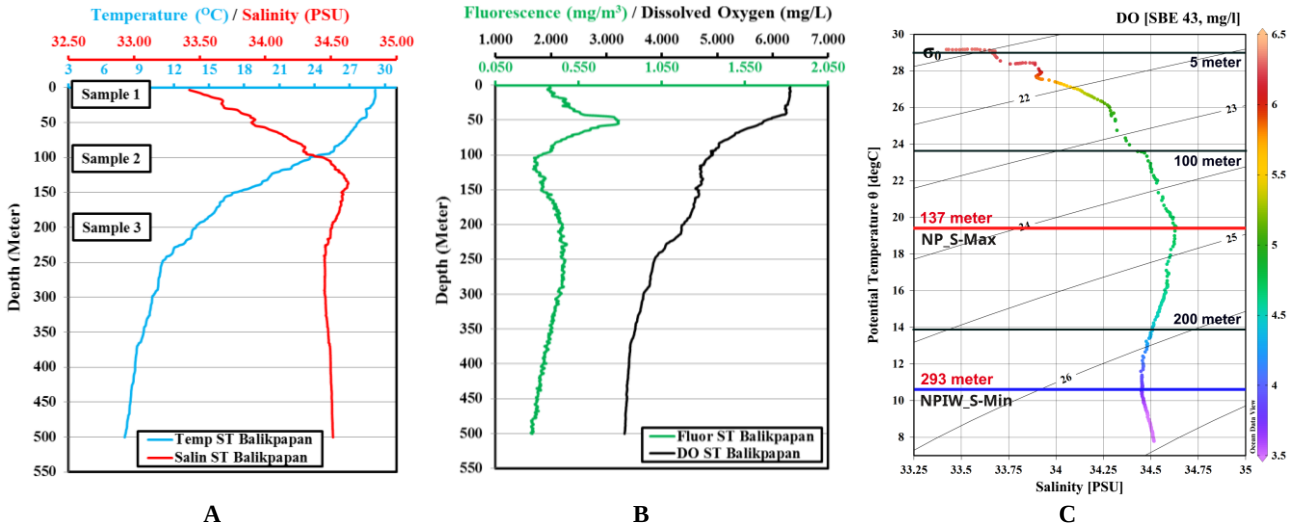


Figure 8. Vertical profile of seawater at Station Balikpapan from 0-500 ms depth. A. Temperature (°C) and Salinity (PSU), B. Fluorescence mg/m^3 and Dissolved oxygen (mg/L), C. TS (Temperature and Salinity) diagram combined with Dissolved Oxygen concentration (colorbar)

The three depths of water sampling had similar characteristics in terms of penetration of light intensity. The biodiversity of microorganisms at this depth was still relatively high, supported by environmental conditions suitable for microorganisms. The biodiversity of bacteria was very dynamic because changes influence the characteristics of the physics and chemistry of seawater, such as upwelling and downwelling events in the water column. The unique area among these three depths was 100 m because the highest bacterial diversity was found in this zone. This zone has unique characteristics because it has a mixing area of the upper and lower layers and forms a transitional area. There were significant temperature, salinity, turbidity, and dissolved oxygen changes. On the other hand, a remarkable bacterial biodiversity similarity was observed between 5 m and 200 m.

The extreme variations of the marine environment, including pressure, salinity, temperature, and nutrients, allow marine microorganisms to develop unique biochemical and physiological competencies for survival. This potentially offers an abundance of secondary metabolites that may differ from the metabolites produced by terrestrial microorganisms. In general, microorganisms are used to make drugs today. Bacteria have important value and benefits for human life because of their ability to produce secondary metabolites that are used as raw materials to make drugs in pharmaceuticals, antibiotics, probiotics, and even producing enzymes. Based on the results, several genera of bacteria may be a good prospect for development in biotechnology, namely *Streptomyces, Paenibacillus, Bacillus, Psychrobacter, Arthrobacter, Bradyrhizobium, Gemmatimonas,* and *Acidobacteria*. *Streptomyces* is a genus of bacteria well known as a producer of antibiotic compounds (Manjusha et al. 2013; Tenebro et al. 2021), while *Paenibacillus* is known to have antifungal abilities (Santiago et al. 2016). *Bacillus* is known to be able to produce antifouling compounds (Ortega-Morales et al. 2008). *Psychrobacter* is known to have enzymatic activity in the form of lipases (Petrovskaya et al. 2021) and esterase (Amato and Christner 2009). *Arthrobacter* is known to have potential as a bioremediation agent (Bjerketorp et al. 2018). *Bradyrhizobium* is a biofertilizer because it can fix nitrogen (Purwani et al. 2021). *Gemmatimonas* is known to produce secondary metabolites, namely carotenoids (Takaichi et al. 2010), and *Acidobacteria* are known as one of the genera of bacteria that produce exopolysaccharide (EPS) compounds (Costa et al. 2020).

As a general summary, the vertical structural profile of the bacterial community at the three depths differed, but the dominant phylum that appeared at the three depths did not significantly differ. Firmicutes were the largest group at each depth, followed by Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Verrucomicrobia, Rokubacteria, Nitrospirae, and Bacteroidetes. The profile of the bacterial community structure at a depth of 5 m differs from 100 m and 200 m depth; different environmental conditions may influence this at each depth. Based on the metagenomic data obtained from the present study, several genera of bacteria could be explored as sources of marine natural products, including *Streptomyces, Paenibacillus, Bacillus, Psychrobacter, Arthrobacter, Bradyrhizobium, Gemmatimonas, Acidobacteria*. It is necessary to conduct further studies on the bioprospection of secondary metabolites that each bacteria can produce.

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REFERENCES

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215: 403-410

Al-Amoudi S, Essack M, Simões MF, Bougouffa S, Soloviev I, Archer JAC, Lafi FF, Bajic VB. 2016. Bioprospecting red sea coastal ecosystems for culturable microorganisms and their antimicrobial potential. Mar Drugs 14 (9): 1-14. DOI: 10.3390/md14090165.

Amato P, Christner BC. 2009. Energy metabolism response to low-temperature and frozen conditions in *Psychrobacter cryohalolentis*. Appl Environ Microbiol 75 (3): 711-718. DOI: 10.1128/AEM.02193-08.

Bjerketorp J, Röling WFM, Feng XM, Garcia AH, Heipieper HJ, Håkansson S. 2018. Formulation and stabilization of an *Arthrobacter* strain with good storage stability and 4-chlorophenol-degradation activity for bioremediation. Appl Microbiol Biotechnol 102 (4): 2031-2040. DOI: 10.1007/s00253-017-8706-6.

Bokulich NA, Subramanian S, Faith JJ, Gevers D, Gordon JI, Knight R, Mills DA, Caporaso JG. 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat Methods 10 (1): 57-59. DOI: 10.1038/nmeth.2276.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA. 2010. Correspondence QIIME allows analysis of high-throughput community sequencing data intensity normalization improves color calling in SOLiD sequencing. Nat Publishing Group 7 (5): 335-336. DOI: 10.1038/nmeth.f.303.

Costa OYA, Costa OYA, De Hollander M, Pijl A, Liu B, Kuramae EE. 2020. Cultivation-independent and cultivation-dependent metagenomes reveal genetic and enzymatic potential of microbial community involved in the degradation of a complex microbial polymer. Microbiome 8 (1): 1-19. DOI: 10.1186/s40168-020-00836-7.

Cui G, Li J, Gao Z, Wang Y. 2019. Spatial variations of microbial communities in abyssal and hadal sediments across the challenger deep. Peer J 7: 1-17. DOI: 10.7717/PEERJ.6961.

da Silva MAC, Cavalett A, Spinner A, Rosa DC, Jasper RB, Quecine MC, Bonatelli ML, Pizzirani-Kleiner A, Corção G, de Souza Lima AO. 2013. Phylogenetic identification of marine bacteria isolated from deep-sea sediments of the eastern South Atlantic Ocean. SpringerPlus 2 (1): 1-10. DOI: 10.1186/2193-1801-2-127.

Dionisi H, Lozada M, Olivera NL. 2012. Bioprospection of marine microorganisms: Biotechnological applications and methods | Bioprospección de microorganismos marinos: Aplicaciones biotecnológicas y métodos. Rev Argent Microbiol 44 (1): 49-60. DOI: 10.1590/S0325-75412012000100010.

Edgar RC. 2013. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. Nat Methods 10 (10): 996-998. DOI: 10.1038/nmeth.2604.

Gordon AL, Fine RA. 1996. Pathways of water between the Pacific and Indian oceans in the Indonesian seas. In Nature 379 (6561): 146-149. DOI: 10.1038/379146a0.

Haas BJ, Gevers D, Earl AM, Feldgarden M, Ward DV, Giannoukos G, Ciulla D, Tabbaa D, Highlander SK, Sodergren E, Methé B, DeSantis TZ, Petrosino JF, Knight R, Birren BW. 2011. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. Genom Res 21 (3): 494-504. DOI: 10.1101/gr.112730.110.

Han D, Ha HK, Hwang CY, Lee BY, Hur HG, Lee YK. 2015. Bacterial communities along stratified water columns at the Chukchi Borderland in the western Arctic Ocean. Deep-Sea Res Part II: Topical Stud Oceanogr 120: 52-60. DOI: 10.1016/j.dsr2.2015.01.018.

Kai W, Peisheng Y, Rui M, Wenwen J, Zongze S. 2017. Diversity of culturable bacteria in deep-sea water from the South Atlantic Ocean. Bioengineered 8 (5): 572-584. DOI: 10.1080/21655979.2017.1284711.

Kapellos GE, Eberl HJ, Kalogerakis N, Doyle PS, Paraskeva CA. 2022. Impact of microbial uptake on the nutrient plume around marine organic particles: High-resolution numerical analysis. Microorganisms 10 (10): 1-22. DOI: 10.3390/microorganisms10102020.

Kennedy J, Marchesi JR, Dobson ADW. 2008. Marine metagenomics: Strategies for the discovery of novel enzymes with biotechnological applications from marine environments. Microb Cell Factories 7: 1-8. DOI: 10.1186/1475-2859-7-27.

Kodzius R, Gojobori T. 2015. Marine metagenomics as a source for bioprospecting. Mar Genom 24: 21-30. DOI: 10.1016/j.margen.2015.07.001.

Ludwig W, Schleifer K, Whitman WB. 2009. Systematic Bacteriology. In Systematic Bacteriology (Issue January). DOI: 10.1007/978-0-387-68489-5.

Magoč T, Salzberg SL. 2011. FLASH: Fast length adjustment of short reads to improve genome assemblies. Bioinform 27 (21): 2957-2963. DOI: 10.1093/bioinformatics/btr507.

Mahapatra GP, Raman S, Nayak S, Gouda S, Das G, Patra JK. 2019. Metagenomics approaches in discovery and development of new bioactive compounds from marine actinomycetes. Curr Microbiol 77 (4): 645-656. DOI: 10.1007/s00284-019-01698-5.

Manjusha WA, Anusha WA, Amritha Krishna BV, Lavanya L, Rejin Prasad JJ, Remya RA, Surya V. 2013. Molecular bioprospecting of *Streptomyces* sp. *(ES5)* from marine alga *Hypnea musiformis*. Indian J Biotechnol 12 (2): 218-224.

Moreno-Letelier A, Olmedo G, Eguiarte LE, Martinez-Castilla L, Souza V. 2011. Parallel evolution and horizontal gene transfer of the pst operon in firmicutes from oligotrophic environments. Intl J Evol Biol 1-10. DOI: 10.4061/2011/781642.

Ortega-Morales BO, Chan-Bacab MJ, Miranda-Tello E, Fardeau ML, Carrero JC, Stein T. 2008. Antifouling activity of sessile bacilli derived from marine surfaces. J Ind Microbiol Biotechnol 35 (1): 9-15. DOI: 10.1007/s10295-007-0260-2.

Petrovskaya L, Novototskaya-Vlasova K, Komolova A, Rivkina E. 2021. "6 Biochemical adaptations to the permafrost environment: lipolytic enzymes from *Psychrobacter cryohalolentis K5*^{T}". Microbial Life in the Cryosphere and Its Feedback on Global Change, edited by Susanne Liebner and Lars Ganzert. De Gruyter 141-152. DOI: 10.1515/9783110497083-006.

Purwani J, Sucahyono D, Wardana IP. 2021. The efficacy of biofertilizer contains *Bradyrhizobium japonicum* isolates on soybean yields grown in Inceptisols, Bogor, West Java, Indonesia. IOP Conf Ser: Earth Environ Sci 648 (1). DOI: 10.1088/1755-1315/648/1/012201.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner F O. 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res 41 (D1): D590-D596. DOI: 10.1093/nar/gks1219.

Rachman A, Purwandana A, Fitriya N. 2021. Phytoplankton community structure of the Makassar Strait, Indonesia. IOP Conf Ser: Earth Environ Sci 789 (1). DOI: 10.1088/1755-1315/789/1/012006.

Radjasa OK, Nasima D, Sabdono A, Kita-Tsukamoto K, Ohwada K. 2007. Characterization of psychrotrophic bacteria from sea waters of Makasar Strait, Indonesia. J Biol Sci 7 (4): 658-662. DOI: 10.3923/jbs.2007.658.662.

Ren Z, Qu X, Peng W, Yu Y, Zhang M. 2019. Nutrients drive the structures of bacterial communities in sediments and surface waters in the river-lake system of Poyang Lake. Water (Switzerland) 11 (5): 1-16. DOI: 10.3390/w11050930.

Santiago R, Huiliñir C, Cottet L, Castillo A. 2016. Microbiological characterization for a new wild strain of *Paenibacillus polymyxa* with antifungal activity against *Botrytis cinerea*. Biol Control 103: 251-260. DOI: 10.1016/j.biocontrol.2016.10.002.

Schauer R, Bienhold C, Ramette A, Harder J. 2010. Bacterial diversity and biogeography in deep-sea surface sediments of the South Atlantic Ocean. ISME J 4 (2): 159-170. DOI: 10.1038/ismej.2009.106.

Sea-Bird Scientific. 2017. Software Manual Seasoft V2 : SBE Data Processing. 177.

Seymour JR. 2014. A sea of microbes: the diversity and activity of marine microorganisms. Microbiol Aust 35 (4): 183. DOI: 10.1071/ma14060.

Suh SS, Park M, Hwang J, Lee S, Chung Y, Lee TK. 2014. Distinct patterns of marine bacterial communities in the South and North Pacific Oceans. J Microbiol 52 (10): 834-841. DOI: 10.1007/s12275-014-4287-6.

Susanto RD, Ffield A, Gordon AL, Adi TR. 2012. Variability of Indonesian throughflow within Makassar Strait, 2004-2009. J Geophys Res Oceans 117 (9): 2004-2009. DOI: 10.1029/2012JC008096.

Takaichi S, Maoka T, Takasaki K, Hanada S. 2010. Carotenoids of *Gemmatimonas aurantiaca* (Gemmatimonadetes): Identification of a novel carotenoid, deoxyoscillol 2-rhamnoside, and proposed biosynthetic pathway of oscillol 2,2′-dirhamnoside. Microbiology 156 (3): 757-763. DOI: 10.1099/mic.0.034249-0.

Tenebro CP, Trono DJVL, Vicera CVB, Sabido EM, Ysulat JA, Macaspac AJM, Tampus KA, Fabrigar TAP, Saludes JP, Dalisay DS. 2021. Multiple strain analysis of *Streptomyces* species from Philippine marine sediments reveals intraspecies heterogeneity in antibiotic activities. Sci Rep 11 (1): 1-14. DOI: 10.1038/s41598-021-96886-4.

Valcheva N, Palazov A. 2010. Quality control of CTD observations as a basis for estimation of thermohaline climate of the western black sea. J Environ Prot Ecol 11 (4): 1504-1515.

Wang J, Kan J, Borecki L, Zhang X, Wang D, Sun J. 2016. A snapshot on spatial and vertical distribution of bacterial communities in the eastern Indian Ocean. Acta Oceanol Sin 35 (6): 85-93. DOI: 10.1007/s13131-016-0871-4.

Wang Q, Garrity GM, Tiedje JM, Cole JR. 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73 (16): 5261-5267. DOI: 10.1128/AEM.00062-07.

Yin Q, Fu B, Li B, Shi X, Inagaki F, Zhang XH. 2013. Spatial variations in microbial community composition in surface seawater from the ultra-oligotrophic center to rim of the south Pacific Gyre. PLoS ONE 8 (2). DOI: 10.1371/journal.pone.0055148.

Yu SX, Pang YL, Wang YC, Li JL, Qin S. 2018. Distribution of bacterial communities along the spatial and environmental gradients from Bohai Sea to northern Yellow Sea. Peer J 2018 (1): 1-21. DOI: 10.7717/peerj.4272.