Iraqi Journal of Veterinary Sciences

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Fecal and gastric fluid microbiome profiles in the indopacific bottlenose dolphins (*Tursiops aduncus*)

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Article information

Article history:
Received April 01, 2022
Accepted May 08, 2022
Available online December 19, 2022

Keywords:
Emerging zoonotic pathogens
Gastrointestinal microbiota
Shewanella xiamenensis

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Abstract

The microbiota of the gastrointestinal system of dolphins has received significant interest recently. Moreover, little is understood about the microbiomes found in the stomachs of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*). This study aimed to evaluate the biodiversity of bacterial microbiota in the digestive system of *T. aduncus*. In the present study, 18 samples were obtained from an ex-situ conservation area, Wersut Seguni Indonesia, Kendal, Indonesia, and processed for bacterial DNA extraction. A total of 7 samples were qualified as representative samples for the 16S metagenomic sequencing. The bacterial composition revealed that the Shewanellaceae was significantly higher in the stomach than in the gut. As a result, the abundance of the microbiome in gastric and stool samples showed significant differences. In contrast, the Peptostreptococcaceae was found in greater abundance in the gut than in the stomach. At the species level, we successfully found emerging zoonotic pathogens involving *Shewanella algae* and *Shewanella xiamenensis*. This report is the first study to explore the bacterial diversity in gastro of *T. aduncus*.

DOI: 10.33899/ijvs.2022.135058.2440,

Introduction

The stability and ecological integrity of marine ecosystems are increasingly threatened, owing primarily to the long-term effects of global warming, habitat destruction, and the impact of human activities (1). Significantly, a decline in the dolphin population is of great concern during the killing festival. In addition, because of ocean pollution, environment degradation, fishing, overfishing, parasites, and emerging infectious diseases, the health of many marine mammals, including Indo-Pacific bottlenose dolphins (*Tursiops aduncus*), is jeopardized (2). The intestine of a dolphin typically harbours a diverse community of non-pathogenic, infectious agents and symbiont bacteria, which can significantly contribute to a host's overall health and disease outbreak. Some microbiota is established in a healthy animal, while others are transient in the intestine (3).

The immune response and the numerous microbes that live on mammal's body surfaces have a long history of coevolution. Normal flora associates of the host are essential for the functioning of the immune system. Even though most bacteria play crucial roles in host physiology. The mammal's immune response is vital for maintaining homeostasis with native microbial populations, which ensures the mutualism characteristics of the symbiont correlation. Simultaneously, resident bacteria substantially impact mammalian immunity (4). Groups of bacterial diversity can strongly influence the development of an infection and attenuate the host's immune response (5). A recent study has highlighted the microbiome's capacity to act actively and substantially for host health with a crucial role in disease presentation and immune system function in dolphins (6). The gastrointestinal tract represents the most significant interface between the organism and the external environment. In the lumen and

upper part of the mucus layer, this organ hosts many microorganisms whose composition affects the functions of the epithelial barrier and the gut immune system. Consequentially, the microorganisms in the gastrointestinal tract influence the health status of the organism. Microflora is living microorganisms that confer a health benefit to the host in specific conditions. Among others, microflora has immunomodulatory properties that usually act directly by: (a) increasing the activity of macrophages or natural killer cells, (b) modulating the secretion of immunoglobulins or cytokines; or indirectly by (c) enhancing the gut epithelial barrier, (d) altering the mucus secretion, and (e) competitive exclusion of other (pathogenic) bacteria.

The gut and bacteria have vital roles in dolphin's nutrition, digestion, absorption, and health. Microorganism manipulation of the microbiota inside the dolphins' digestive tract and live feed microbial assemblages will receive much attention. Understanding the microbiota composition in this context could provide helpful information for managing the feeding habit requirements in developing sustainable dolphins. It could also be beneficial in manipulating microbiota in dolphin systems at various stages of development to prevent pathogenic infection or improve nutrition (7). The gut microbiota may contribute to a variety of diseases. As a result, improving the intestinal flora by increasing the number of helpful bacteria while suppressing disease development might be a viable solution to these problems (8). Alternative disease control methods have been requested in other aquatic animals; including modification of bacterial diversity in the rearing ecosystem and linking it to the host to reduce the existence of pathogenic organisms while concurrently enhancing the host immune reactions. In marine mammals, *Escherichia coli* as opportunistic bacteria were abundant, though some strains of this bacteria are considered a pathogen, such as *Escherichia coli* O157:H7. The increase of marine pathogens like *Vibrio* sp., *Clostridium* sp., and *Salmonella* spp. is a big concern for marine mammals.

Inferior information and study, regarding the microbiome of Indo-Pacific bottlenose dolphins, are still inconclusive. The microbiome structure, as a species-specific in several vertebrates, is affected by host phylogenetics during millions of years of coevolution (9). In 1998, *T. aduncus* were recognized as a separate species from the widely known bottlenose dolphin *T. truncatus* (10). *T. aduncus* perform specific ancestor's genotypes and studying their associated microbes can aid in studying genetic variation. *T. aduncus* are categorized as near threatened on the IUCN Red List of endangered species (11). Thus; environmental destruction, decisive exploitation, and troubles related to locale (12) owing to climatic changes, reduced food and supplies, habitat destruction, and toxic contamination, vulnerability to infection may indicate that the stakes are incredibly high for *T. aduncus* (13). Also, continuation processes, that occur after such a primary bacterial infection, contribute to dysbiosis, and changes in the host's microbiota could be a more vital indicator of the development of the disease than of the existence of specific bacterial pathogens (14). To identify abnormalities, we must provide a preliminary study on microbes typically associated with *T. aduncus*.

Techniques for gene studies have produced a previously undiscovered variety of organisms in various environments over the last years. Culturally independent techniques, based on next-generation sequencing (NGS) technology, have recently acquired significant recognition for defining host-associated microbiomes in marine mammals. This study investigated the microbiome information in *T. aduncus*; focusing on the digestive microbiome. Since dolphins have a unique multi-chambered stomach for a carnivore; therefore, the digestive microbial community of dolphins may provide a novel and distinctive gastrointestinal microbiome host arrangement formed in marine mammals and particularly adapted to the carnivorous diet. The study of the gut microbes subject matter of threat or susceptible species could serve as an effective monitoring tool; revealing pathogenic microorganism presence and indicating overall environmental health (15). In this paper, we describe the gastrointestinal bacterial of Indo-Pacific bottlenose (*T. aduncus*) dolphins.

Materials and Methods

Sample collection

In the current study, nine adults of *T. aduncus* (four females and five males), from Wersut Seguni, Indonesia, were used. Regularly, animals are allowed in private environment pools with LSS (life support system) and chlorine. In indoor pools, the dolphins were kept in separate areas. Diets were composed of a whole frozen fish including: Mackerel scad (*Decapterus macarellus*), Oxeye scad (*Selar boops*), Short mackerel (*Rastrelliger brachysoma*), Rainbow sardine (*Dussumieria acuta*), and Indian oil sardine (*Sardinella longiceps*) to meet individual animal requirements. The dolphins, used in this study, were not receiving any antibiotics treatment before the sample collection.

Animal Ethics Research Committee, at IPB University, approved the sample collection procedures of 017/KEH/SKE/XI/2020. Specimen collection consisted of two samples per individual: faecal and gastric content samples (n=18). All samples were collected from the dolphins' conservation area, Wersut Seguni, in Kendal, Jawa Tengah, Indonesia (16). Physical examination and ultrasound were used to determine the animals' health status (17). Samples' description of nine adults of the Indo-Pacific bottlenose dolphins (*T. aduncus*). All samples were aseptically collected by two dolphin experienced veterinarians. Gastric fluid is obtained by inserting a sterile stomach tube into the dolphin's stomach. Fresh feces are collected by inserting a sterile rectal tube into the anus. The

collected samples were placed into a 15 mL tube containing 2 mL of RNA/DNA Shield. The tubes were then placed directly in an icebox until they could be moved to the Laboratory of Microbiology, Faculty of Veterinary Medicine, IPB University.

Extraction and sequencing of DNA

QIAamp® Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) was applied to extract total microbial DNA by following the procedure. Following the manufacturer's instructions, QIAamp Mini Spin Columns (QIAGEN) were used to purify the DNA further. The NanoDrop™ 2000/2000c Spectrophotometers (Thermo Scientific™, USA) were used to measure the concentrations of the purified bacterial DNA samples.

The CTAB/SDS method extracted total genome DNA from samples. On 1% agarose gels, DNA concentration and purity were measured. DNA was diluted to 1 ng/L in sterile water according to the concentration. 16S rRNA genes from different regions (16SV3-V4) were amplified using the barcoded primers 341F (5-CCTAYGGGRBGCASCAG-3) and 806R (5-GGACTACNNGGGTATCTAAT-3). Phusion® High-Fidelity PCR Master Mix (New England Biolabs) was used for all PCR reactions. The same volume of 1X loading buffer (SYB green) with the PCR products was mixed, and electrophoresis was run on a 2% agarose gel for detection. For the following experiments, samples, with a 470bp strong main strip, were selected. The Amplicons were combined at the same density. The purification of PCR products was performed by Qiagen Gel Extraction Kit (Qiagen, Germany). The sequencing libraries were created using the NEBNext® Ultra DNA Library Pre-Kit for Illumina. The manufacturer's instructions added index codes. The Qubit@ 2.0 Fluorometer (Thermo Scientific) and the Agilent Bioanalyzer 2100 platform were used to assess the library's performance. Eventually, the library was sequenced on an Illumina system, producing paired-end reads of 2 x 250 bp.

Assembly and quality control of paired-end readings

Paired-end reads were assigned to samples using unique barcodes and truncated by discarding the barcode and primer sequences. FLASH (V1.2.7) was used to merge paired-end reads (18), a quick and accurate analysis tool for combining paired-end reads, while at least a few of the needs to read overlap the read produced from of the opposite end of the same DNA fragment, as well as the splicing sequences, have been made reference to it as raw tags. Performance filtering on raw tags has been conducted under particular filtering settings to obtain high-quality clean tags (19) according to the Qiime (V1.7.0) (20). The tags have been compared with the reference database by using the UCHIME algorithm (UCHIME Algorithm, view description (21) to detect chimera sequences and it was removed (22). Finally, The Effective Tags were obtained.

***OTU* cluster and taxonomic annotation**

Uparse software was used to analyze the sequences (23) using all the effective tags. OTUs were assigned to sequences that shared 97% of their similarities. A representative sequence was screened for further annotation for each OTU. Qiime (Version 1.7.0, see details at http://qiime.org/scripts/assign taxonomy.html) allocates a taxonomy to every representative sequence (24) in the Mothur method was conducted against by the SSUrRNA database of SILVA Database (25) for species annotation at each taxonomic rank (threshold:0.81) (26) (kingdom, phylum, class, order, family, genus, species). MUSCLE (27) Version 3.8.31, can quickly compare various sequences to determine all OTU representative sequences' phylogenetic connection. The abundance of OTUs was standardized by applying a sequence number standard that corresponded to the samples with the minimum sequences. Based on this output normalized data, subsequent analyses of alpha and beta diversity were carried out.

Data analysis

The richness of biodiversity in a sample from the observed species was monitored using several parameters, which are Chao1, Shannon, Simpson, ACE, and Good-coverage indices. These indices were calculated and presented using R software (Version 2.15.3) in our samples using QIIME (Version 1.7.0). Two indices of community richness calculation were chosen: Chao - the Chao1 estimator and ACE - the ACE estimator. Then, the Shannon - the Shannon index and Simpson - the Simpson index, also performed by the same software to identify community diversity, including coverage. The coverage - the Good's coverage is one metric used to describe the sequencing depth.

The Arithmetic Means Unweighted Pair-Group Method (UPGMA) QIIME software brings clustering as a hierarchical clustering method to analyze the distance matrix using average linkage (Version 1.7.0). The QIIME tool was used to determine beta diversity on both weighted and unweighted unifrac (Version 1.7.0) to assess differences in sample species complexity. R software was used to perform the ANOSIM. An analysis of molecular variance (AMOVA) was performed to determine whether the difference of microbial community structure among gut and stomach is significant (28).

Results

The gastrointestinal microbiota's compositional structure was successfully investigated, gastric content and fecal samples were collected twice from seven adult Indo-Pacific bottlenose dolphins. All dolphins sampled lived in a controlled environment at WSI (Kendal, Indonesia). This work applied next-generation sequencing of the V3-V4 gene region of the bacterial 16S rDNA to define the stomach and

intestinal microbiota diversity from seven samples (Figure 1). Operational Taxonomic Units (OTUs) were obtained and recognized with 97% similarity on the Effective Tags of all samples to analyze the microbial community composition in each sample. Basic information from various samples, such as effective tags, low-frequency tags, and Tags annotation data, was collected during the OTU construction process. By analyzing the diversity of a single sample (Alpha diversity), we can reflect the richness and diversity of microbial communities in each sample, including species accumulation boxplots, biodiversity curves (Figure 2), and a series of statistical analyses, indicating a high level of total ecosystem diversity coverage. The differences in Alpha Diversity indices between groups were examined by boxplots chart analysis. T-tests and Wilcox tests are used to determine the significance of differences between groups. Figure 3 depicts boxplots based on observed species and Shannon indices.

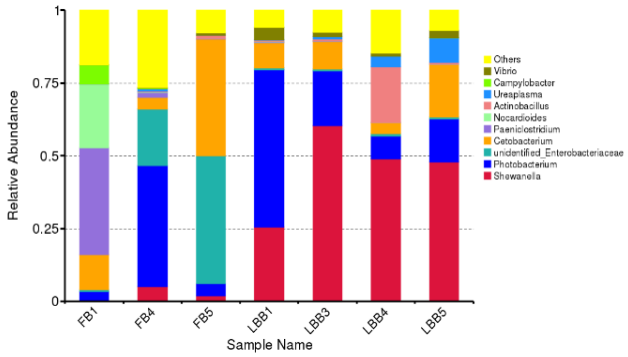

Figure 1: The distribution histogram of relative abundance of taxa in genus level of each sample. Several samples were collected from feces (FB1, 4 and 5) and gastric content (LBB 1, 3, 4, and 5).

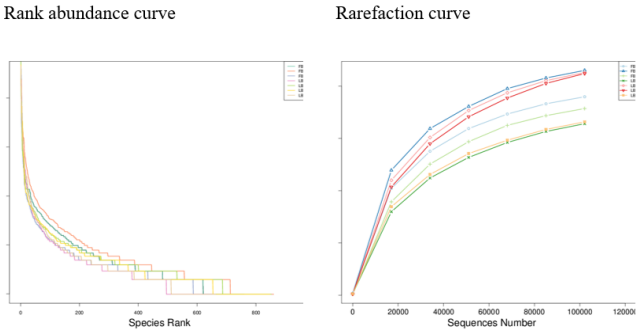

Figure 2: Biodiversity curves. Several samples were collected from feces (FB1, 4 and 5) and gastric content (LBB 1, 3, 4, and 5).

Beta diversity represents a comprehensive comparative assessment of microbiomes based on their diversity. As a result, the distinctions among microbiomes are assessed using beta-diversity metrics. To express the dissimilarity of samples, a square matrix of "distance" or "dissimilarity" was created, such as Unweighted Unifrac and Weighted Unifrac distance, to contrast the microbial diversity of each pair of community samples. The Unweighted Pair-group Method with Arithmetic Means can graphically depict the information in this distance matrix (UPGMA). To investigate the similarities of multiple specimens, a cluster tree was created using clustering analysis. We computed the Weighted Unifrac distance matrix and the Unweighted Unifrac distance matrix. Figures 4 and 5 were shown with the integration of cluster analysis results and the relative abundance of each sample by phylum. The ANOSIM analysis was performed to determine if the variance between groups is considerably more significant than the variability within groups, which helps in assessing the justification for group classification. Rank was obtained from the sorted distance between samples, according to ANOSIM results ($R = 0.7407$; $p = 0.03$). Figure 6 depicts boxplots based on rank (Between-group and Within-group). However, microbial community structure among fecal and gastric content is not significantly different (p-value 0.066) based on the AMOVA result.

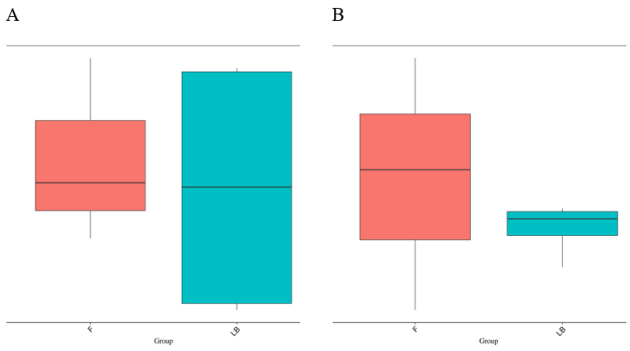

Figure 3: Difference of alpha diversity indices between groups: A. Box plot of the difference of observed species; B. Boxplots for the difference of Shannon indices. Feces (F) and gastric content (LB).

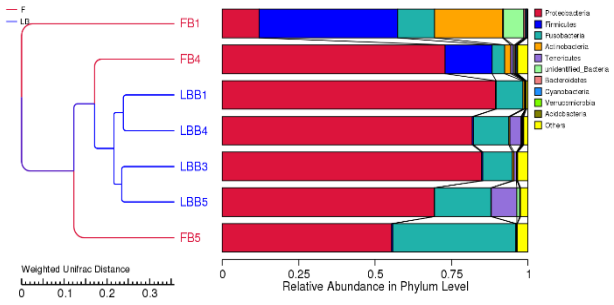

Figure 4: UPGMA cluster tree based on Weighted Unifrac distance. Feces (FB1, 4 and 5) and gastric content (LBB 1, 3, 4, and 5).

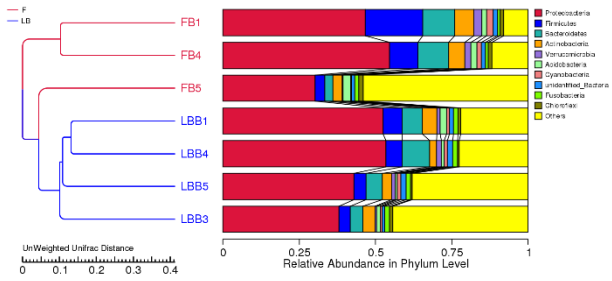

Figure 5: UPGMA cluster tree based on Unweighted Unifrac distance. Feces (FB1, 4 and 5) and gastric content (LBB 1, 3, 4, and 5)

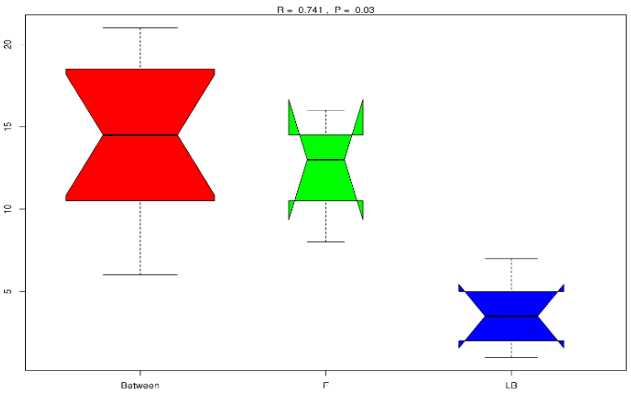

Figure 6: ANOSIM result. That figure summarizes the R-value based on ANOSIM from different sampling points. The rank value plotted them on the Y-axis and the Between-group and Within-group on the X-axis. R-value is between -1 and 1. A positive R+ value means that inter-group variation is considered significant, while a negative R-value suggests that inner-group variation is more prominent than the inter-group one. Therefore; no significant differences. The confidence degree is represented by P-value, whose value less than 0.05 suggests statistical significance.

Based on OTU identification and taxonomic annotation results, the total bacteria percentage in the gut and stomach are 82.4% and 91.3%, respectively. At genus level, unidentified Enterobacteriaceae 25.97% was present at the highest abundance in the gut microbiota ecosystem, followed by *Cetobacterium* 22.78%, *Photobacterium* 19.84%, *Paeniclostridium* 15.59%, *Nocardioides* 8.94%, *Shewanella* 2.84%, *Campylobacter* 2.76%, *Ureaplasma* 0.47%, *Vibrio* 0.38% and *Actinobacillus* 0.46%. In the stomach microbiota ecosystem, the most represented in genus level were *Shewanella* 50.09%, *Photobacterium* 26.12%, *Cetobacterium* 10.93%, *Actinobacillus* 5.73%, *Ureaplasma* 3.55%, *Vibrio* 2.60%, unidentified Enterobacteriaceae 0.79%, *Nocardioides* 0.11% and *Paeniclostridium* 0.07%. Figure 7 depicts the taxon relative abundance distribution graph in each group's genus levels.

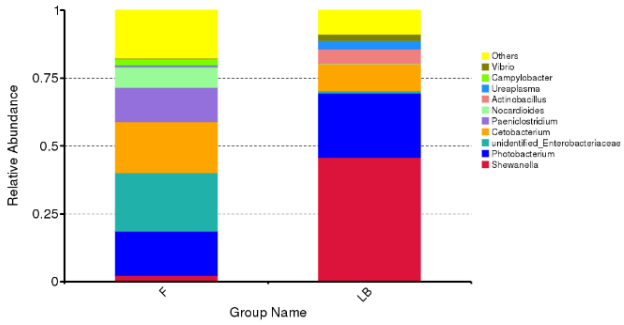

Figure 7: The Indo-Pacific bottlenose dolphins' microbiota at the genus level. Bar charts summarizing the genus-level microbiota composition in the feces (F) and gastric content (LB) from seven adult Indo-Pacific bottlenose dolphins.

The heatmap was created using the abundance information of the top 35 genera of all samples to assess whether samples with comparable processing are clustered and the identity and diversity of samples. *Actinobacteria*, *Cyanobacteria*, *Firmicutes*, *Fusobacteria*, *Proteobacteria*, and *Tenericutes* were detected in the heatmap (Figure 8).

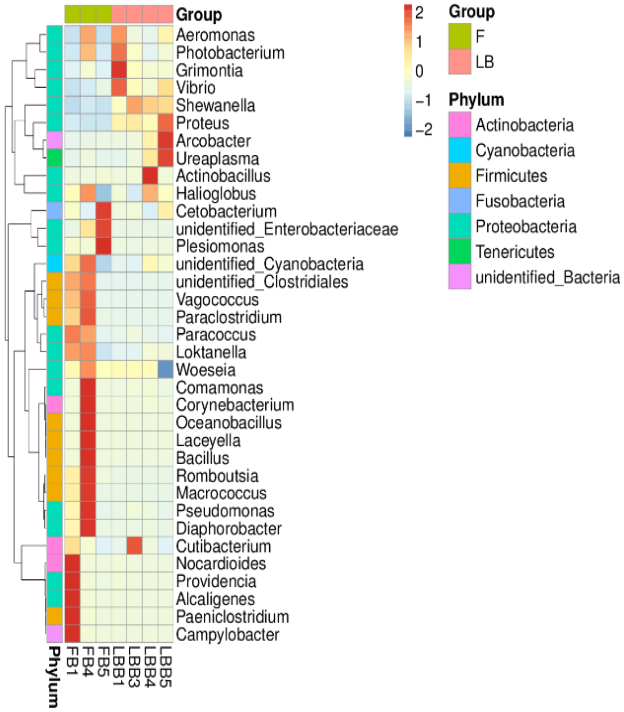

Figure 8: Taxonomic abundance cluster heatmap has been plotted. That figure summarizes the genus-level microbiota composition in the feces (F) and gastric content (LB) from seven adult Indo-Pacific bottlenose dolphins. The absolute value of Z represents the distance between the raw score and the standard deviation mean. The Z is negative when the raw score is below the mean and vice versa.

Interestingly, the result showed that unidentified bacteria could not be assigned to any phylum outlining the high score of *Arcobacter* and *Campylobacter*. *Arcobacter* was detected in a high score in gastric content of the individual sample (LBB5) 96%, while *Campylobacter* showed high 98% in feces of the individual sample (FB1). Additively, Enterobacteriaceae, *Cyanobacteria*, and *Clostridiales* were also detected but could not be identified to any genus, but these unidentified bacteria were present in high scores in the fecal group.

Discussion

This paper successfully presents gastrointestinal bacterial communities from a well-studied community of Indo-Pacific bottlenose dolphins. The Rarefaction curves and Rank abundance curves are two main methods for indicating sample biodiversity. The rarefaction curve reflects the rationality of the sequencing data volume and, in turn, the richness of the microbial community in the fecal and gastric content of Indo-Pacific bottlenose dolphins. In contrast, the Rank abundance curve reflects the richness and evenness of species in samples. These curves have been reported in other mammals, including humans (29), wildlife species (30), and Sunda pangolin (*Manis javanica*) (31). ANOSIM analysis is a nonparametric test that determines whether group variation is significantly larger than group variation, which aids in determining the reasonability of group division. An R test statistic ranging from 1 to 1 is generated by ANOSIM. A positive R-value indicates a within-group similarity higher than similarity with larger R values indicating stronger sample clustering. R-value of zero indicates that no sample tends to cluster. In contrast, a negative R-value indicates more between-group similarity than within-group similarity. (ANOSIM, $R = 0.7407$; $p = 0.03$), demonstrating the distinct microbial signature for each group in this study.

In this study, an unidentified genus of the Enterobacteriaceae family, *Cetobacterium*, and *Photobacterium* were dominant in the feces of *T. aduncus*. In a previous study (32), *Clostridium* sensu stricto, *Cetobacterium*, and *Paeniclostridium* accounted for above 98% of all identified genera in captive dolphin fecal. In contrast, wild dolphin stool contained five genera: *Actinobacillus*, *Haemophilus*, *Photobacterium*, *Vibrio*, and *Ureaplasma*. The current study was fascinating because it looked into the bacterial genera distinguishing gut microbes between *T. aduncus.* The feeding habit of dolphins influences the structure of intestinal microbiota. Previous researchers (32) fed their dolphins with chub mackerel, *Scomber japonicus*, Japanese flying fish, *Cypselurus agoo*, Shishamo smelt, *Spirinchus lanceolatus*. In the present study, the dolphins were fed with Mackerel scad (*Decapterus macarellus*), Oxeye scad (*Selar boops*), Short mackerel (*Rastrelliger brachysoma*), Rainbow sardine (*Dussumieria acuta*), and Indian oil sardine (*Sardinella longiceps*). Diet, antimicrobial use, water chlorine, and contact with humans may influence the microbiota composition. Thus, the gut microbiota variations were compositional between Indo-Pacific bottlenose dolphins from Japan and dolphins from Indonesia. *T. aduncus* from Japan were shown to have a diet very high in fish protein obtained from the Japan Sea. Their microbiota was highly enriched in *Clostridium* sensu stricto 1 bacteria. This exploratory study has shown that the healthy gut microbiota primarily consists of anaerobic and fermenting bacteria. *Clostridium* sensu stricto 1 is a significant anaerobe in the human gut. Carbohydrates, amino acids, alcohols, and purines are among the compounds they can metabolize. Butyric acid is a fermented metabolite that is genus-specific. Various amounts of acetic acid, lactic acid, ethanol, propanol, or butanol are generated as fermentation products. Meanwhile, our dolphins from Indonesia were fed with fish from Indonesia Sea origin, showing the predominance of the unidentified genus of the family Enterobacteriaceae. The predominance of the unidentified genus of family Enterobacteriaceae has also been reported in the feces of broiler chickens (33), human stool with chronic spontaneous urticaria (34), the premature neonate's stool (35), and the cloacal of North American Colubrids (36). The unidentified genus of the family Enterobacteriaceae was relatively increased 25.97% compared to the stomach 0.79% in this study. Many family members are ordinary members of the gut microbiota in mammals. However, this study found that Enterobacteriaceae had the highest relative abundance in *T. aduncus* gut while this family was lower in the bottlenose dolphin *T. truncates* (37). We investigated the species that contribute to the unidentified genus of the family Enterobacteriaceae from the heatmap interactive web page presentation of taxonomic annotation corresponding to OTUs and successfully found that *Escherichia coli* is the most species-rich unidentified genus in the Enterobacteriaceae family. *E. coli* is not always found in marine environments, but it always can be found in the intestines of warm-blooded animals. *E. coli*, on the other hand, may respond to environmental changes that are unusual for this type of bacterium. Under significant nutrient loading, *E. coli* cannot only live in the marine environment for long periods of time, but also stay physiologically active.

Cetobacterium was the third most prevalent microbiota in the stomach 10.93% and became the second most prevalent bacteria 22.78% in the gut. This finding is slightly higher than previous work (38) which is *Cetobacterium* found to be dominant 20% in the intestine of striped dolphin (*Stenella coeruleoalba*), higher than in *T. truncatus* gut 8.13% (16) but lower than in wild *T. aduncus* fecal 38.7% (32). It has been confirmed that *Cetobacterium* isolated from the intestine generates vitamin B-12 (cobalamin) which is involved in erythrocyte development and fatty acid metabolism, emphasizing the importance of this genus in vitamin B-12 production and in the contribution of this genus to host nutrition and health.

Photobacterium showed a higher contribution 26.12% in the stomach microbiome than it did in the gut 19.84% in the other microbiota. *Photobacterium* is prevalent in the marine ecosystem, on dolphin surfaces, and in their intestinal contents (39). Recently, some *Photobacterium* spp. has been considered a pathogen species in dolphins (40-42). *Photobacterium* species frequently interact with marine creatures in unspecified microbes, protozoa, and saprophytes. *Photobacterium* can be isolated from the surface, the fluids of the gastrointestinal tract, decomposing animal material, diseased amphipods, and other crustaceans' hemolymph, and saltwater. These general and harmful relationships oppose the highly particular luminescent symbiosis with zebrafish and octopus and mutually beneficial interactions of several Photobacterium species but understanding the symbiosis of *Photobacterium* species in dolphins is unknown. We evaluated the species of *Photobacterium* in this study and found out that *P. leiognathi* shares a high contribution in the gastrointestinal microbiome of *T. aduncus*. *P. leiognathid*, which inhabits warm coastal waters to create symbiotic interactions with shallow-dwelling fish. *P. leiognathi* is provided with shelter and a nutrient-rich habitat, allowing it to grow. The zebrafish and octopus may get benefit from employing bioluminescent light to attract and seduce prey. Other *Photobacterium* species, aside from *P. leiognathi*, have been harmful to aquatic life and animals. ToxR, a transmembrane DNA binding protein, and ToxS, a related membrane protein, are found in many ocean species harmful to people or fish. ToxR is present in *P. leiognathi*, but no proof of pathogenicity has been discovered in the organisms.

This study is the first work to investigate the bacterial community in the stomachs of Indo-Pacific bottlenose dolphins. *Shewanella* was the dominant bacterial genus found in the gastro specimens 50.09%, followed by *Photobacterium* 26.12%, *Cetobacterium* 10.93%, *Actinobacillus* 5.73%, *Ureaplasma* 3.55%, *Vibrio* 2.60%, unidentified *Enterobacteriaceae* 0.79%, *Nocardioides* 0.11% and *Paeniclostridium* 0.07%. *Shewanella* bacteria are saprophytic gram-negative bacteria found in warm and temperate climate zones, and they are part of the normal marine microbiota. In this study, *Shewanella* dominated the Indo-Pacific bottlenose dolphin gastric fluid 50.09%, but in contrast, *Shewanella* was not dominant in fecal 2.84%. Interestingly, *Shewanella* was not reported in the bacterial population of wild and captive Indo-Pacific bottlenose dolphins studied before (32) and in the bacterial population of *Stenella coeruleoalba* (43). However, *Shewanella* spp. was isolated in the previous study (44) from the blow of a dead-stranded juvenile Risso's dolphin (*Grampus griseus*) and a dead captive-born common bottlenose dolphin (*Tursiopss truncatus*). Further, *Shewanella putrefaciens* was reported in bottlenose dolphins *T. truncatus* and associated with clinical illness in humans (45). Several *Shewanella* species were identified in the current study as new sources of soft tissue and invasive infections following seawater contact, including *Shewanella algae* 0.17% and *Shewanella xiamenensis* 0.04%. Tropical locations such as Southeast Asia, Southern Europe, South Africa, and the Caribbean have recorded the bulk of human *Shewanella* infections. *S. putrefaciens* and *S. algae* are the only *Shewanella* spp. found in human clinical samples, with *S. algae* contributing to more than 80% of isolates documented in the research. The most virulent species was *S. algae*, resistant to penicillin and first- and second-generation cephalosporins (46). It has been proposed that this species' haemolytic activity might play an important role in its pathogenesis. A close interaction between human and the sea environment or its components is a serious health concern for *Shewanella* infection. It suggests that *S. algae* may play a pathogenic role in the marine environment for mammal. *S. algae* was isolated from the hearts of free-roaming Atlantic bottlenose dolphins (*T. trucantus*) that had meningo-encephalitis (47). Surprisingly, *Shewanella xiamenensis* has never been reported in dolphins. To the best of our ability, *S. xiamenensis* was first discovered and identified in *T. aduncus* in our study. Further work is needed to determine whether the microbial community in seawater is associated with the high abundance of *Shewanella* in *T. aduncus*. Furthermore, the present study needs to explore the skin microbiome of Indo-Pacific bottlenose dolphins and other organ systems.

Acknowledgments

Dwi Restu Seta and Muhammad Elmanaviean provided technical assistance in sample collection. This work was done as part of the MICROBIOME project, funded by PT. WSI in Indonesia.

Conflict of interest

There is no conflict of interest.

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الصورة المظهرية للاحياء المجهرية في براز ومعدة دلافين المحيط الهندي-الهادي قارورية الانف

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الخلاصة

في الآونة الأخيرة، حظيت الكائنات الحية الدقيقة في الجهاز الهضمي للدلافين باهتمام كبير، علاوة على ذلك، لايوجد الكثير من المعلومات عن صورة وتركيبة الاحياء المجهرية الموجودة في معدة دلافين المحيط الهندي-الهادي . هدفت الدراسة إلى تقييم التنوع البيولوجي للكائنات الحية البكتيرية في الجهاز الهضمي لمعدة الدلافين. فقد تم الحصول على ١٨ عينة من منطقة محمية خارج الموقع الطبيعي، وارسوت سغوني، كاندل، إندونيسيا. عوملت العينات لاستخلاص الحمض النووي البكتيري. تم تأهيل ما مجموعه ٧ عينات كعينات تمثيلية للتسلسل الميتاجينوم المعتمدة على (16S). أظهرت التركيبة البكتيرية أن مجموعة بكتريا الشوانيلا كانت أعلى بشكل ملحوظ في المعدة منها في الأمعاء ونتيجة لذلك، فقد أظهرت وفرة بكتريا الشوانيلا في عينات المعدة والبراز اختلافات معنوية، في المقابل، تم العثور على انواع اخرى من بكتريا السبحية حيث كان تواجدها في الأمعاء أكثر من المعدة. على مستوى الأنواع، كذلك نجحت الدراسة في التوصل الى مسببات الأمراض الحيوانية المنشأ الناشئة والتي تشمل *Shewanella algae* و *Shewanella xiamenensis* . واعتبرت هذه الدراسة هي الاولى من نوعها في تحديد التنوع البكتيري في معدة الدلافين *T. aduncus*.

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