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Metagenomic analysis of deep-sea bacterial communities in the Makassar and Lombok Straits

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The extreme conditions of the deep-sea environment, including limited light, low oxygen levels, high pressure, and nutrient scarcity, create a natural habitat for deep-sea bacteria. These remarkable microorganisms have developed unique strategies to survive and adapt to their surroundings. However, research on the diversity of deep-sea bacteria, both culture-dependent and culture-independent, in Indonesian waters remains insufficient. This study focused on exploring the biodiversity of deep-sea bacteria, specifically in the Makassar and Lombok Strait, the main Indonesian throughflow pathway characterized by relatively fertile water, which serves as an important deep-sea region. High-throughput DNA sequencing of full-length 16S rRNA was employed to construct a genomic database. The results of the bioinformatic analysis revealed that two stations, 48 and 50 (Makassar Strait), exhibited a more similar community structure of deep-sea bacteria than did station 33 (Lombok Strait). Among the predominant phyla found at a depth of 1000 m, the top ten were Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Planctomycetes, Acidobacteria, Nitrospinae, Verrucomicrobia, Candidatus Melainabacteria, and Cyanobacteria. Furthermore, the genera *Colwellia*, *Moritella*, *Candidatus Pelagibacter*, *Alteromonas*, and *Psychrobacter* consistently appeared at all three stations, albeit with varying relative abundance values. These bacterial genera share common characteristics, such as psychrophilic, halophilic, and piezophilic tendencies, and are commonly found in deep-sea ecosystem. The environmental conditions at a depth of 1000 m were relatively stable, with an average pressure 10 MPa, temperature 4.68 °C, salinity 34.58 PSU, pH 8.06, chlorophyll-a 0.29 µg/L, nitrate 3.19 µmol/L, phosphate 6.32 µmol/L and dissolved oxygen (DO) 2.90 mg/L. The bacterial community structures at the three sampling stations located at the same depth (1000 m) exhibited similarities, as indicated by the closely aligned similarity index values.

Keywords Indonesian throughflow, 16S rRNA gene, Diversity, Deep-sea bacteria, Proteobacteria

The deep sea is a vast and mysterious realm, distinguished by its immense depths that stretch far beyond coastal regions. This enigmatic region of the ocean is often referred to as the "deep sea," encompassing those portions of the ocean that descend to considerable depths, often exceeding hundreds to thousands of meters. To be more precise, deep-sea ecosystems are typically defined as ocean areas with a depth of more than 200 m from the surface, although this depth threshold can vary in different contexts[1]. The physical characteristics of the deep sea differ from those of shallow waters. The deep sea is characterized by high pressure, an absence of sunlight, low and stable temperatures, and limited food sources.

Bacteria are diverse microorganisms that can adapt to various habitats or environmental conditions, including the deep sea. In the deep ocean, bacteria can be found around hydrothermal vents[2,3] in deep-sea sediments[4–7] or in deep-sea organisms such as sea sponges[8,9]. Marine bacteria play an important role in biogeochemical processes in the deep sea, such as nutrient cycling, decomposition of organic matter, and methane formation.

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Deep-sea bacteria have wide metabolic diversity, enabling them to utilize various chemical compounds in their surroundings. Some deep-sea bacteria can breakdown complex compounds, such as hydrocarbons, methane, or sulfur compounds, and use them as a source of energy and nutrients. Moreover, deep-sea bacteria have developed the ability to obtain energy from alternative sources, such as the oxidation of inorganic compounds (chemosynthesis)[10]. They can thrive and expand in highly dim environments. Some bacteria produce enzymes and proteins that can function effectively at low temperatures[11]. Bacteria might also have defenses against cold temperatures that harm cell structures.

Mimicking deep-sea environmental conditions in a laboratory setting can be difficult and requires sophisticated high-pressure equipment. Therefore, a nonculture or metagenomics approach is a possible option for studying deep-sea bacteria. Metagenomic studies of deep-sea bacteria are used to understand the genetic diversity and functional potential of deep-sea microbes without prior isolation or culture. Several metagenomic studies of deep-sea bacteria have been conducted, which provide insight into microbial communities and their functional role in deep-sea ecosystems[12–14]. The diversity of marine bacteria is an exciting research topic because knowledge of this diversity can provide insight into the ecology and potential of marine microbial bioprospecting[15–17]. The assessment of bacterial diversity through the sequencing of 16S ribosomal RNA (16S rRNA) genes has been widely used in environmental microbiology, particularly since the advent of high-throughput sequencing technology. Full-length 16S rRNA sequences provide a higher level of taxonomic and phylogenetic resolution for bacterial identification because they take into account all of the informative sites of the 16S rRNA genes[18].

The Makassar Strait, situated between Indonesia's western and eastern waters, holds strategic significance as a pivotal junction between the Pacific Ocean to the north and the Indian Ocean to the south, forming the vital Indonesian Throughflow (ITF)[19]. Approximately 80% of the ITF is transported through the Makassar Strait, 25% of which exits directly into the Lombok Strait, while the remainder exits into the Banda Sea before exiting into the Indian Ocean[20]. Hence, it is hypothesized that there is a diversity of microbial relationships among the ITF pathways in the Makassar–Lombok Strait based on metagenomic data, specifically in the deep-sea water column. The biodiversity in the southern Lombok Strait may differ slightly due to internal tide-induced mixing where the ITF waters are mixed with the Indian Ocean water[21]. These geographical conditions create an exceptional and diverse habitat that sustains a wide array of marine life, including deep-sea bacteria. The exploration of metagenomics in the study of deep-sea bacteria within the Makassar Strait is particularly intriguing. To date, there is a notable absence of data pertaining to this specific location. Consequently, the primary objective of this research was to determine the richness and variety of deep-sea bacteria by leveraging the 16S ribosomal RNA approach. This undertaking serves as an inaugural step in characterizing the deep-sea bacterial diversity within Indonesian waters, paving the way for subsequent bioprospecting initiatives based on genomic information. The hope is that this research will make a valuable contribution to our broader understanding of marine microbiology while shedding light on the pivotal role of marine bacteria in maintaining equilibrium within deep-sea ecosystems.

Materials and methods

Study area

This research was undertaken in the Makassar Strait and Lombok Strait in December 2019 during the TRIUMPH Expedition (Throughflow Indonesian seas, Upwelling and Mixing Physics), an international collaborative study among scientists from the National Research and Innovation Agency of Indonesia (BRIN), University of Maryland-USA, and First Institute of Oceanography-China. Seawater samples were collected from 1000 m deep at Makassar Strait (Stations 48 and 50) and Lombok Strait (Station 33) for bacterial community analysis (Fig. 1a). Additionally, vertical profiles of seawater properties were obtained at the same location to analyze oceanographic conditions. The specific sampling points for the bacterial community are provided in Supplementary Table 1. The map study area (Fig. 1a) was created using QGIS software version 3.4.4-Madeira (https://www.qgis.org/en/site/forusers/download.html). The bathymetric data (Fig. 1b) used in this study was created from the Indonesian National Bathymetry (https://tanahair.indonesia.go.id/demnas/#/).

Sample collection and CTD measurement

Deep-sea water samples were collected from a 1000 m depth using a Carousel Water Sampler type SBE 32 equipped with a conductivity temperature depth (CTD) type SBE 911 + (Sea-Bird Scientific) to measure environmental parameters of seawater (pressure, temperature, pH, salinity, chlorophyll-a, and dissolved oxygen (DO). Phosphate and nitrate concentrations were measured using a spectrophotometer (UV–Vis Shimadzu 1700, Kyoto, Japan). CTD casts were deployed from the surface to a depth of 1000 m. The recording interval was set to 32 measurements/second, and the casting speed was kept at a maximum of 50 m/min to minimize noise during the measurements. On board, 5 L of seawater was filtered through 0.22 µm pore-size cellulose nitrate membrane filters with diameter of 47 mm. The filters were stored in falcon tubes at − 20 °C onboard and in the laboratory.

DNA extraction

DNA extraction was conducted in the laboratory by first cutting the filter membrane into small pieces. The extraction process was conducted according to the protocol provided with the ZymoBIOMICS™ DNA Mini Kit (Zymo Research Corp., Irvine, CA, USA), with one modification: the shaking step was performed at maximum speed for approximately 40–60 min during the initial phase[22]. DNA concentration and purity were assessed using 1% agarose gels. Based on the measured concentration, the DNA was subsequently diluted to 1 ng/µL using sterile water.

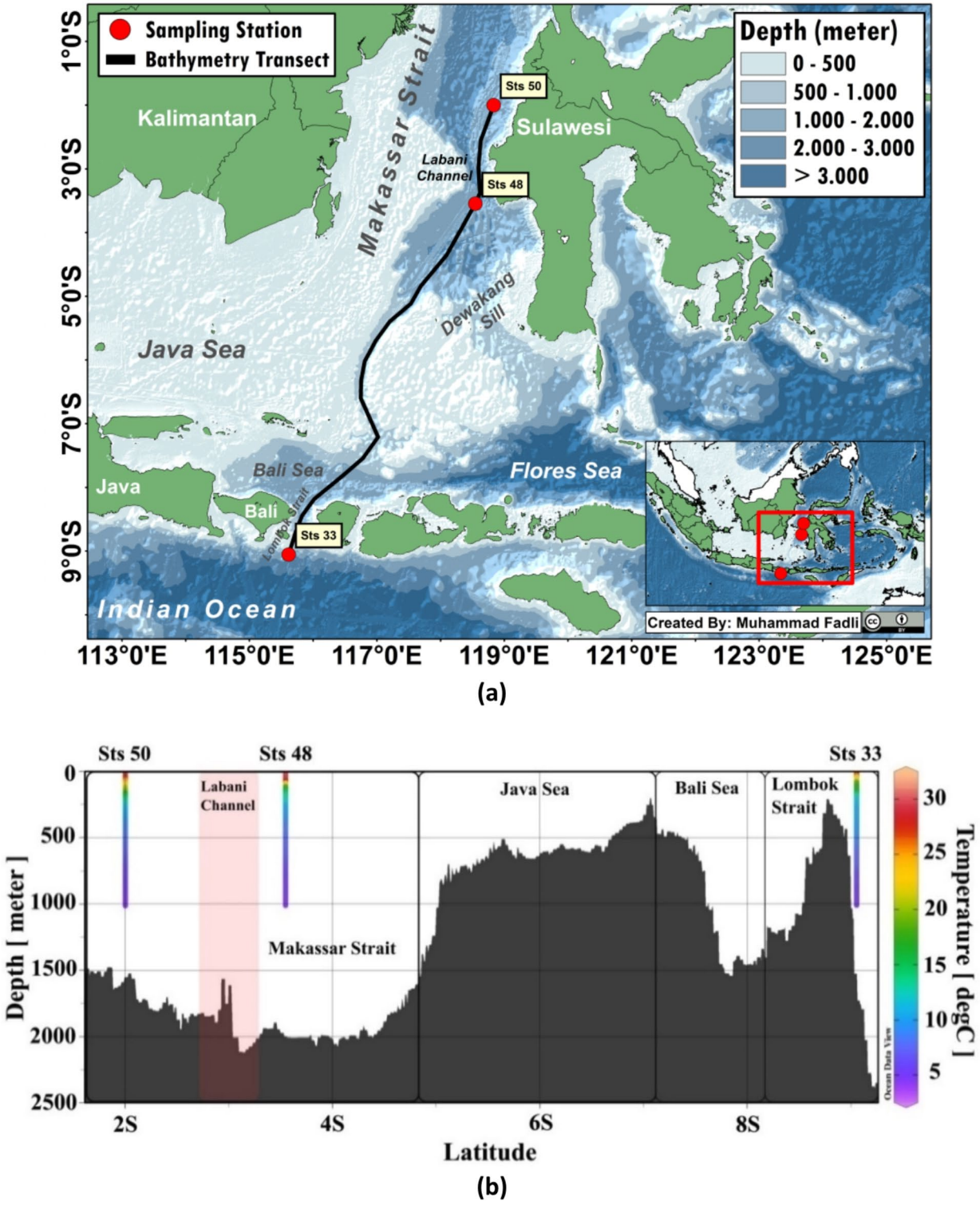

Fig. 1. (**a**) The sampling location within the Makassar Strait represents a pivotal route for water movement from the tropical Pacific Ocean to the Indian Ocean, signifying the primary pathways. The microorganism station is marked by the red circle. (**b**) The bathymetric transect from station 50 and 48 (Makassar Strait) to station 33 (Lombok Strait), with the transect line shown in black.

PCR amplification

DNA was amplified using the universal bacterial primers 27F-YM (5'-AGAGTTTGATYMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). All PCRs were conducted with Phusion® High-Fidelity PCR Master Mix (New England Biolabs) and run on a Peltier Thermal Cycler (Bio-Rad) with the following conditions:

an initial denaturation at 95 °C for 2 min; 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 40 s; followed by a final extension at 72 °C for 7 min[22]. The same volume of 1 × loading buffer (SYBR Green) was mixed with the PCR products, which were then subjected to electrophoresis on a 2% agarose gel for detection. The PCR products were combined in equal density ratios and purified using a Qiagen Gel Extraction Kit (Qiagen, Germany).

Sequencing and data analysis

The DNA concentration was determined using both NanoDrop spectrophotometers and Qubit fluorometer. For 16S rRNA gene sequencing, libraries were prepared using the 16S Barcoding Kit (Oxford Nanopore Technologies, Oxford, UK), following the manufacturer's protocol. Nanopore sequencing was operated by MinKNOW software version 22.05.7. Basecalling was performed using Guppy version 6.1.5 with a *high-accuracy model*[23]. The quality of the FASTQ files was visualized using NanoPlot[24]. Reads were classified using the centrifuge classifier[25]. The bacterial and archaeal indices were determined using the NCBI 16S RefSeq database (https://ftp.ncbi.nlm.nih.gov/refseq/TargetedLoci/). Downstream analysis and visualizations were performed using Pavian (https://github.com/fbreitwieser/pavian), Krona Tools (https://github.com/marbl/Krona), and R Studio in R version 4.2.0 (https://www.R-project.org/). Sequencing depth was determined by using rarefaction curve (Fig. 5a) and data points were visualized using UPGMA-clustered dendrograms based on the Bray–Curtis dissimilarity[26]. Sankey diagrams were used to visualize microbial species in microbiome studies[27].

The data from the CTD underwent seven steps of quality control (QC) methods, including data conversion, wild editing, filtering, CTD alignment, cell thermal mass assessment, loop editing, derivation of variables based on the EOS 80 standard, and bin averaging. The SBE Data Processing software provided by Sea Bird Electronics was used for this process[28]. The final activity in the QC was a screening check conducted by the researcher to assess the results of the quality control procedure. Vertical profiles of physical parameters are presented as variables (temperature, salinity, chlorophyll-a, and dissolved oxygen) versus depth (Fig. 6). Water mass characteristics were analyzed using a temperature versus salinity diagram (TS diagram) (Fig. 6a) facilitated by Ocean Data View Software (ODV 5.6.2) (https://odv.awi.de/), which enhanced the environmental analysis.

Results

Deep-sea water samples were collected at a depth of 1000 m from the Makassar Strait (Stations 48 and 50) and the Lombok Strait (Station 33) in early December 2019 during the boreal winter. These two locations, situated within the Indonesian Throughflow (ITF) pathway, represent the middle inflow (Makassar Strait) and outflow regions (Lombok Strait). The distance from Station 33 to Station 48 was about 740 km, and from Station 48 to Station 50 was about 177 km (Fig. 1a). Based on environmental parameter data measured at a depth of 1000 m, the average pressure was 10 MPa, temperature 4.68 °C, salinity 34.58 PSU, pH 8.06, chlorophyll-a 0.29 µg/L, DO 2.90 mg/L, nitrate 3.19 µmol/L, and phosphate 6.32 µmol/L (Suppl. Table 2).

The alpha diversity index (Table 1) serves as a metric for characterizing the average species diversity within a specific location or habitat on a local scale. This index quantifies the number of distinct species present within a given community or ecosystem at a particular location. The highest diversity index was found in the Makassar Strait, particularly at Station 50, followed by Station 33 in the Lombok Strait, and then Station 48. The Shannon index, which ranged from 4.25 to 4.57, in conjunction with the Simpson index approaching 1, collectively indicates a well-balanced and diverse community structure, characterized by a high degree of species richness and an equitable distribution of individuals among species.

Based on the relative abundance diagram data at the phylum level (Fig. 2a), the phylum Proteobacteria was dominant, followed by Firmicutes, Bacteroidetes, Actinobacteria, Planctomycetes, Acidobacteria, Nitrospinae, Verrucomicrobia, Candidatus Melainabacteria, and Cyanobacteria. All three stations shared a similar level of dominance, particularly at the class level, with Alphaproteobacteria and Gammaproteobacteria being predominant (Fig. 2b). The relative abundances at the genus level (Fig. 3a) showed that station 33 was predominantly represented by *Colwellia, Photobacterium, Candidatus Pelagibacter, Moritella, Cognaticolwellia, Alteromonas, Pseudomonas, Pseudoalteromonas, and Psychrobacter*. Station 48 was dominated by *Halomonas, Colwellia, Candidatus Pelagibacter, Stutzerimonas, Alteromonas, Psychrobacter, Pseudomonas, Moritella, and Cognaticolwellia. Station 50 featured Moritella, Halomonas, Candidatus Pelagibacter, Colwellia, Psychrobacter, Alteromonas, Pseudomonas, Marinobacter,* and *Pseudoalteromonas*. The genera *Colwellia, Moritella, Candidatus Pelagibacter, Alteromonas*, and *Psychrobacter* consistently appeared at all three stations. The UPGMA-clustered dendrograms based on Bray curtis dissimilarity analysis indicated no significant differences in deep-sea bacterial profiles across the three stations (Fig. 3). However, Stations 48 and 50 demonstrated a higher degree of similarity relative to Station 33, which is likely attributed to the geographic proximity of stations 48 and 50 within the same region.

Sankey diagrams (Fig. 4) were used to illustrate the flow or distribution of the 10 most abundant deep-sea bacterial species at each station, as identified through metagenomic analysis (16S rRNA). Station 33 (Fig. 4a)

Sample name	Observed	Chao1	ACE	Shannon	Simpson	InvSimpson	Fisher
Sta 33	3396	5139.75	127.61	4.49	0.95	22.69	660.94
Sta 48	3436	5218.38	131.21	4.25	0.94	18.89	659.62
Sta 50	3370	5035.01	123.43	4.57	0.96	30.16	650.60

Table 1. Alfa diversity index.

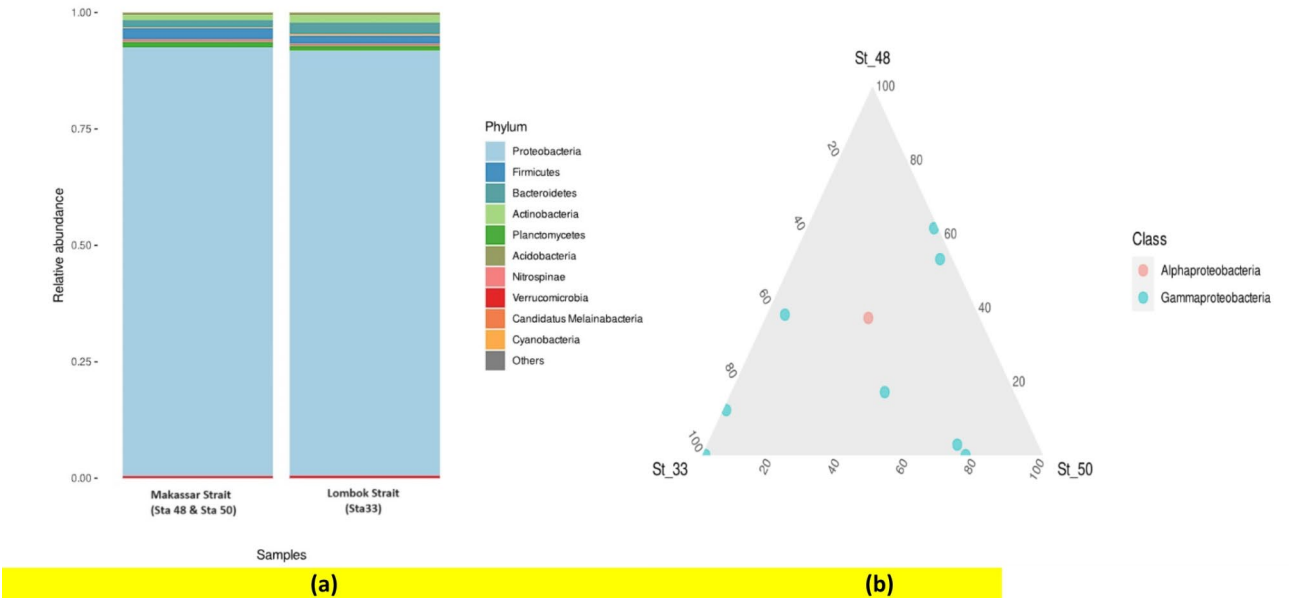

Fig. 2. (**a**) Relative abundance of deep-sea bacteria at the Phylum between two location Makassar Strait (sta 48 & sta 50) and Lombok Strait (sta 33) (**b**)Ternary plot station 33, 48 and 50.

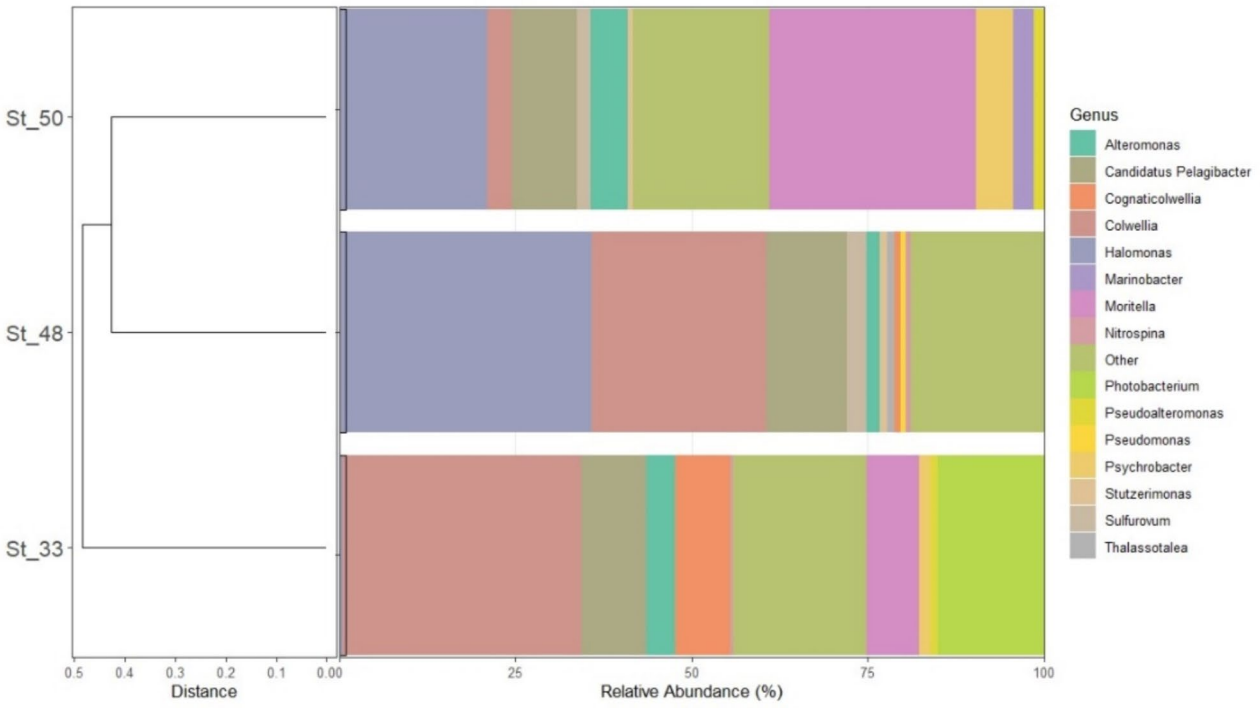

Fig. 3. UPGMA-clustered dendrograms based on Bray–Curtis dissimilarity and Relative abundance of deep-sea bacteria at the Genus between three different station.

consists of *Colwellia psychrerythraea, Photobacterium frigidiphilum, Candidatus Pelagibacter ubique, Colwellia echini, Cognaticolwellia mytili, Colwellia hornerae, Moritella marina, Colwellia piezophile, Alteromonas macleodii, and Photobacterium indicum*. Station 48 (Fig. 4b) consisted of *Halomonas axialensis, Halomonas aquamarine, Candidatus Pelagibacter ubique, Colwellia psychrerythraea, Colwellia maris, Halomonas meridiana, Colwellia piezophile, Sulfurovum aggregans, and Pseudomonas stutzeri*. Station 50 (Fig. 4c) consisted of *Moritella marina,*

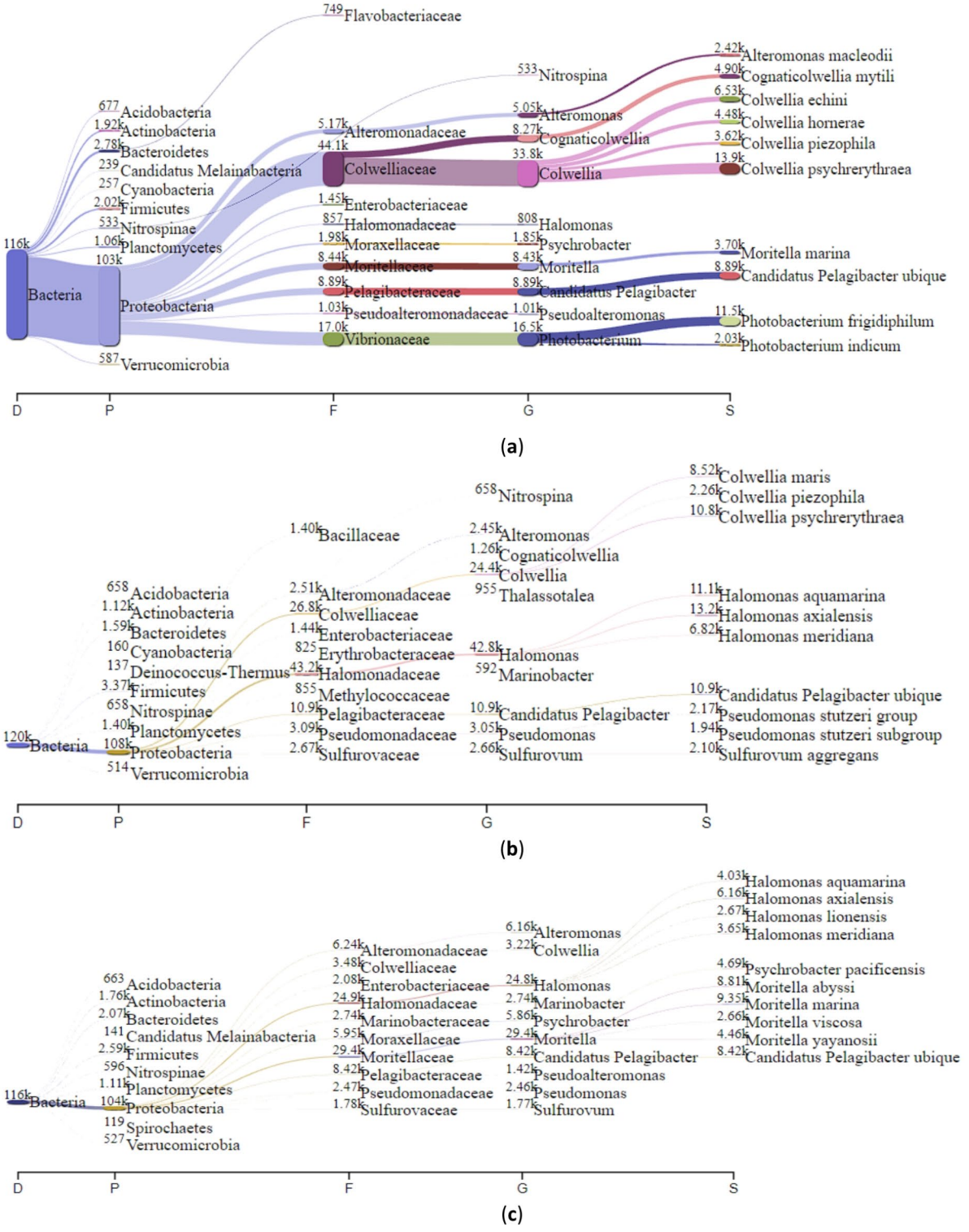

Fig. 4. Sankey diagram visualization of bacterial diversity at the species level (**a**) station 33, (**b**) station 48 and (**c**) station 50.

Moritella abyssi, Candidatus Pelagibacter ubique, Halomonas axialensis, Psychrobacter pacificensis, Moritella viscosa, Moritella yayanosi, Halomonas aquamarine, Halomonas meridiana, and Halomonas lionensis.

Venn diagrams serve as valuable tools for comparing species diversity or organism groups across different geographical locations or under different environmental conditions[29]. The three stations share 1736 OTUs (Operational Taxonomic Units), indicating the presence of a widespread and consistent core bacterial community across these locations (Fig. 5b). Station 33 shows lower OTU overlap with Station 48 (420 OTUs) and Station 50

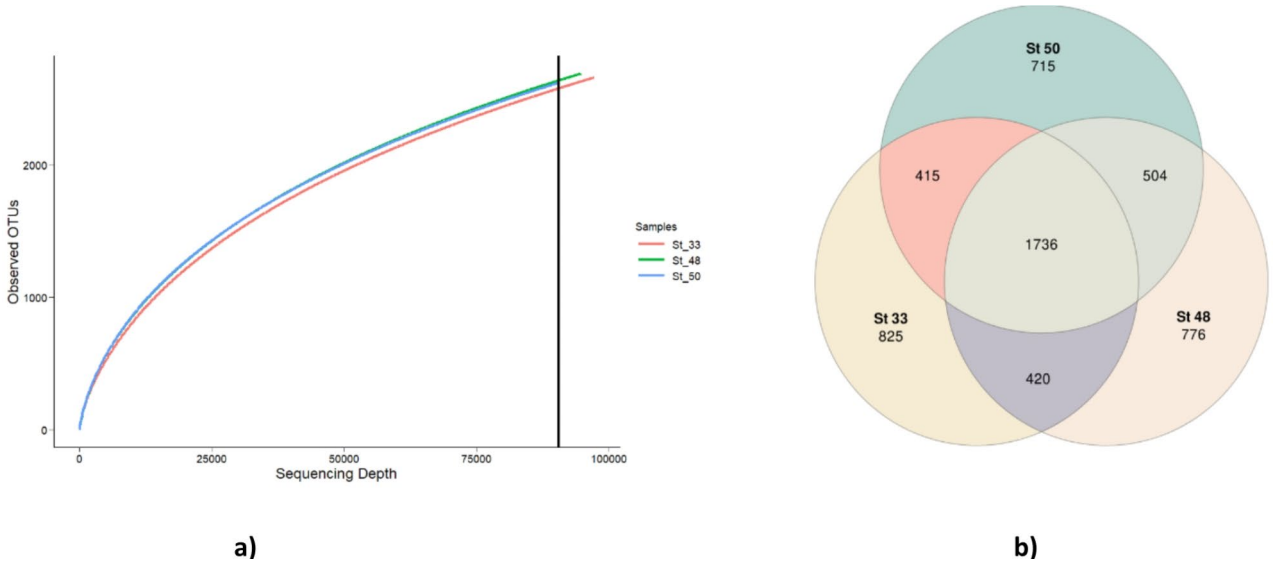

Fig. 5. (**a**) Rarefaction curve, (**b**) Diagram vent.

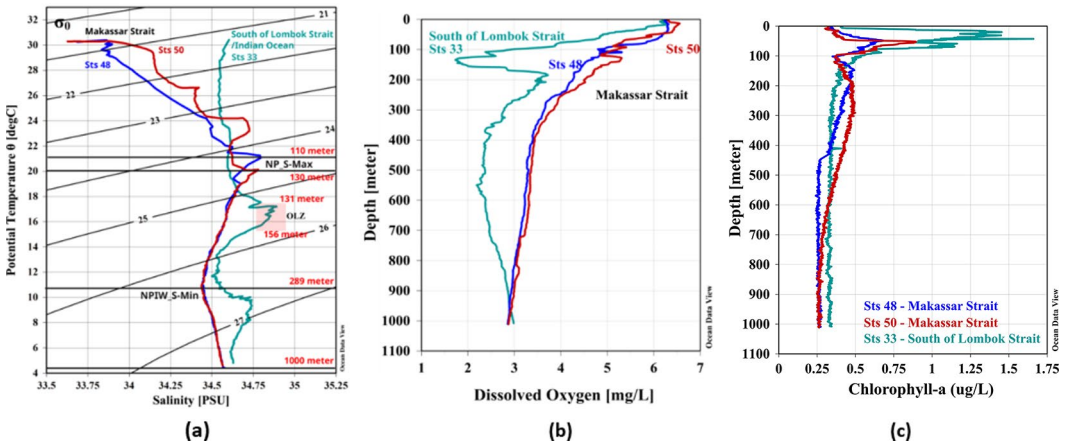

Fig. 6. (**a**) Temperature and Salinity (TS) diagram, and (**b**) Vertical profile of Dissolve Oxygen, (**c**) Vertical profile of chlorophyll-a.

(415 OTUs), suggesting that its bacterial community may be more distinct or subject to greater environmental variation compared to Stations 48 and 50.

Discussion

The Makassar Strait located between Kalimantan Island to the west and Sulawesi Island to the east, serves as a vital conduit linking the Sulawesi Sea in the north with the Java and Flores Seas in the south. This strait is influenced by material inputs from the Mahakam River and fresh water masses from the Java Sea during boreal winter[30]. The Makassar Strait also acts as a crucial channel for water masses flowing from the Pacific to the Indian Ocean, where the Labani Channel's narrow and deep structure generates strong currents and tidal mixing, significantly modifying the ITF in the upper layers[19]. Stations 48 and 50, situated in proximity to the Sulawesi Sea and the northern Pacific Ocean, exemplify the dynamic interactions between water masses in these areas. Based on water mass conditions, only the layer above 200 m in the Makassar Strait connects to the Lombok Strait, though its characteristics have significantly altered due to internal wave activity[31]. The Makassar Strait's vertical water mass structure comprises three layers: surface water above the thermocline, North Pacific Subtropical Water (NPSW) at the thermocline, and North Pacific Intermediate Water (NPIW) in the deep layer[32]. At Stations 48 and 50, the surface water layer extends to a depth of 110 m. The NPSW characterized by maximum salinity, occupies the 110–220 m range, with its salinity peaking between 110 and 130 m. The NPIW identified by its minimum salinity, was found between 220 and 513 m, with the lowest salinity recorded at 289 m. Beyond 513 m, the deep water predominates characterized by a more uniform temperature and salinity profile, as well as reduced variability in other oceanographic parameters (Fig. 6a–c).

The Lombok Strait located between Bali and Lombok Islands, channels 20–25% of the total ITF water mass from the Makassar Strait[31,33]. At station 33, salinity was higher and the NPSW signature from the Makassar Strait was absent. A high-salinity layer at 131–156 m, coinciding with low oxygen concentrations, indicates origins from the Northern Indian Ocean (Arabian Sea Water/ASW)[34]. The lowest salinity layer, found around 260 m, corresponds to Indonesian Upper Water, while a second high-salinity layer at approximately 300 m suggests origins from the Central Indian Ocean. Dissolved oxygen profiles in the Makassar and Lombok Straits were similar, with oxygen-rich upper layers decreasing with depth (Fig. 6b). Therefore, based on the oceanography analysis, the summary is that the water mass at a depth of 1000 m in the Makassar Strait is not connected with the water mass at the same depth in the Lombok Strait and is subject to significantly different oceanographic dynamics.

Despite distinct water mass characteristics due to differing bathymetry (Fig. 1b) and dynamics, environmental parameters measured at 1000 m depth in the Makassar Strait and the Lombok Strait exhibit notable stability, as evidenced by consistent profiles of temperature, salinity, pH, chlorophyll-a, nitrate, phosphate and dissolved oxygen (suppl. Table 2). This stability likely plays a significant role in influencing the deep-sea bacterial communities at this depth. Analysis of bacterial diversity across three distinct stations at this depth reveals high diversity, with diversity index values remaining relatively consistent among the stations. This consistency in bacterial diversity is indirectly related to the environmental conditions of the deep-sea water column, which are conducive to microbial life. Bacterial community at 1000 m depth rely on organic matter and nutrient inputs from the surface layers[35]. The fertility of the surface layers, modulated by processes such as upwelling, mixing, and riverine inputs, impacts the availability of essential materials for deeper layers, thus sustaining microbial life in the deep-sea[35,36].

The bacterial communities observed at the three stations share significant similarities. Proteobacteria dominate across all stations (33, 48, 50), followed by Firmicutes, Bacteroidetes, Actinobacteria, Planctomycetes, Acidobacteria, Nitrospinae, Verrucomicrobia, Candidatus Melainabacteria, and Cyanobacteria. This is consistent with previous studies showing dominance of Gammaproteobacteria and Alphaproteobacteria in deep-sea waters near the Ninetyeast Ridge in the Indian Ocean[37]. These findings also align with reports from the Arctic and Pacific Oceans, which highlight Proteobacteria as prevalent[38]. The presence of genera such as *Colwellia, Moritella, Candidatus Pelagibacter, Alteromonas,* and *Psychrobacter* across all stations suggests these deep-sea bacterial genera are well-adapted to high salinity, low temperature, and high hydrostatic pressure.

According to the analysis of the specific bacterial species at Station 33(Suppl. Figure 2a), *Colwellia psychrerythraea* and *Photobacterium frigidiphilum* were more dominant than the other species. *Colwellia psychrerythraea* is a marine psychrophilic bacterium that is adaptable to cold conditions. Strains of *Colwellia psychrerythraea* have been shown to adapt to local deep-sea environments[39]. *Photobacterium frigidiphilum*, a gram-negative, *Psychrophilic* bacterium, was isolated from deep-sea sediments in the western Pacific Ocean[40]. *Candidatus Pelagibacter* ubique, an Alphaproteobacteria, was identified at Stations 33, 48, and 50 (Suppl. Figure 2b). This bacterium is known for its ability to thrive in low-nutrient conditions[41]. The genus Moritella dominated stations 33, and 50 (Suppl. Figure 2a,c), with *Moritella marina* being one of the most commonly isolated psychrophilic organisms from marine environments. *Moritella abyssi* was characterized as a gram-negative, non-spore-forming, strictly psychrophilic bacterium[42].

At the Stations 48 and 50, the *Halomonas* genus predominated (Suppl. Figure 2b,c). Some of the species found included *Halomonas axialensis*, *Halomonas aquamarine*, and *Halomonas meridiana*. *Halomonas axialensis* is psychrotolerant and piezotolerant and has the capacity to grow under cold, deep-sea conditions[43]. *Halomonas aquamarine* is a slightly halophilic bacterium found in deep-sea sediments at significant depths[44,45]. *Halomonas meridiana* is a gram-negative halophilic organism isolated from the Red Sea and recognized for its active L-glutaminase production[46]. Additionally, the genera *Alteromonas* and *Psychrobacter* also exhibited dominance. *Alteromonas macleodii* is a gram-negative, aerobic marine bacterium found throughout various oceanic environments. It inhabits waters from temperate to tropical zones, encompassing both coastal and pelagic regions[47]. *Psychrobacter pacificensis* is a deep-sea psychrophile capable of growing in the absence of NaCl, particularly within deep seawater layers[48].

This study provides a significant contribution to our understanding of deep-sea bacterial biodiversity within the 1000 m water column along the Indonesian Throughflow (ITF) route, specifically in the Makassar and Lombok Straits. The findings reveal a high level of biodiversity, reflecting the complexity of the deep-sea ecosystem in these regions. This ecosystem is characterized by extreme environmental conditions, including low temperatures, low oxygen levels, elevated pH, high salinity, and intense hydrostatic pressure, which collectively create a habitat for a highly specialized and exclusive bacterial community. To the best of our knowledge, this study is the first to document the presence of *Colwellia psychrerythraea, Moritella marina, Halomonas meridiana, Photobacterium frigidiphilum, Candidatus Pelagibacter, Alteromonas macleodii,* and *Psychrobacter pacificensis* in the deep-sea waters of Indonesia, specifically within the 1000-m water column, using 16S rRNA gene. Building on these findings, future research should explore the ecological roles and metabolic pathways of these microorganisms, as well as their interactions within the tropical deep-sea ecosystem. Additionally, bioprospecting could uncover novel enzymes, bioactive compounds, or metabolites with applications in biotechnology, medicine, and industry.

Conclusion

According to the analysis of the metagenomic data, the bacterial community profiles at the three stations, all located at a depth of 1000 m, exhibited relatively similar bacterial types. The primary phyla most prevalent at this 1000-m depth across all three stations were Proteobacteria, followed by Firmicutes, Bacteroidetes, Actinobacteria, Planctomycetes, Acidobacteria, Nitrospinae, Verrucomicrobia, Candidatus Melainabacteria, and Cyanobacteria. Within this depth range, the dominant genera included *Halomonas, Colwellia, Moritella, Candidatus pelagibacter, Photobacterium, Alteromonas, Pseudomonas, Cognaticolwellia*, and *Psychrobacter*.

Notably, these bacterial genera share common characteristics, such as psychrophilic, halophilic, and piezophilic tendencies, and are commonly found in deep-sea environments. The physicochemical conditions of the water at these three locations, measured at a depth of 1000 m, exhibit relatively consistent values. However, a more comprehensive study is necessary to discern the influence of these environmental conditions on the distribution of bacterial communities and other factors that have yet to be investigated.

Data availability

The raw read sequences have been deposited in GenBank (NCBI) under the Accession Number PRJNA1046651.

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Author contributions

Z.L.S., O.K.R. and F.M.D. conceptualized and designed the research. Z.L.S., C.E.D.F., R.D.A.O. and M.F. play a role in the process of sampling and measuring environmental data on board. Z.L.S. and H.N. performed the bioinformatics analysis. Z.L.S. and M.F. drew the figures in the manuscript. O.K.R., F.M.D., H.N., V.S.H.S., R.D.S., and Z.W. supervised, reviewed, and revised the manuscript. All authors have read and agreed to the final version of the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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