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Research Article

Metagenomic Analysis of Bacterial Communities in the Musi River Estuary, South Sumatra, Indonesia

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ABSTRACT

Understanding the diversity, function, and ecological dynamics of bacterial communities depends on unique transitional environments, estuaries. Knowledge on bacterial taxa in the Musi River estuary, South Sumatra still eludes, however. This effort aims to identify bacterial species in estuary waters using metagenomic analysis based on 16S rRNA gene. Surface water samples from three study locations were analysed by DNA isolation, nanodrop spectrophotometer qualitative assessment, 16S rRNA gene amplification, electrophoresis, and Illumina NovaSeq sequencing. Results showed that Proteobacteria predominated at all sites, followed by Campilobacterota, Cyanobacteria, and Bacteroidota. At the class level, Gammaproteobacteria was most common, followed by Alphaproteobacteria and Campylobacteria. Dominant bacterial orders were Campylobacterales, Rhodobacterales, and Pseudomonadales while the most common families were Arcobacteraceae, Rhodobacteraceae, and Pseudomonadaceae. The most plentiful genera were *Rheinheimera*, *Pseudomonas*, and *Pseudarcobacter*. Variations in bacterial spread among stations suggest environmental factors including salinity, nutrient availability, and human activities influencing microbial community composition. Ternary plots, heat maps, and krona diagrams were employed to disclose distinct patterns of bacterial community dispersion in the estuary. This paper underscores the importance of metagenomic research in illuminating microbial diversity in estuarine environments and its impact on ecological dynamics and water quality.

1. Introduction

Estuaries are dynamic ecosystems that serve as critical interfaces where freshwater from rivers meets saline ocean water, resulting in diverse ecological niches and distinct environmental gradients. These conditions foster diverse microbial communities essential for biogeochemical cycling, nutrient cycling, and ecosystem integrity (Zhang *et al.* 2019; Bao *et al.* 2023; Henson & Thrash 2024; Yang *et al.* 2024; Wu *et al.* 2024). Understanding the structure and function of these microbial communities has become increasingly important due to escalating human-induced pressures on aquatic ecosystems, such as pollution, habitat degradation, and climate change, in recent years. Traditional culture-based approaches frequently struggle to capture the full microbial diversity found in natural environments (Wang *et al.* 2021; Li *et al.* 2024; Yang *et al.* 2024). In contrast, metagenomic analysis offers a more accurate and comprehensive assessment. This approach enables researchers to study microbial composition, functional potential, and ecological relationships among species. Studies by Ghosh *et al.* (2022), Nam *et al.* (2023), Amin *et al.* (2024), Selvarajan *et al.* (2024), Wu *et al.* (2024), and Li *et al.* (2025) highlight the

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suitability of metagenomics for investigating estuarine systems like the Musi River, where high salinity, nutrient loads, and human-induced changes influence microbial communities.

The Musi River estuary is not only important for local fisheries and ecosystem services, but it also faces significant environmental challenges such as increased sedimentation, pollution from agricultural runoff, and urbanisation. Urban development exacerbates these issues by introducing pollutants from household waste and industrial discharges into estuarine waters. Urbanisation can destroy habitat, degrade water quality, and increase exposure to heavy metals and other pollutants, threatening the estuary's ecological integrity and ability to support marine life (Melki *et al.* 2018a; Agustriani *et al.* 2019; Melki *et al.* 2019; Tjahjono *et al.* 2022; Rahutami *et al.* 2022).

Metagenomic analyses in this domain aim to clarify the current state of microbial communities, identify dominant taxa such as *Pseudomonas, Vibrio*, and *Cyanobacteria*, and understand how environmental factors influence their distribution and function (Zhu *et al.* 2019; Birrer *et al.* 2021; Erazo & Bowman 2021). The provided explanations serve to inform and direct water management and conservation initiatives aimed at preserving the ecological integrity and vitality of this essential estuarine ecosystem. Metagenomics has profoundly influenced aquatic ecology through the analysis of community genomes, enabling the identification of microorganisms, clarification of their functions, and the discovery of novel proteins (Behera *et al.* 2020; Ahmad *et al.* 2021; Vijayan *et al.* 2023). Metagenomic techniques have undergone rapid development and have demonstrated considerable potential for establishing links between microbial community dynamics and biogeochemical processes (Grossart *et al.* 2020) and for identifying entire microbial communities from the environment (Rusiñol *et al.* 2020; Vijayan *et al.* 2023). The field of metagenomics has become very important for revealing the complex genetic abilities of microorganisms. It provides a deeper understanding of microbial diversity than is possible with traditional methods (Sharma *et al.* 2021; Hisham *et al.* 2022). Restoring polluted bodies of water and then studying the bacteria that are involved in this process are very important.

Microbial populations in any ecosystem support a wide range of ecological processes (Moopantakath *et al.* 2020; Vijayan *et al.* 2023). This is an important role that microbial populations play in every ecosystem. Bacteria, the earliest forms of life on Earth, are ubiquitous and essential components of all aquatic systems. They occupy a wide range of ecological niches (Vijayan *et al.* 2023). Bacteria are ubiquitous and necessary components of all aquatic ecosystems. Research on bacterial communities in estuaries can elucidate how these microorganisms respond to environmental pollution (Hongxia *et al.* 2021) and implications for human health (Brumfield *et al.* 2020). Microbial communities are widely acknowledged as essential indicators of river system health (Brumfield *et al.* 2020; Rajeev *et al.* 2021). Microbial analysis is essential in aquatic ecology, as it is crucial for evaluating the contributions of microorganisms to food web dynamics and biogeochemical processes (Brumfield *et al.* 2020; Grossart *et al.* 2020; Ahmad *et al.* 2021; Wang *et al.* 2021; Vijayan *et al.* 2023).

This study lays the foundation for a detailed exploration of the microbial diversity in the Musi River estuary through metagenomic analysis. It's a thrilling opportunity to highlight the importance of this community in relation to environmental conditions and human influences. It also emphasizes the need for further research on estuarine microbiomes (Nandan & Sajeevan 2020). These findings are set to make a significant impact on our understanding of ecological studies, paving the way for the development of effective conservation policies that will safeguard the remarkable biodiversity of this delicate ecosystem.

2. Materials and Methods

2.1. Research Location and Sampling

Water samples were collected from the surface water (Zhou *et al.* 2022) in the Musi River Estuary, South Sumatra Province, at a total of three research stations, as illustrated in Figure 1. The description of these research stations is shown in Table 1. Water samples were obtained using 1,000 ml sterile glass bottles, which were submerged in the water against the current at a depth of 50 cm (Liu *et al.* 2020). The samples were meticulously placed in a cool box for transport to the National Research and Innovation Agency laboratory in Cibinong, Bogor, West Java We took measurements of water quality, including salinity, pH, dissolved oxygen, and temperature, at research stations using a hand refractometer (ATAGO Co. Ltd, Tokyo, Japan), a pH meter (SM101, Milwaukee Instruments, Romania), and a dissolved oxygen meter (HI 98193, Hanna Instruments Inc, USA).

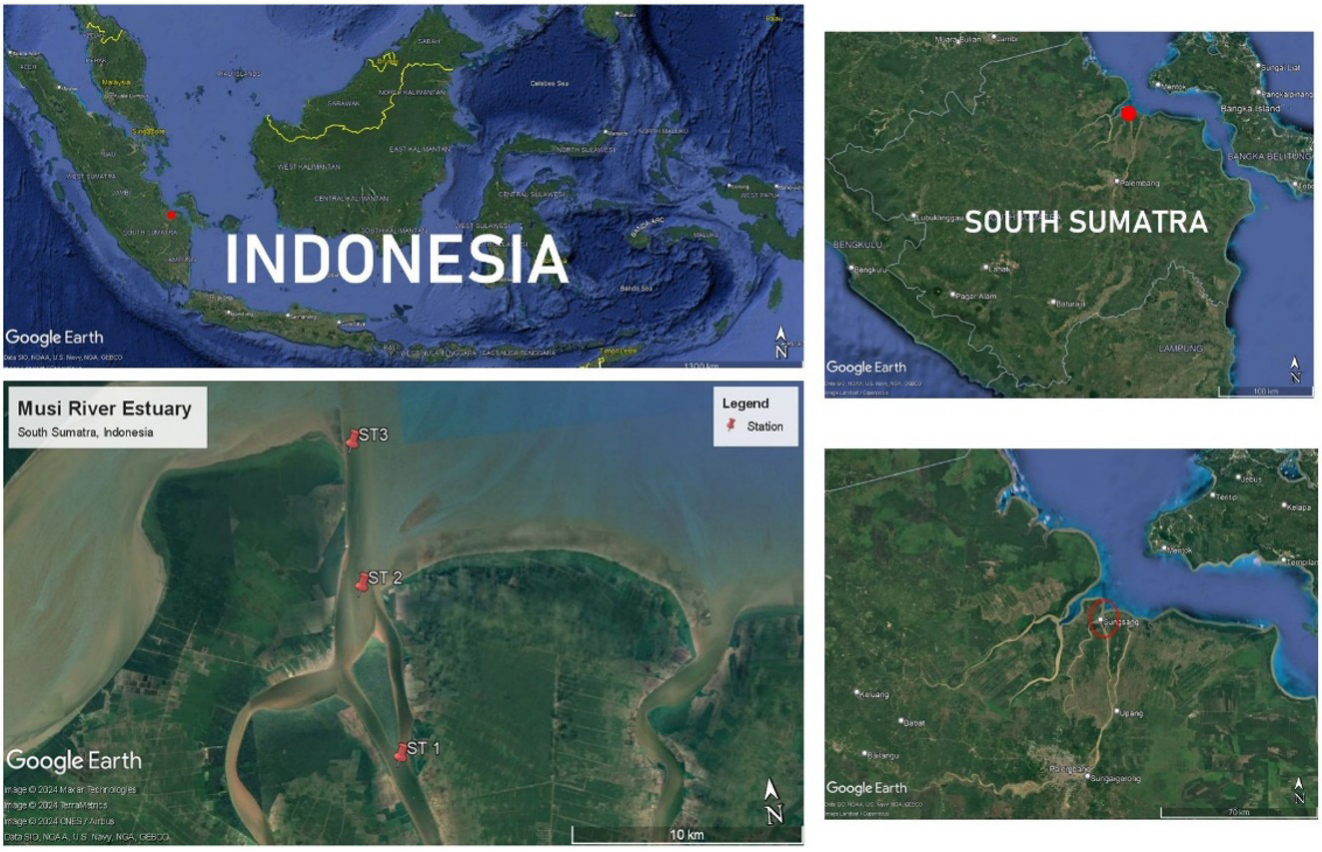

Figure 1. Sketch of the study area in the Musi River estuary

Table 1. Description of research stations in the Musi River estuary

Research stations	Position		Description
	Longitudes (E)	Latitudes (S)	
ST 1	104° 92' 87.2"	2° 40' 87.9"	Upstream from the Musi River, there is a mangrove ecosystem and a fishing boat route
ST 2	104° 91' 85"	2° 34' 2"	This station is the mouth of the Musi estuary, influenced by the activities of the Sungsang village community and the boat route
ST 3	104° 92' 6.3"	2° 28' 76.4"	Stations directly affected by the sea and fishing areas

2.2. Metagenomic Identification of Bacteria

The preliminary stage in the identification process of metagenomic bacteria is the crucial step of DNA isolation, which continues until the samples are shipped. To ensure the accuracy of the results, it's essential to follow a strict set of protocols during the DNA isolation process (Hong *et al.* 2020). This study employed an incredible DNA isolation method that adhered to the protocol outlined in the ZymoBIOMICSTM DNA miniprep Kit. The process of extracting bacterial genomic DNA from water samples is truly remarkable. It involves the filtration of 1,000 ml of water using 0.22 µm filter paper. This is a key step in the process of separating bacteria from large particles, sediment, and debris. These impurities can interfere with the analysis process, but the filtration method ensures a clean and accurate extraction of DNA (Melki *et al.* 2018b). We were thrilled to conduct a qualitative assessment using a nanodrop spectrophotometer. This was done to ensure the purity of PCR products (Huang *et al.* 2022).

The 16S rRNA gene amplification process is a complex biochemical procedure that involves the combination of various reagents into a reaction mixture. The precise composition of this mixture includes 5 µL of 2x my Taqred, 0.5 µL of each primer, 1 µL of DNA sample, and 3 µL of NFW. The amplification process employed 16S rRNA primers with a target position of 1,500 base pairs (bp). This approach aligns with the

findings of previous studies utilizing the 16S rRNA gene, as evidenced by the research conducted by Acharya *et al.* (2020), Narayan *et al.* (2020), Moopantakath *et al.* (2020), Betiku *et al.* (2021), Cabello-Yeves *et al.* (2021), Mamindlapelli *et al.* (2021), Hisham *et al.* (2022), and Ye *et al.* (2022). Electrophoresis was performed on a 1.5% agarose gel, and DNA visualization was subsequently conducted under UV light using a UV transilluminator. The 16S rRNA gene amplicon sequencing was performed using the Illumina Novaseq System, and a commercial sequencing company performed the subsequent bacterial identification.

2.3. Data Analysis

The Divisive Amplicon Denoising Algorithm 2 (DADA2) pipeline was used to evaluate the demultiplexed raw data generated by the Illumina Novaseq Sequencing platform (Callahan *et al.* 2016). The adapter and PCR primer sequences present in the paired-end reads were efficiently removed using the Cutadapt tool. DADA2 was then used to identify chimeric faults, remove low-quality sequences, and resolve sequencing errors. The amplicon data was then taxonomically classified using the Silva database (silva_nr99_v138.1). For the subsequent analysis and visualization, such as alpha and beta diversity analysis, R Studio packages were utilized in conjunction with Krona Tools and PICRUSt2. These R Studio packages include dada2, ggplot2, ggpicrust2, MicEco, microbiomeMarker, microbiome utils, microbiota process, phyloseq, and vegan.

3. Results

3.1. Water Quality

The water quality of the samples from all the research stations in the Musi River estuary is shown in Table 2. ST1, located upstream near mangrove ecosystems and fishing boat routes, had the highest temperature (32.9°C), lowest DO (6.4 mg/L), neutral pH (7.18), and freshwater conditions (0 ppt salinity), likely influenced by reduced tidal mixing and anthropogenic activities. ST2 at the mouth of the estuary near Sungsang village had cooler water (29.8°C), higher DO (7.2 mg/L), acidic pH (6.40), and brackish conditions (16 ppt salinity), reflecting mixing processes and community activities. ST3, directly exposed to marine influences in fishing areas, had intermediate temperature (30.6°C), DO (6.9 mg/L), more acidic pH (6.62), and the highest salinity (20 ppt), clearly showing the seawater intrusion gradient. These results highlight how water quality correlates with geographic position along the estuary, with increasing marine influence downstream leading to higher salinity and acidity, while upstream areas exhibit warmer, oxygen-depleted freshwater characteristics, all modified by local ecological and anthropogenic factors.

Table 2. Water quality of research stations in the Musi River estuary

Research stations	Temperature (°C)	DO (mg/L)	pH	Salinity (ppt)
ST 1	32.9	6.4	7.18	0
ST 2	29.8	7.2	6.40	16
ST 3	30.6	6.9	6.62	20

3.2. Profile of Bacterial Diversity

The relative abundance of the top 10 bacterial phyla in Musi River estuary water is shown in Figure 2. The microbial communities of the Musi River estuary stations showed a clear spatial partitioning driven by salinity gradients and anthropogenic influences. Proteobacteria dominated all stations, but showed the highest abundance in marine influenced ST3, demonstrating their adaptability to saline conditions. The freshwater-dominated ST1 was characterized by Cyanobacteria and Bacteroidota, indicating photosynthetic activity and organic matter degradation, while the brackish transition zone (ST2) was dominated by sulfate-reducing Desulfobacterota and Campilobacterota. Notably, the marine station (ST3) uniquely harbored Crenarchaeota, likely involved in nitrification processes. The presence of Firmicutes in ST1 and ST2 suggests potential fecal contamination from boat traffic and village activities. These microbial patterns strongly correlate with the observed physicochemical gradients, particularly the increasing salinity (0-20 ppt) and decreasing pH (7.18-6.62) from upstream to marine zones, highlighting how environmental conditions shape estuarine microbial ecology.

The relative abundance of the top 10 bacterial classes in the Musi River estuary is shown in Figure 3. This figure shows a clear salinity-driven zonation of bacterial classes across the Musi River estuary, with Gammaproteobacteria dominating all stations, but showing peak abundance in the marine influenced ST3 (20 ppt salinity), highlighting their ecological versatility in saline conditions. The freshwater ST1 (0 ppt) was characterized by Cyanobacteria and Bacteroidia, indicating photosynthetic activity and organic matter degradation in low salinity

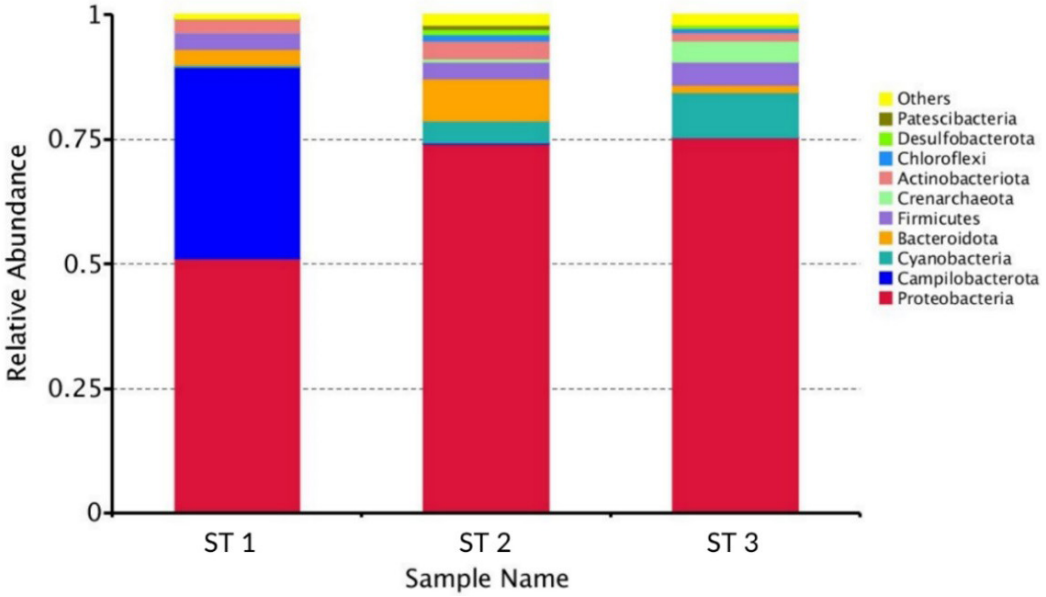

Figure 2. Relative abundance of the top 10 bacterial phylums in the Musi River estuary water

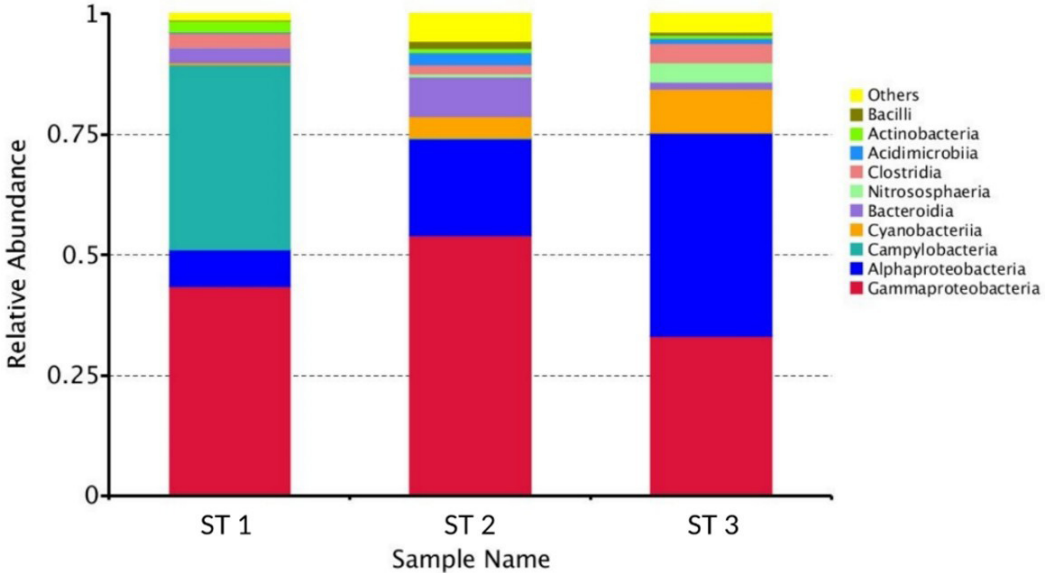

Figure 3. Relative abundance of the top 10 bacterial classes in the Musi River estuary water

environments, while the brackish ST2 (16 ppt) showed unique signatures of Campylobacteria (sulfur oxidizers) and Clostridia, the latter possibly reflecting anthropogenic influence from nearby village activities. Notably, the marine ST3 harbored Nitrososphaeria, suggesting active nitrification processes in high salinity waters. These class-level patterns provide a finer taxonomic resolution to phylum-level trends (Figure 2), revealing how the estuarine salinity gradient (0-20 ppt) and human activities collectively shape microbial community structure and function, with distinct functional guilds emerging in freshwater (ST1), transitional (ST2), and marine (ST3) zones, including photosynthetic primary producers, sulfur cyclers, and nitrifying archaea, respectively.

The relative abundance of the top 10 bacterial orders in the Musi River estuary is shown in Figure 4. This figure shows distinct order-level microbial community patterns across the salinity gradient of the Musi River estuary, with clear zonation of functional groups. Campylobacterales dominated the brackish ST2 (16 ppt), likely driving sulfur cycling

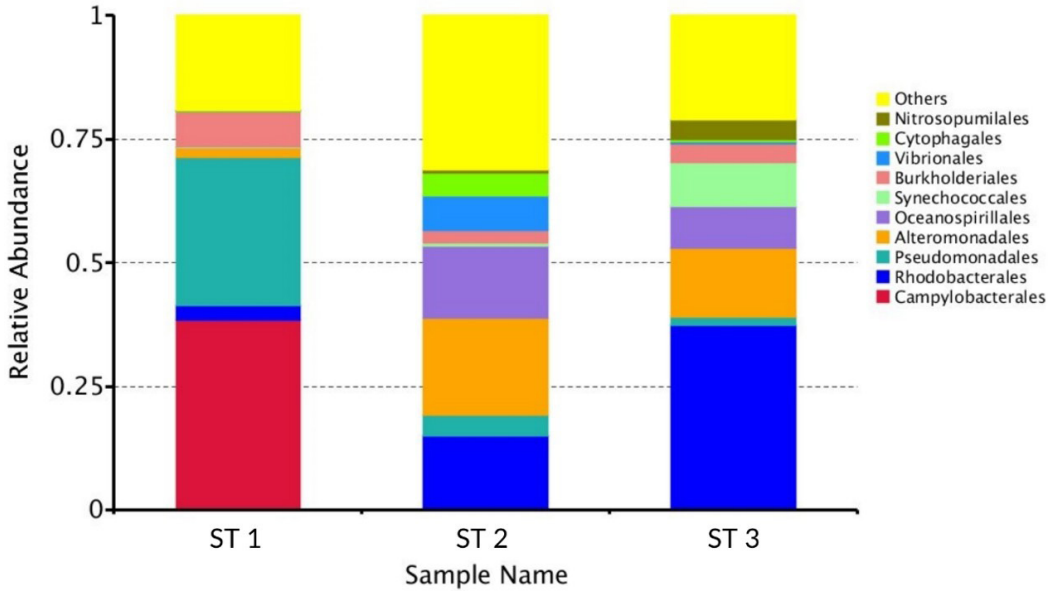

Figure 4. Relative abundance of the top 10 bacterial orders in the Musi River estuary water

in this transitional zone, while Pseudomonadales and Alteromonadales were most abundant in the marine ST3 (20 ppt), reflecting their known halotolerance and role in organic matter degradation. The freshwater ST1 (0 ppt) was characterized by photosynthetic Synechococcales and organic degrading Cytophagales, consistent with its low salinity conditions. Notably, the presence of Nitrosopumilales (ammonia-oxidizing archaea) exclusively in ST3 suggests active nitrification in marine waters, while Vibrionales (including potential pathogens) in ST2 may indicate anthropogenic influence from nearby village activities. These order-level distributions provide higher taxonomic resolution than previous analyses and demonstrate how the physicochemical gradient of the estuary (0-20 ppt salinity) selects for specific functional groups from freshwater-adapted primary producers (ST1) to marine specialists (ST3), with ST2 representing a unique ecotone hosting both sulfur-cycling Campylobacterales and potential fecal indicators (Vibrionales).

The relative abundance of the top 10 bacterial families in the Musi River estuary is shown in Figure 5. This figure shows distinct family-level microbial distribution patterns across the salinity gradient of the Musi River estuary, revealing specialized functional adaptations to each zone. The marine-influenced ST3 (20 ppt salinity) was dominated by the halotolerant families Alteromonadaceae and Pseudomonadaceae, known for their role in organic matter degradation under saline conditions, while Rhodobacteraceae peaked at this station, suggesting active phototrophic sulfur oxidation. Brackish ST2 (16 ppt) showed high abundance of Arcobacteriaceae (sulfur/sulfide metabolizers) and Vibrionaceae (potential pathogens), the latter reinforcing earlier evidence of anthropogenic influence from Sungsang village. Freshwater ST1 (0 ppt) was characterized by Cyanobiaceae (photosynthetic cyanobacteria) and Cyclobacteriaceae (organic degraders), consistent with its low-salinity, high-light conditions. In particular, the exclusive presence of Saccharospirillaceae in ST3 and Pseudohongleilaceae in ST2 highlights niche-specific adaptations at the family level. These patterns provide the finest taxonomic evidence to date of how the estuarine physicochemical gradient (0-20 ppt salinity) structures microbial communities, from freshwater primary producers (Cyanobiaceae) to specialized marine heterotrophs (Alteromonadaceae), with transitional zones harboring both sulfur cycling (Arcobacteriaceae) and potential fecal indicator taxa (Vibrionaceae).

The relative abundance of the top 10 bacterial genera in the Musi River estuary is shown in Figure 6. This figure shows genus-level microbial specialization across the salinity gradient of the Musi River estuary, with different functional groups dominating each zone. Freshwater ST1 (0 ppt salinity) was characterized by *Cyanobium*_PCC-6307 (photosynthetic Cyanobacteria) and *Algoriphagus* (organic degraders), reflecting its autotrophic and terrestrial input characteristics. In the

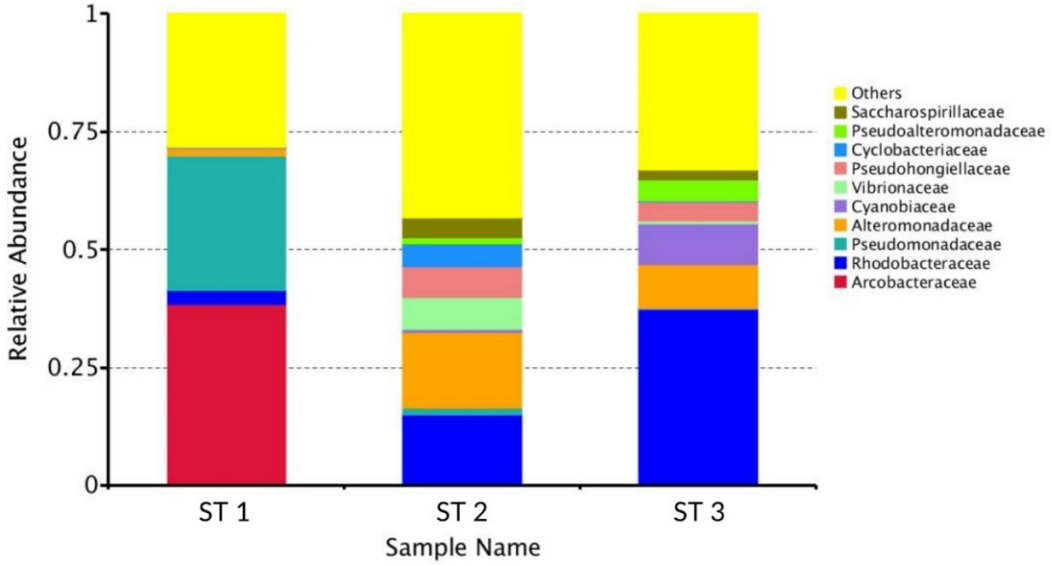

Figure 5. Relative abundance of the top 10 bacterial families in the Musi River estuary water

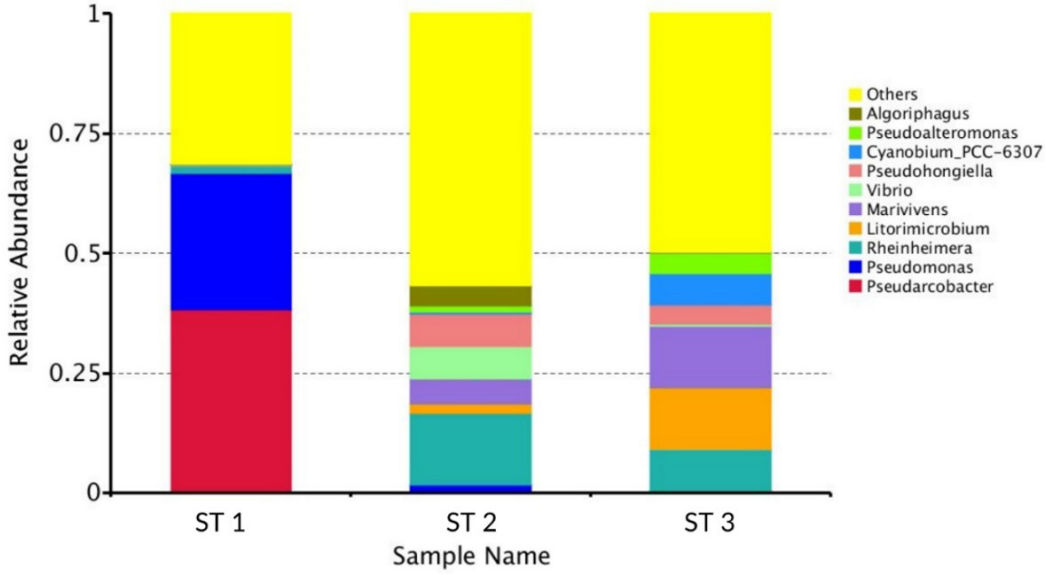

Figure 6. Relative abundance of the top 10 bacterial genera in the Musi River estuary water

brackish ST2 (16 ppt), *Vibrio* (potential pathogens) and *Pseudarcobacter* (sulfur cyclers) dominated, confirming previous evidence of anthropogenic influence and sulfur cycling in this transitional zone. The marine ST3 (20 ppt) showed a predominance of *Pseudoaiteromonas* and *Pseudomonas* (halotolerant organic degraders) along with *Marivivens* (adapted to high salinity conditions), highlighting specialized marine adaptations. In particular, the exclusive presence of *Rheinheimera* (hydrocarbon degraders) in ST3 suggests potential petroleum hydrocarbon metabolism, possibly related to boat traffic. These genus-level patterns provide the highest taxonomic resolution and demonstrate how niche partitioning drives microbial community structure from freshwater (Cyanobium) to marine (*Pseudoaiteromonas*), with transitional zones maintaining both anthropogenic (*Vibrio*) and sulfur-cycling (*Pseudarcobacter*) specialists, completing the salinity-driven ecological continuum observed at higher taxonomic levels.

The ternary plot illustrating the relative composition of several dominant bacterial genera at three stations (ST1, ST2, and ST3) in the Musi River estuary is shown in Figure 7. At ST1, located upstream near a mangrove ecosystem with freshwater conditions, *Pseudomonas* is the dominant genus. In

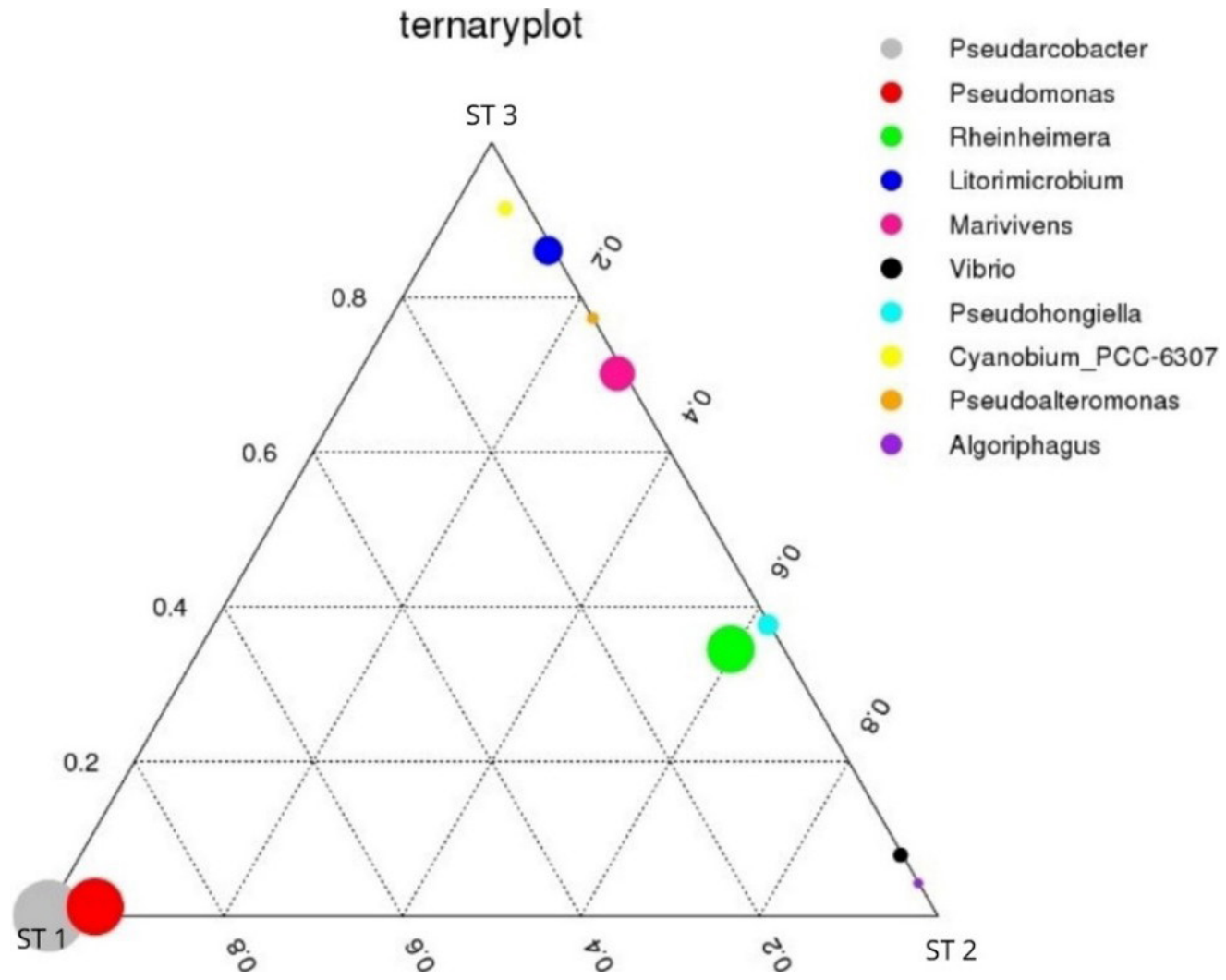

Figure 7. Ternary plot of abundant genera at three stations in the Musi River estuary

ST2, located at the mouth of the estuary with brackish water conditions (16 ppt salinity), there is a more balanced distribution of bacteria with a predominance of *Rheinheimera* and *Pseudomonas*. In ST3, directly exposed to the sea with the highest salinity (20 ppt), *Rheinheimera* and *Vibrio* become more dominant. This ternary plot shows how the bacterial composition changes with differences in water quality along the estuary, with *Pseudomonas* dominating in freshwater conditions, *Rheinheimera*, and *Vibrio* more dominant in brackish and seawater conditions.

Figure 8 shows a heatmap analysis of bacterial genus distributions across the three stations of the Musi River estuary, revealing a clear salinity-driven zonation of microbial communities at genus level resolution. The heatmap clusters show distinct genus assemblages corresponding to the environmental conditions of each station: (1) Freshwater ST1 (0 ppt) is characterized by *Cyanobium*_PCC-6307 (Cyanobacteria), *Flavobacterium*, and *Polynucleobacter*, reflecting photosynthetic and organic matter degradation processes under low salinity conditions; (2) Brackish ST2 (16 ppt) shows high abundance of potentially pathogenic genera (*Vibrio*, *Escherichia-Shigella, Aeromonas*) in addition to sulfur-cycling *Pseudarcobacter*, strongly suggesting anthropogenic influence from nearby village activities; (3) Marine ST3 (20 ppt) is dominated by halophilic genera (*Pseudoalteromonas, Marivivens, Psychrobacter*) and hydrocarbon-degrading *Rheinheimera*, suggesting specialized adaptations to high salinity and potential exposure to petroleum hydrocarbons. Notably, the archaeal genus *Candidatus_Nitrosopumilus* appears exclusively

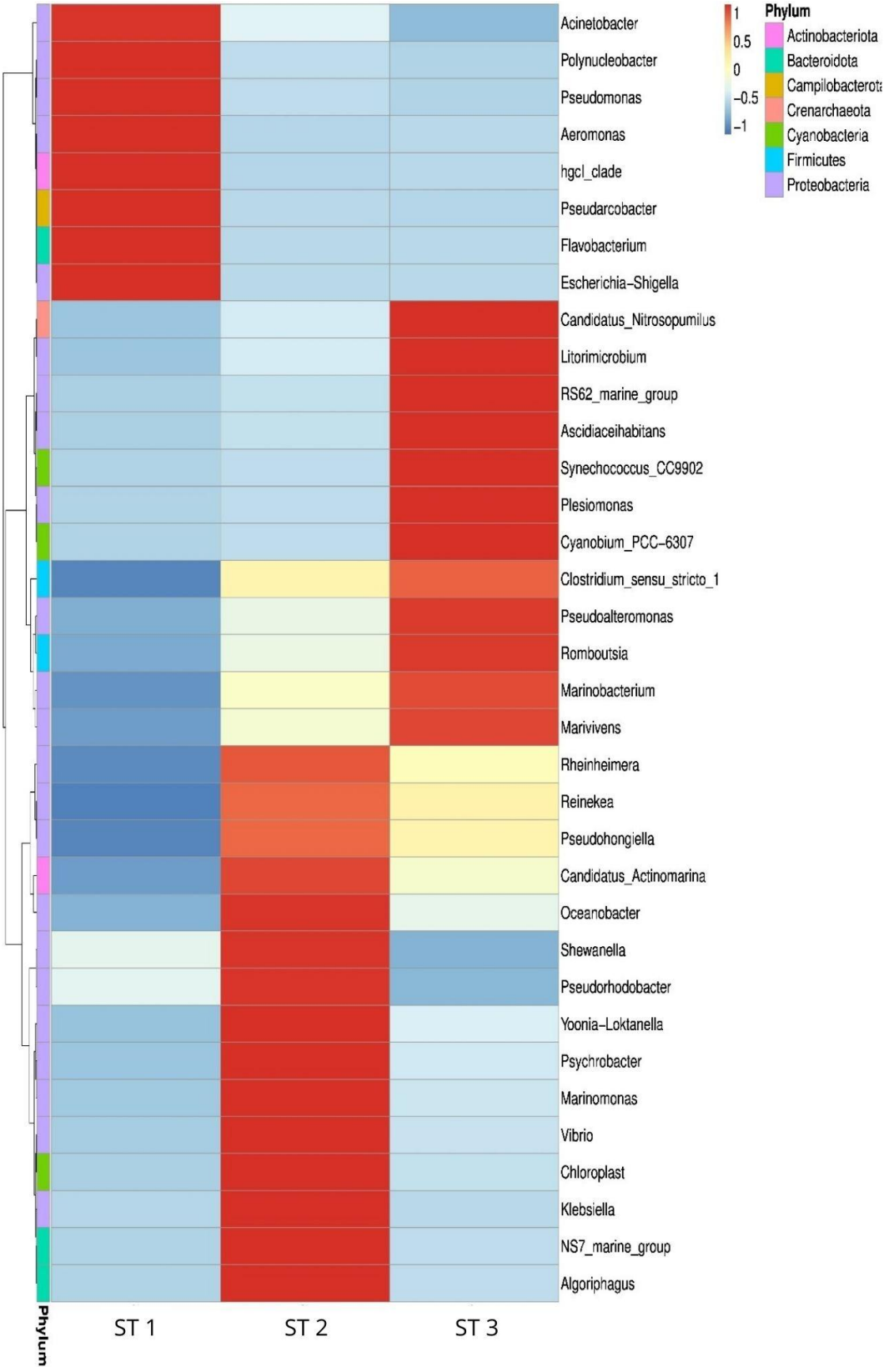

Figure 8. Heat map of the relative abundance of bacterial genera identified in metagenomes from three stations in the Musi River estuary

in ST3, reinforcing the nitrification potential in marine waters observed in previous analyses. The color coding of the phylum level of the heatmap shows that the Proteobacteria genera dominate in all stations, but show ecological specialization (e.g, gamma-Proteobacteria in marine ST3 vs. beta-Proteobacteria in freshwater ST1), while Bacteroidota and Cyanobacteria are preferentially abundant in ST1. This visualization powerfully synthesizes the microbial biogeography of the estuary, demonstrating how the salinity gradient (0-20 ppt) and anthropogenic factors collectively structure genus-level community composition from freshwater to marine zones.

3.3. Phylogenetic

This Krona visualization illustrates the distribution and relative abundance of 100 bacterial species identified in the waters of the Musi River estuary (Figure 9). The visualization is divided into concentric rings, each representing different bacterial groups, with color-

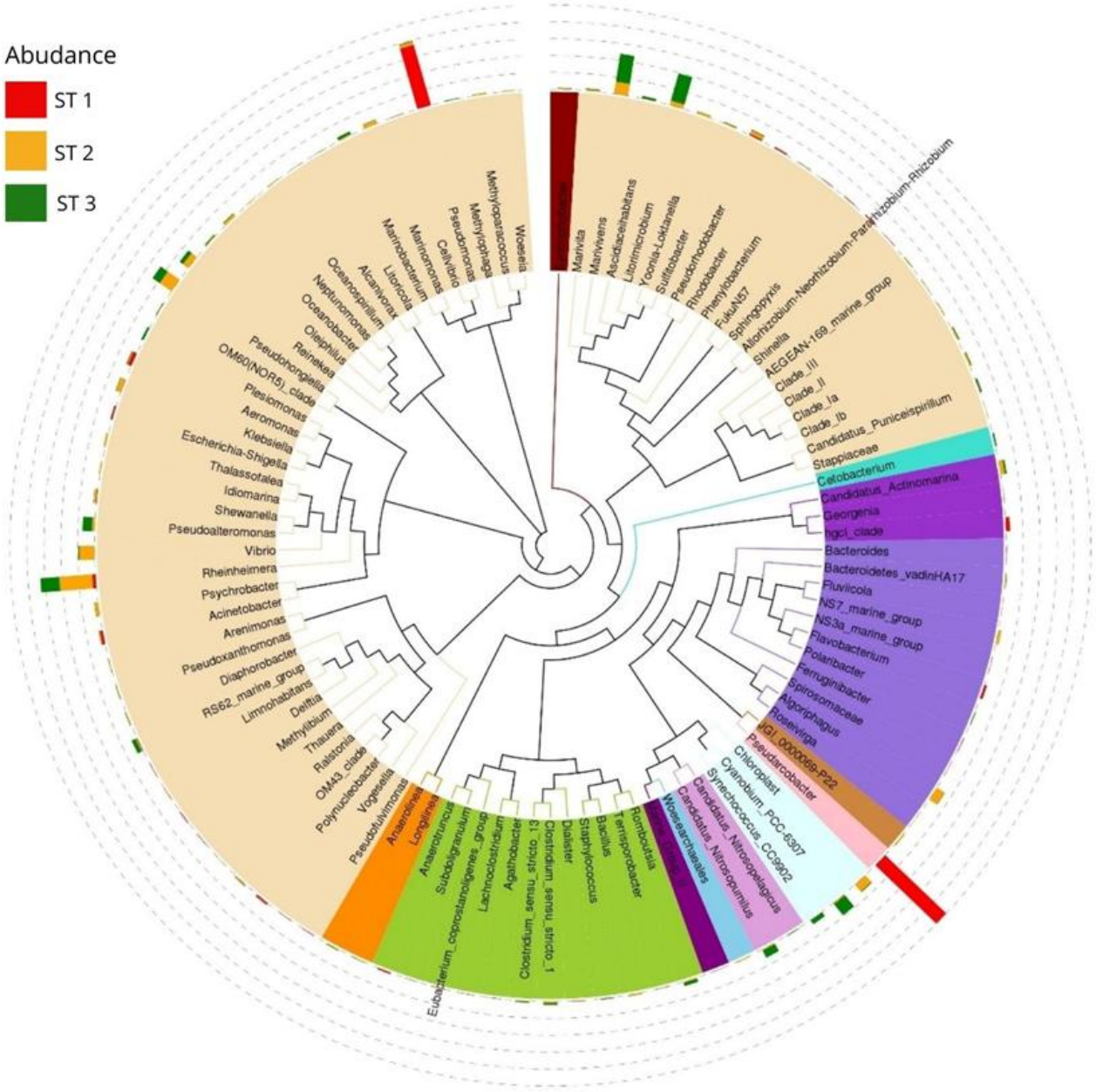

Figure 9. Krona visualization of 100 bacteria types from Musi River estuary water

coded bars indicating the abundance of these groups at three different research stations: ST1 (upstream, near mangrove ecosystems) are likely to favor bacterial groups adapted to low-oxygen environments, such as *Vibrio* and *Pseudomonas* species, which are more prominent in the upstream areas; ST2 (near the mouth of the estuary), the bacterial community becomes more diverse, showing a mix of freshwater and marine-adapted species. In contrast, ST3, influenced by marine conditions, shows a higher abundance of marine-tolerant bacteria, reflecting seawater intrusion into the estuary. In both ST2 and ST3, the dominant bacteria found were *Vibrio, Pseudomonas,* and *Rheinheimera* species. This Krona visualization helps to illustrate how bacterial communities along the estuary are structured by the different water quality conditions, with upstream areas supporting freshwater bacteria and downstream areas showing a shift towards marine bacteria, influenced by local ecological factors and anthropogenic activities.

The taxonomic tree of bacteria in the waters of the Musi River estuary (Figure 10) visually represents the

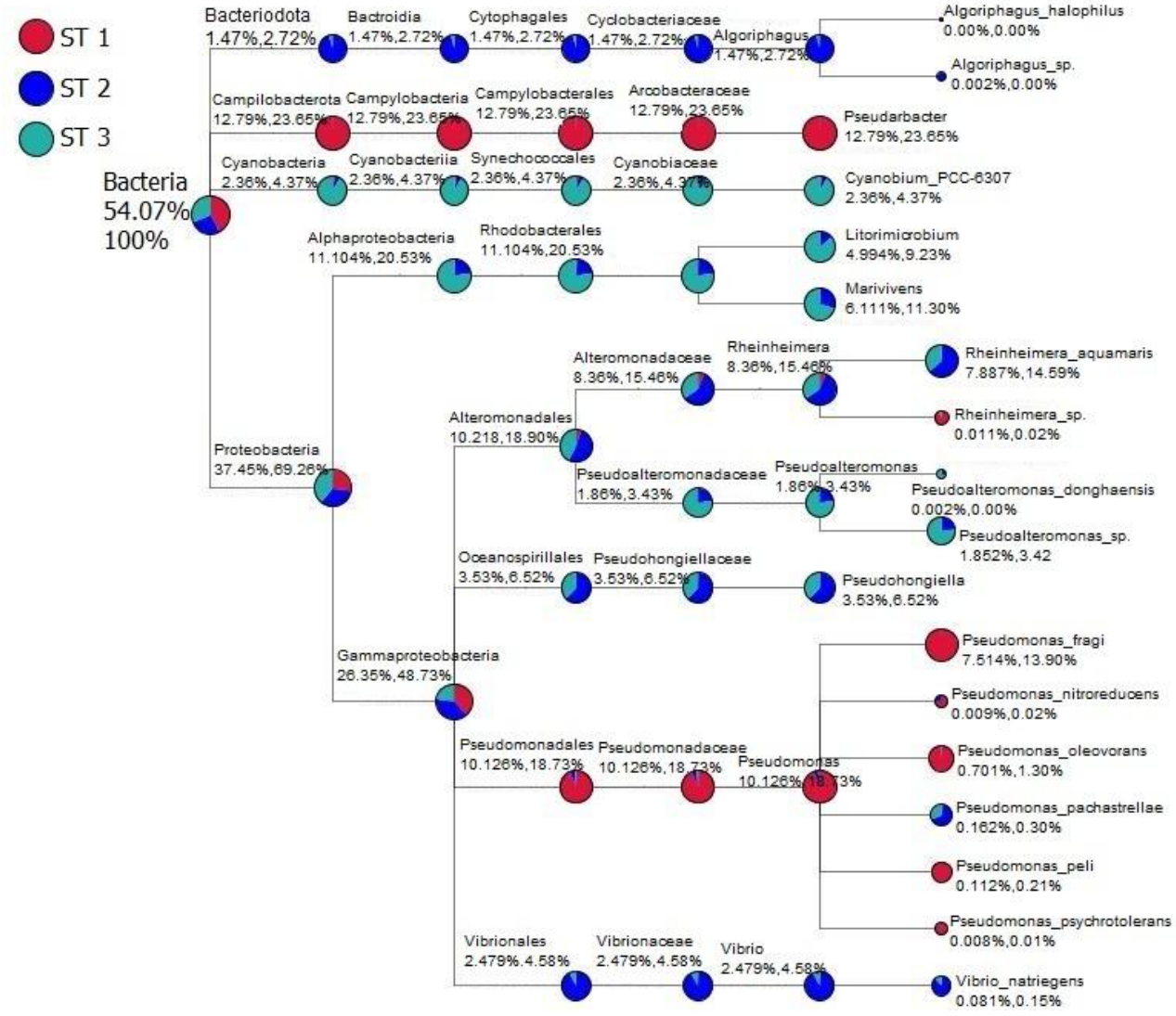

Figure 10. Taxonomic tree of bacteria in the Musi River estuary waters

hierarchical classification and relative abundance of bacterial taxa across three sampling stations (ST1, ST2, and ST3). This tree shows the relative abundance of several families of bacteria, including Proteobacteria, Gamma-Proteobacteria, *Pseudomonas*, Vibrionales, and others, color-coded to show their distribution across the three stations. ST1 shows a higher proportion of Proteobacteria (37.45%) and lower diversity in other groups, reflecting the warmer, oxygen-depleted freshwater conditions found here. This station is influenced by reduced tidal mixing and anthropogenic activity, which is more likely to support bacterial species tolerant to low oxygen, such as *Pseudomonas* species. ST2, here the taxonomic distribution shifts, with the appearance of *Pseudomonas* (10.12%) and other groups. The conditions of brackish water, with higher salinity and dissolved oxygen (DO), support a more diverse bacterial community than in ST1. This combination of freshwater and saltwater influences is a perfect environment for bacterial species that can thrive in fluctuating salinity levels, as seen in the genus *Pseudomonas*. Furthermore, in ST3, the highest salinity and more acidic pH are reflected in a shift towards marine adapted bacteria, as seen in *Pseudomonas* (7.14%), which thrive in higher salinity conditions. The presence of these bacteria in higher abundance in ST3 is a remarkable discovery, indicating a strong marine influence favoring species that are more tolerant to salt and adapted to seawater.

When contextualized with water quality data, the bacterial community structure exhibits a gradient from freshwater conditions upstream (ST1) to brackish (ST2) and marine influence (ST3). This shift in bacterial diversity underscores the direct impact of water quality, encompassing temperature, DO, pH, and salinity, on bacterial communities along the estuary. The downstream region exhibits an increased prevalence of marine species, while the upstream region is characterized by a predominance of freshwater species.

4. Discussion

Metagenomic research of water samples from ST1, ST2, and ST3 at the Musi River estuary revealed their microbial makeup. From genus to kingdom, complete taxonomic classification (Ahmad *et al.* 2021). Sequencing analysis revealed the bacterial diversity of the three Musi River estuary water samples as well as the relative abundance of the top 10 bacterial taxa at each taxonomic level-phylum, class, order, family, and genus. Displaying bacterial taxa (Azli *et al.* 2022), the resulting bar chart shows the microbial taxon patterns in the water. Distance between data points shows the degree of variation; samples in the same group have the same colour (Sari *et al.* 2023). One of the most popular techniques in microbial ecology for estimating microbial diversity and abundance is 16S rRNA gene profiling in metagenomics (Brumfield *et al.* 2020; Hong *et al.* 2020; Toole *et al.* 2021). Finding correlations between final taxonomic data in terms of relative microbial abundance can be done through microbial community profiling (Han *et al.* 2020).

The Musi River estuary in South Sumatra has a diverse bacterial community that is influenced by environmental factors. A recent study revealed that Proteobacteria is the most dominant phylum across all sampling stations, with the highest abundance at ST3 and ST2. These findings are consistent with those of previous studies, which indicate that Proteobacteria thrive in estuarine environments due to their ability to adapt to variations in salinity and nutrient levels (Zhu *et al.* 2018; Nikhitha *et al.* 2021). The second most prevalent phylum was Campylobacterota, which exhibited notable concentrations in ST1, followed by a substantial decrease in ST2 and ST3. This indicates that environmental conditions at each station influence the distribution of bacteria (Ma *et al.* 2016; Parvathi *et al.* 2021). Cyanobacteria were most abundant in ST3, while Bacteroidota were more abundant in ST2, reflecting their association with nutrient-rich water (Xian *et al.* 2024). In addition, Firmicutes and Actinobacteriota showed stable abundance across all stations, indicating a consistent presence despite environmental fluctuations. Other phyla, such as Patescibacteria, Desulfobacterota, Chloroflexi, and Crenarchaeota, were found in lower numbers, highlighting the diverse but uneven distribution of bacterial communities (Jeffries *et al.* 2016; Aguirre *et al.* 2017; Hu *et al.* 2023).

The ternary plot visually represents the distribution of the ten dominant bacterial genera across three sampling stations in the Musi River estuary. *Pseudarcobacter* is primarily found at ST1 and is virtually absent at other stations, indicating that ST1 provides particularly favorable conditions for its growth (Shao *et al.* 2019; Nandan & Sajeevan 2020; Meiyerani *et al.* 2024). *Pseudomonas* has been detected in elevated concentrations within ST1. A decrease in its levels was noted in ST2 and ST3, presumably due to nutrient enrichment from urban runoff (Zhang *et al.* 2019; Wang *et al.* 2023). *Rheinheimera*

has been found to be more abundant in ST2 and ST3, suggesting that these stations fulfill its growth conditions. Organic matter has been identified as the definitive cause of this phenomenon (Aiman *et al.* 2020; Yamamoto *et al.* 2020). The presence of *Litorimicrobium* and *Marivivens* in ST3 serves as conclusive evidence of a site-specific microbial community structure. *Vibrio* is more abundant in ST2, which is associated with the influx of organic matter due to human activities (Hereira-Pacheco *et al.* 2021; Zheng *et al.* 2022). *Cyanobium*, a fascinating genus of Cyanobacteria, thrives in ST3, exemplifying its remarkable adaptation to the unique environmental conditions present at this locale (Furtado *et al.* 2019; Wang *et al.* 2020). This distribution pattern unequivocally demonstrates the substantial influence of environmental factors, such as nutrient availability, salinity, and human activities, on the microbial community in the estuary. Ternary plots are imperative for elucidating bacterial diversity and comprehending the ecological functions and health of estuarine ecosystems. As indicated in the works of Soetignya *et al.* (2021), Huliselan *et al.* (2023), and Pulido-Chávez *et al.* (2023).

The heatmap offers significant insight into the relative abundance of bacterial genera at three different water sampling stations. The data indicate distinct patterns of bacterial distribution shaped by local environmental factors. *Pseudomonas* has been recognized as a more dominant species in ST1, indicating that the environmental conditions in this context may promote its proliferation (Liang *et al.* 2023). This finding aligns with previous research that has demonstrated the proliferation of *Pseudomonas* in nutrient-rich, contaminated environments. In contrast, *Rheinheimera*, *Litorimicrobium*, and *Marivivens* demonstrate increased prevalence in ST2 and ST3, presumably attributable to variations in ecological niches or resource availability (Gardade & Khandeparker 2024). The significantly increased presence of *Vibrio* in ST2 is remarkable, as this genus is generally associated with estuarine environments and nutrient-rich conditions (Udoh *et al.* 2022). The clustering on the left side of the heatmap indicates that specific bacterial genera display similar abundance patterns, possibly reflecting phylogenetic relationships and ecological interactions. These patterns could facilitate the delineation of the functional roles of these genera within the ecosystem (Tian *et al.* 2023). The application of color-coded bars in the heatmap serves to underscore the variation among bacterial phyla across multiple sites. The functionality of ecosystem processes, including nutrient cycling and microbial stability, is contingent upon this variety. A growing body of research suggests that the presence of a greater variety of microorganisms can enhance the resilience of ecosystems to external changes in the environment. This is particularly important for estuarine ecosystems that are subjected to stressors resulting from human activity (Gao *et al.* 2021).

Krona's depiction of bacterial distribution in the Musi River estuary offers an insightful analysis of environmental forcing, microbial diversity, and ecosystem health. These visualizations facilitate the monitoring of microbial changes and the direction of conservation efforts, particularly in the context of ongoing changes in human activity and climate, which are increasingly impacting ecosystems. (Naveed *et al.* 2024; Xian *et al.* 2024). The circular diagram effectively represents bacterial phylogeny, with branching structures showing relationships among taxa. Color coding differentiates bacterial groups, while bar lengths indicate abundance across sampling stations (ST1, ST2, ST3), providing a clear view of spatial distribution patterns (Nikhitha *et al.* 2021; Yue *et al.* 2022). The dominance of certain bacterial genera at specific locations suggests that variables such as salinity, nutrient concentrations, and anthropogenic activities affect the structure of the microbial community. The dominance of Proteobacteria, Bacteroidota, and Cyanobacteria at various stations highlights their adaptability and significance in estuarine biogeochemical processes (Quero *et al.* 2020; Nikhitha *et al.* 2021; Bao *et al.* 2023). Proteobacteria facilitate the decomposition of organic matter, whereas Cyanobacteria enhance primary production via photosynthesis (Scanes *et al.* 2020; Wu *et al.* 2024).

The observed bacterial distributions also reflect environmental dynamics influenced by pollution and nutrient enrichment from agriculture and municipal waste. A mounting body of research has demonstrated that elevated levels of nitrogen and phosphorus can result in a shift in the composition of microbial communities. These communities have been observed to exhibit a tendency to favor taxa that are adapted to eutrophic conditions. These interactions give rise to complex microbial habitats in estuaries, where freshwater meets seawater (Baker *et al.* 2023; Yang *et al.* 2024). In addition to promoting diversity, visual aids have been shown to aid in the assessment of ecosystem health. The presence or absence of specific genera has been shown to be an indicator of water quality, with an abundance of opportunistic pathogens possibly indicating pollution risks (Zhou *et al.* 2020; Yi *et al.* 2021). Consequently, Krona visualization not only enhances comprehension of microbial communities but

also functions as a pivotal instrument for environmental monitoring and the formulation of conservation strategies for the Musi River estuary (Dolbeth *et al.* 2021; Nikhitha *et al.* 2021).

This study determined the microbial composition of water samples from the Musi River estuary and demonstrated how environmental factors influence bacterial diversity. The analysis revealed a preponderance of Proteobacteria across all stations, with the availability of nutrients, salinity levels, and human activities exerting a significant influence on the distribution of genera. The use of visualisations such as ternary plots, heatmaps, and krona charts helped to highlight these patterns. However, the small sample size may not adequately capture the microbial diversity present. Researchers should consider incorporating multi-omics approaches and seasonal sampling into future studies to improve our understanding of microbes' roles and impact on ecosystem health and water quality.

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