



# Taxonomic profiling of bacteria in *Oreochromis* sp. floating-net cage aquaculture system at Bangka Belitung University, Indonesia

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**Abstract.** This research aimed to provide information related to the bacteria presence in water samples from the Nile tilapia (*Oreochromis* sp.) floating net cage aquaculture system at Bangka Belitung University, Indonesia. It also determined the bacterial energy flow described by the Sankey diagram to gain information on the linkage between bacterial occurrence and relative abundance. The determined physical-chemical parameters of pond water included pH, temperature, dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), total ammonia (NH<sub>3</sub>-N), orthophosphate (PO<sub>4</sub>-P), nitrate (NO<sub>3</sub>-N), nitrite (NO<sub>2</sub>-N), free ammonia (NH<sub>3</sub>), C-organic. The metagenomic analysis of bacteria was carried out using next-generation sequencing through 16S rRNA gene sequencing. We have found the presence of Gram-negative bacteria in the pond water. The phylum Pseudomonadota (Proteobacteria), class Betaproteobacteria, order Burkholderiales, family Comamonadaceae, genus *Acidovorax*, and *Acidovorax soli* was the predominant bacterial group in the pond water. The water parameter values were: 27-30°C temperature, 5.4-5.6 pH, 5.6-5.7 mg L<sup>-1</sup> DO, 1.6-1.8 mg L<sup>-1</sup> BOD, 8.47-10.95 mg L<sup>-1</sup> COD, 2.72-3.36 mg L<sup>-1</sup> Al, and 416-429 mg L<sup>-1</sup> Fe.

**Key Words:** bacterial, diversity, Gram negative, metagenome.

**Introduction.** One of the world's fastest-growing industries for producing food is aquaculture (Sumaila et al 2022). The quality of fish pond water is essential for the proper functioning of the ecosystem, fish, other organisms, and overall aquaculture productivity (Ajayi & Okoh 2014; Tumwesigye et al 2022). Healthy aquatic ecosystems are supported by good physiochemical properties and biological diversity (Leong et al 2018), and the presence of microorganisms in water can influence water quality (Fister et al 2016).

Microorganisms are the most abundant and diverse biological resources on Earth (Rosenberg & Zilber-Rosenberg 2022). Bacteria are the oldest and most biodiverse group, followed by archaea and fungi (Vitorino & Bessa 2018). Bacteria play important roles in biogeochemical and nutrient cycles (Meng et al 2022), being an important part of ecosystem function (Zhou et al 2023).

The culture and non-culture methods are the two main approaches used to research microorganisms (Akram et al 2023). The culture method is a well-established, low-cost, and simple-to-use approach to bacterial isolation. The culture method has been conventionally used for the identification of bacterial communities, however, can only be used to culture a small number of species present in an environment with the estimation that only 5-20% of bacteria species are cultivable (Eckburg et al 2005; Costa & Weese 2019).

The modern molecular biology technique, the non-culture method, uses the gene sequences of bacteria to investigate the composition and diversity of communities. The technique offers the advantage of identification processing a huge number of data (An et al 2022; Chen et al 2022). Compared to culture methods, next-generation sequencing

(NGS)-based microbial identification is more comprehensive, has a higher accuracy, and it accelerated metagenomic studies (Ashraf et al 2022; Navgire et al 2022).

Assessment of the bacteria diversity through sequencing of 16S ribosomal RNA (16S rRNA) genes has been an approach widely used in environmental microbiology, particularly since the advent of high-throughput sequencing technology. Oxford Nanopore Technology (ONT) provides the long reads sequencing that covers the full-length sequence of 16S rRNA gene (V1-V9 regions) through a fast, cheap, and high throughput process. Since all the informative sites of 16S rRNA genes are considered, the full-length 16S rRNA sequences offer a higher level of taxonomic and phylogenetic resolution for bacteria identification (Bahram et al 2018).

In this study, the bacteria diversity and their energy flow in the Nile tilapia (*Oreochromis* sp.) floating-net cage aquaculture system at Bangka Belitung University, Indonesia were investigated. This study aimed to provide information related to the bacteria presence and their energy flow described by the Sankey diagram to determine the linkage between bacterial occurrence and relative abundance.

**Material and Method.** This research took place from March to June 2023. The study was conducted to analyze the physical-chemical properties and metagenome diversity of bacteria as biological properties of water in a Nile tilapia floating net cage aquaculture system. Water samples were randomly collected from fish ponds in Bangka Belitung University, Indonesia.

2 L of water samples from the pond were collected for physico-chemical properties analysis (Table 1). The physico-chemical properties determined include pH, temperature, dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), total ammonia (NH<sub>3</sub>-N), orthophosphate (PO<sub>4</sub>-P), nitrate (NO<sub>3</sub>-N), nitrite (NO<sub>2</sub>-N), free ammonia (NH<sub>3</sub>), C-organic, and metals or heavy metals. The pH was measured with a pH meter, temperature with a digital thermometer, DO with a DO meter, BOD was determined by the gravimetric method, while COD, total ammonia, free ammonia, orthophosphate, nitrate, and nitrite were measured with spectrophotometry. C-organic was determined with the Walkey & Black method, while the metals or heavy metals were measured with portable X-Ray Fluorescence. However, these results have been already published in a previous scientific article (Kurniawan et al 2023) and are presented in Table 1.

1 L of water sample from the pond was also collected for metagenome analysis. The sample was sent to the Laboratory of Genetica Science Indonesia for metagenome analysis.

The metagenomic analysis through 16S rRNA gene sequencing was investigated by amplicon full length sequencing with nanopore sequencing. Genomic DNA was extracted using ZymoBIOMICS DNA Miniprep Kit (Zymo Research, D4300). DNA concentration was determined using both NanoDrop spectrophotometers and Qubit fluorometer. Library preparations were conducted using kits from Oxford Nanopore Technology. 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 (Wick et al 2019). The quality of FASTQ files was visualized using NanoPlot, and quality filtering was performed using NanoFilt (De Coster et al 2018; Nygaard et al 2020). Reads were classified using a centrifuge classifier (Kim et al 2016). The bacterial index was 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 RStudio using R version 4.2.0 (<https://www.R-project.org/>).

Table 1

Physical and chemical properties of water in Nile tilapia (*Oreochromis* sp.) floating net cage aquaculture system, Bangka Belitung University, Indonesia

<i>Description</i>	<i>Values (Kurniawan et al 2023)</i>
Physical properties	
Temperature (°C)	27-30
Total dissolved solids (mg L <sup>-1</sup> )	254-256
Chemical properties	
pH	5,4-5,6
Dissolved oxygen (mg L <sup>-1</sup> )	5.6 -5.7
Biological oxygen demand (mg L <sup>-1</sup> )	1.6-1.8
Chemical oxygen demand (mg L <sup>-1</sup> )	8.47-10.95
Total ammonia (NH <sub>3</sub> -N) (mg L <sup>-1</sup> )	0.018
Orthophosphate (PO <sub>4</sub> -P) (mg L <sup>-1</sup> )	<0.003
Nitrate (NO <sub>3</sub> -N) (mg L <sup>-1</sup> )	<0.08
Nitrite (NO <sub>2</sub> -N) (mg L <sup>-1</sup> )	<0.002
Free ammonia (NH <sub>3</sub> ) (mg L <sup>-1</sup> )	0.020
C-organic (mg L <sup>-1</sup> )	0.50-0.52
Chemical elements	
Al (mg L <sup>-1</sup> )	2.72-3.36
As (mg L <sup>-1</sup> )	289-317
Bi (mg L <sup>-1</sup> )	27-31
Cl (mg L <sup>-1</sup> )	1470-1710
Ca (mg L <sup>-1</sup> )	1.012-1.502
Fe (mg L <sup>-1</sup> )	416-429
Se (mg L <sup>-1</sup> )	
Si (mg L <sup>-1</sup> )	28.49-29.96
Sr (mg L <sup>-1</sup> )	2-8
Zr (mg L <sup>-1</sup> )	191-202

**Results and Discussion.** The color of pond water was light to dark brown. Water parameters are limiting factor in fish culture (Munni et al 2013). Kritzberg et al (2020) explain that the browning of surface waters is a result of increasing dissolved organic carbon and iron concentrations. Furthermore, Pećzuła et al (2022) explain that when waters turn brown, the ecosystems may change from clear-water, autotrophic, benthic-dominated to brown-water, heterotrophic, and pelagic-dominated.

The taxonomic profiling and the relative abundance of bacteria composition in the Nile tilapia floating net cage aquaculture system is presented in Figure 1. The relative abundance showed phylum Pseudomonadota (Proteobacteria), class Betaproteobacteria, order Burkholderiales, family Comamonadaceae, and genus *Acidovorax* to be the predominant bacteria of the pond water.

Pseudomonadota is the correction of the synonym Proteobacteria (sic) (Garrity et al 2005; Oren & Garrity 2021). Pseudomonadota is a diverse group of bacteria that are generally Gram-negative (Zheng et al 2022). It includes some of the most ubiquitous bacterial groups as well as a vast array of opportunistic pathogens (Leão et al 2023). It is the richest phylum in the water pond and all sediments and plays key roles in metabolism and sedimentation (Qin et al 2016; Shen et al 2022). Proteobacteria plays an important role in nutrient cycling processes, such as nitrogen cycling, sulfide cycling, and mineralization of organic compounds in the ponds (Liu et al 2023).

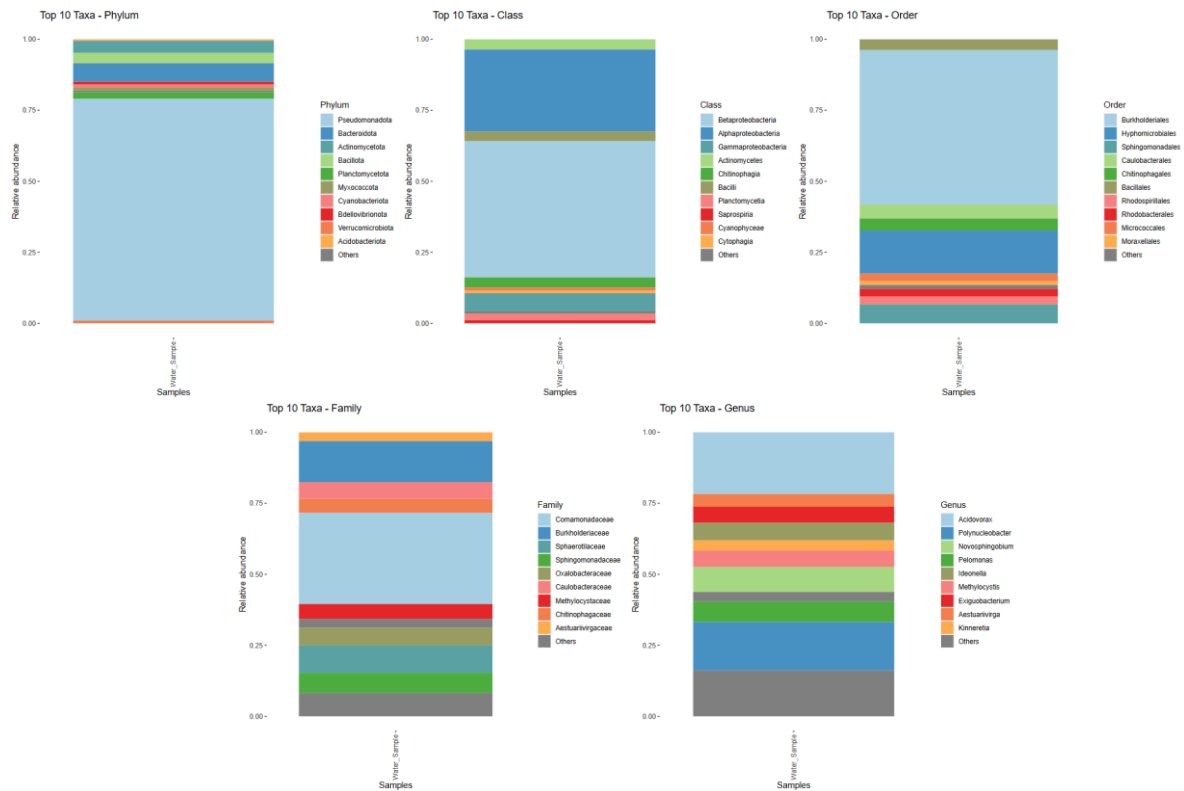


Figure 1. Top 10 phyla, classes, orders, families and genera based on relative abundance compared among samples in barplots.

Furthermore, we observed the contribution of bacteria in the habitat by the Sankey diagram. Zhou et al (2022) explain the contributions of microbial genomes to individual metabolic and biogeochemical processes, with entire elemental cycles described with the Sankey diagram. The transformation and cycling of elements, energy, and matter among the lithosphere, atmosphere, hydrosphere, and biosphere are significantly fueled by microbially mediated biogeochemical processes (Madsen 2011). Freshwater bacteria are at the hub of biogeochemical cycles and control water quality in lakes (Newton et al 2011). In their natural habitats, microbial communities take the shape of intricate networks that share and compete for metabolites (Morris et al 2013; Zelezniak et al 2015; Abreu & Taga 2016).

The top nine species found abundant were from the phylum Pseudomonadota: *Acidovorax soli*, *Acidovorax delafieldii*, *Ideonella paludism*, *Aestuariivirga litoralis*, *Kinneretia asaccharophila*, *Methylocystis parvus*, *Pelomonas puraquae*, *Pelomonas saccharophila*, and *Chitinimonas taiwanensis*. In addition, species *Exiguobacterium indicum* from phylum Bacillota was also found in this habitat (Figure 2).

Based on the Sankey diagram, we described the characteristics of the bacteria for understanding their role in this habitat. *A. soli* was the predominant species in the Nile tilapia floating net cage aquaculture system at Bangka Belitung University. *A. soli* is a Gram-negative, aerobic, rod-shaped (0.56-1.3 mm), non-motile bacteria. The colonies are circular, convex, and grow at 10-42°C (optimum 30°C), 0-1% NaCl (optimum 0 %), and pH between 6-9 (optimum 7). The species of the genus *Acidovorax* such as *A. delafieldii* are found mainly in soil and water habitats, with *A. soli* being isolated from landfill soil (Choi et al 2010). *Acidovorax* are denitrifying bacteria, resistant to antibiotics, responsible for septicemic diseases among freshwater fish, and can produce total mortality of fish. Some of them are phytopathogenic species that infect corn, oats, rice, and many other plants (Ogunyemi et al 2019; Duman et al 2021; Javaid et al 2021; Bruno et al 2023).

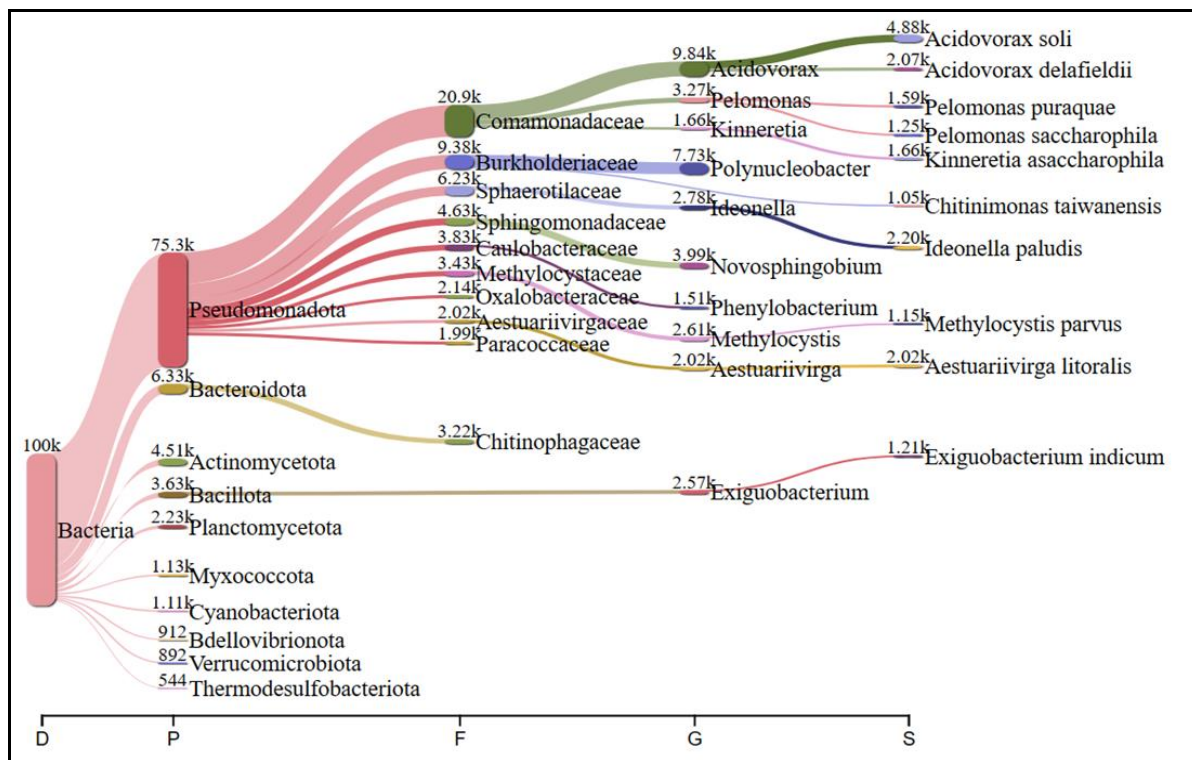


Figure 2. Sankey diagram of the top 10 taxa identified at each taxonomic level in water samples collected from a Nile tilapia (*Oreochromis* sp.) floating net cage aquaculture system; D - domain; P - phylum; F - family; G - genus; S - species.

*A. litoralis* is Gram-negative, non-motile, short rod, and aerobic (Li et al 2019). *C. taiwanensis* is Gram-negative, slightly curved rod, motile using of a single polar flagellum, aerobic and anaerobic (Chang et al 2004). *K. asaccharophila* is Gram-negative, rod-shaped, motile using of a single polarly inserted flagellum, and aerobic (Gomila et al 2010). *P. puraquae* and *P. saccharophila* are Gram-negative, rod-shaped, non-spore-forming, motile using of a single polarly inserted flagellum, and aerobic (Gomila et al 2007). *M. parvus* is Gram-negative, rod-shaped, non-motile, and aerobic methanotroph (Stevens et al 2023). *Ideonella paludis* is Gram-negative, rod-shaped, motile, and aerobic (Sheu et al 2016). *E. indicum* is Gram-positive, motile, rod-shaped, non-spore-forming, and aerobic (Chaturvedi & Shivaji 2006). All species of Pseudomonadota were Gram-negative and species of Bacillota were Gram-positive. Peřkala-Safińska (2018) and Breijyeh et al (2020) explain that Gram-negative bacteria are commonly known to be pathogenic to fish, more resistant than Gram-positive bacteria, and can cause significant morbidity and mortality worldwide. Furthermore, Mekasha & Linke (2021) explain that phylum Proteobacteria is the most prominent Gram-negative bacterial fish pathogen. The pathogens including toxins, adhesins, effectors, and enzymes promote colonization and infection of bacteria.

Based on the description, these bacteria the have potential to harm fish. Overall, the detected bacteria were grouped in Gram-negative bacteria commonly known to be pathogenic to fish (Peřkala-Safińska 2018). Thus, aquaculture management is required to ensure the Nile tilapia floating net cage aquaculture system in Bangka Belitung University, Indonesia runs well. The surveillance and monitoring of water quality and fish health play an important role in ensuring that no infectious diseases produce sudden and unexpected mass mortality of cultured fish.

**Conclusions.** We have investigated the presence of Gram-negative bacteria from pond water of Nile tilapia floating net cage aquaculture system, Bangka Belitung University, Indonesia. The phylum Pseudomonadota (Proteobacteria), class Betaproteobacteria, order Burkholderiales, family Comamonadaceae, genus *Acidovorax*, and species *Acidovorax soli* was the predominant bacteria of the pond water. The presence of the

bacteria may contribute to fish disease, so water management is needed to anticipate the potential of infectious disease.

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**Conflict of Interest.** The authors declare that there is no conflict of interest.

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\*\*\* <https://github.com/marbl/Krona>  
\*\*\* <https://www.R-project.org/>

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