Journal of Advanced Zoology

ISSN: 0253-7214
*Volume **44** Issue **S-7 Year 2023** Page **359:367***

Analyzing The Metagenomics of Bacterial Diversity in The Backwater Mangrove Regions of Pichavaram and Parangipettai, Tamil Nadu, India, Reveals Insights into Sediment Microbial Communities

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Article History

Received: 06 June 2023
Revised: 05 Sept 2023
Accepted: 13 Dec 2023

Abstract

This metagenomic study delves into the microbial diversity and functional potential of sediment samples from the Pichavaram and Parangipettai mangrove ecosystems. The dataset, initially comprising millions of base pairs and sequences, undergoes meticulous quality control, revealing refined characteristics post-artificial duplicate read identification. Taxonomic analyses unveil a rich microbial landscape, dominated by bacteria, with intriguing presence of Eukaryota, Archaea, and unclassified sequences. The intricate community structures, highlighted through phylogenetic distributions, showcase the diversity and unexpected taxa, providing a nuanced understanding of microbial dynamics. Functional gene exploration uncovers key enzymes driving biosynthesis of secondary metabolites and metabolic pathways, shedding light on the microbial community's ecological roles and biotechnological potential. This comprehensive metagenomic analysis serves as a foundation for further ecological assessments, taxonomic refinements, and investigations into the adaptive strategies of these microbial communities in mangrove sediments.

Keywords: *Pichavarma and Parangipettai, Sediment soil, 16s rRNA metagenomics, bacterial community, Functional genes*

1. Introduction

Pichavaram and Parangipettai, located in the coastal state of Tamil Nadu, India, boast distinctive ecological landscapes characterized by their expansive backwater mangrove areas. These regions are renowned for their rich biodiversity and unique sediment conditions that play a crucial role in shaping the local ecosystem [1]. Pichavaram, situated near the historic town of Chidambaram, is renowned for its sprawling mangrove forests and interconnected waterways. The intricate network of canals, estuaries, and tidal channels in Pichavaram forms a complex ecosystem that supports a myriad of plant and animal species. The sediment in this area is influenced by the intricate balance of freshwater and tidal influences, creating a dynamic environment that harbors diverse microbial life. Parangipettai, on the other hand, is a coastal town with a significant presence of mangrove ecosystems along its shoreline. The sediment conditions in Parangipettai are shaped by the intricate interplay of marine and terrestrial influences [2, 3]. The mangrove vegetation not only provides a unique habitat for various species but also contributes to stabilizing the sediment, preventing erosion, and acting as a natural buffer against tidal forces.

Mangrove ecosystems are renowned for their rich biodiversity, and among the diverse array of organisms that thrive in these coastal habitats, bacteria play a crucial role [3]. The bacterial diversity within mangroves is particularly fascinating due to its profound impact on the production of secondary metabolites, compounds that are not directly involved in the growth or reproduction of the organism but often play a pivotal role in ecological interactions. Mangrove bacterial diversity is a complex tapestry of microorganisms adapted to the unique environmental conditions of these

ecosystems. Salinity fluctuations, tidal movements, and nutrient availability create a dynamic and challenging setting, leading to the evolution of bacteria with remarkable adaptive capabilities. This diversity is not merely a biological curiosity; it holds immense significance to produce secondary metabolites [4, 5].

One of the primary roles of mangrove bacteria is their involvement in nutrient cycling. As decomposers, they break down organic matter, recycling nutrients and contributing to the overall health of the ecosystem [6]. This activity, in turn, influences the availability of essential elements for the mangrove vegetation. Some bacteria are capable of nitrogen fixation, converting atmospheric nitrogen into a form usable by plants, promoting the growth of mangrove trees. Beyond nutrient cycling, mangrove bacteria are prolific producers of secondary metabolites [7, 8]. These compounds have garnered attention for their diverse biological activities, including antimicrobial, antiviral, antifungal, and anticancer properties. The ability of mangrove bacteria to thrive in challenging conditions has led to the evolution of unique metabolic pathways, resulting in the synthesis of novel secondary metabolites not found in other environments [9, 10].

Mangrove ecosystems, rich in microbial diversity, offer potential for discovering novel bioactive compounds and secondary metabolites. These bacteria adapt to the harsh conditions of these coastal ecosystems, resulting in unique secondary metabolites with diverse biological activities. Understanding these compounds holds promise for advancements in medicine, biotechnology, and environmental management. Metagenomics analysis of mangrove bacterial communities helps researchers understand the genetic potential of these microorganisms and their role in secondary metabolite production [11, 12]. This allows scientists to identify biosynthetic gene clusters responsible for secondary metabolite production, providing a roadmap for bioprospecting and discovering novel bioactive molecules. This information is crucial for understanding the biochemical processes underlying the production of these compounds. By deciphering the functional potential of mangrove bacterial communities, researchers can optimize conditions to enhance the yield of specific secondary metabolites or even engineer bacteria for improved production. Mangrove bacterial metagenomic analysis is a valuable tool for unlocking the genetic diversity and metabolic capabilities of microbial communities.

2. Materials And Methods

Mangrove sediment sample collection and DNA isolation for metagenomic analysis

Mangrove Sediment soil samples, designated as S1 and S2, were obtained from the ground sediment of the mangrove forests in Pichavaram and Parangipettai (Figure 1), situated at a latitude of 9.319078° and longitude of 79.330245°. Collection was carried out using a core sampler, targeting a depth of 0-10 cm. The surface sediment exhibited a composition of muddy sand, ash, and black elements. To maintain sample integrity, mangrove sediment samples were carefully collected in sterile plastic bags and promptly transported at 4°C to the Biokart lab in Bangalore for subsequent metagenomic analysis. Metagenomic DNA extraction from marine sediment samples was performed utilizing the Nucleospin Soil kit, a commercially available soil kit. The extraction process utilized specific primers (16s rRNA F: GCCTACGGGNGGCWGCAG and 16S rRNA R: ACTACHVGGGTATCTAATCC). Following extraction, the quality of the isolated metagenomic DNA samples was meticulously assessed using NanoDrop for accurate quantification. This systematic approach ensures the reliability and precision of the metagenomic analysis, offering valuable insights into the genetic composition of the mangrove sediment bacterial communities in the Pichavaram and Parangipettai regions [13-15].

Figure 1: Mangrove sediment sample collection site Pichavaram and Parangipettai

Bioinformatic Analysis

The examination of 16S-rRNA involved a single-read gene-level analysis to identify bacteria. This identification was conducted by referencing database files, allowing for detailed investigations into bacterial species and sub-species. To establish phylogeny, the sequence underwent analysis with the basic local alignment search tool (BLAST), aligning it with closely related sequences. Subsequently, multiple sequence alignment was performed. The workflow was specifically tailored for a BLAST baseline sequence, utilizing the national 16S bacterial database. Each sequence reading was categorized based on percent coverage and identity. Out of the initial raw sequences, 8,777 QC-qualified sequences were selected for taxonomic and functional genomic analysis. The 16S workflow serves as a valuable tool for discerning beneficial bacteria within a mixed sample or unveiling the microbial community's composition.

Metagenomic Analysis of Bacterial Community

The microbial metagenomic data's Fastq read files underwent processing on the Metagenomic Rapid Annotations using Subsystem Technology (MG-RAST) server, utilizing default parameters. Genes associated with diverse pathways, encompassing secondary metabolite biosynthesis, as well as amino acid, carbohydrate, and lipid metabolism, energy production, xenobiotic metabolism, and carbon, nitrogen, and sulfur metabolism, were extracted from the Kyoto Encyclopedia of Genes and Genomes (KEGG).

3. Results and Discussion

Isolated metagenomic DNA form the marine mangrove sediment bacteria community quality was checked by gel electrophoresis (Table 1 and Figure 2.

Table 1: Mangrove sediment samples and the names

SI.NO	Sample Name
1	Pichavaram (Bacterial 16s rRNA)
2	Parangipettai (Bacterial 16s r RNA

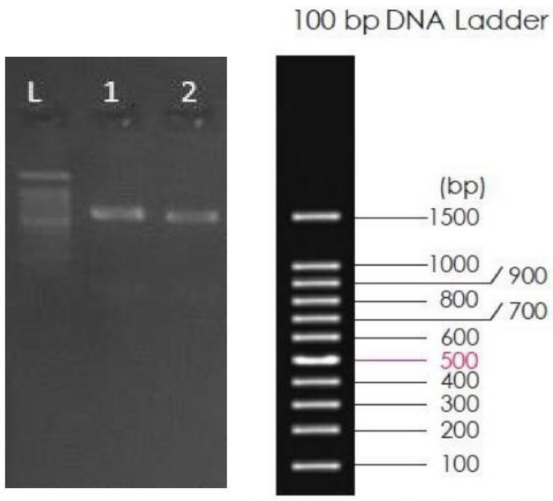

Figure 2: QC report of the 16s rRNA for metagenomic analsysis of mangrove sediment bacteria.

Taxonomic Analysis and Diversity

The metagenomic data analysis of Pichavaram mangrove sediment provides a comprehensive overview of the dataset's characteristics. The initial upload comprised 73,537,611 base pairs (bp) with a sequence count of 244,311, yielding a mean sequence length of 301 ± 0 bp and a mean GC content of 55 ± 3%. Post quality control (QC), artificial duplicate reads were identified at 98,765, resulting in a refined dataset of 43,316,492 bp and 144,653 sequences. The mean sequence length post QC was 299 ± 17 bp, maintaining a consistent GC content of 55 ± 3%. In terms of functional annotation, the analysis predicted 1,362 protein features and 42,537 rRNA features. Through alignment, 210 protein features and 39,167 rRNA features were identified. This detailed breakdown provides valuable insights into the quality and composition of the metagenomic data. The identification of artificial duplicate reads highlights the importance of rigorous QC processes in ensuring the accuracy of subsequent analyses. The abundance of predicted protein and rRNA features underscores the genomic

complexity of the mangrove sediment microbial community, offering a foundation for in-depth exploration of functional pathways and taxonomic composition (Table 2).

Table 2. Details of Pichavaram mangrove sediment metagenomic data analysis

Upload: sequence bp count	73,537, 611 bp
Sequence count (upload)	244,311
Mean sequence length (upload)	301 ± 0 bp
Mean GC (upload)	55 ± 3 %
Artificial duplicate reads: sequence count	98,765
bp count (post QC)	43,316,492 bp
Sequences count (post QC)	144,653
Mean sequence length (post QC)	299± 17 bp
Mean G and C (post QC)	55 ± 3 %
Predicted protein features	1,362
Predicted rRNA features	42537
Identified protein features (alignment)	210
Identified rRNA features (alignment)	39167

The metagenomic data analysis of Parangipettai mangrove sediment reveals key insights into the characteristics of the dataset. The initial upload included 54,846,113 base pairs (bp) with a sequence count of 182,213, showcasing a mean sequence length of 301 ± 0 bp and a mean GC content of 56 ± 3%. Post quality control (QC), 48,370 artificial duplicate reads were identified, resulting in a refined dataset of 40,072,626 bp and 133,373 sequences. The mean sequence length post QC was 300 ± 7 bp, maintaining a consistent GC content of 56 ± 3%. In terms of functional annotation, the analysis predicted 32,00 protein features and 54,249 rRNA features. Through alignment, 418 protein features and 46,336 rRNA features were identified. The abundance of predicted protein and rRNA features suggests a diverse genomic landscape within the Parangipettai mangrove sediment microbial community. The identification of artificial duplicate reads underscores the importance of robust QC procedures in ensuring the reliability of subsequent analyses. The detailed breakdown provides a foundation for exploring the functional potential and taxonomic composition of the microbial community in Parangipettai mangrove sediment (Table 3).

Table 3. Details of Parangipettai mangrove sediment metagenomic data analysis

Upload: sequence bp count	54,846, 113 bp
Sequence count (upload)	182213
Mean sequence length (upload)	301 ± 0 bp
Mean GC (upload)	56 ± 3 %
Artificial duplicate reads: sequence count	48,370
bp count (post QC)	40,072,626 bp
Sequences count (post QC)	133,373
Mean sequence length (post QC)	300± 7 bp
Mean G and C (post QC)	56 ± 3 %
Predicted protein features	32,00
Predicted rRNA features	54,249
Identified protein features (alignment)	418

Identified rRNA features (alignment)	46,336

Microbial community structure pichavaram and Parangipettai mangrove sediment

Distribution of taxa present in the pichavaram sediment sample.

In the Pichavaram sediment sample, the microbial community exhibited a diverse taxonomic distribution, with bacteria dominating at 97.13%, highlighting their pivotal role in this ecological niche. Eukaryota accounted for 2.19%, showcasing the presence of complex organisms, potentially contributing to the ecosystem's overall biodiversity. The presence of unclassified sequences at 0.37% indicates the potential discovery of novel taxa or underscoring the limitations in our current understanding of microbial diversity. Archaea, comprising 0.30%, play a crucial role in sediment biogeochemistry. Notably, the absence of viruses (0.00%) in the dataset raises intriguing questions about viral dynamics or the potential influence of sample processing methods. This comprehensive taxonomic overview underscores the complex interplay of microorganisms in Pichavaram sediment, providing valuable insights for ecological assessments and future research endeavors (Figure 3).

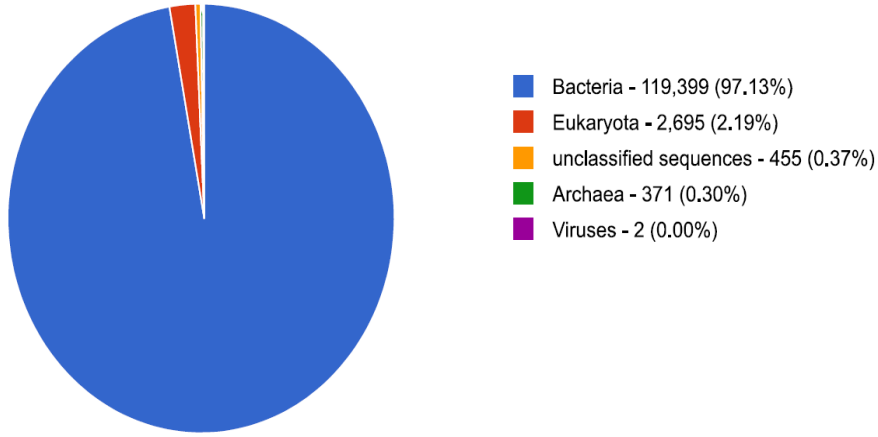

Figure 3: Distribution of taxa present in the pichavaram sediment sample.

Distribution of taxa present in the Parangipettai sediment sample

In the Parangipettai sediment sample, a dynamic taxonomic distribution was observed, with bacteria dominating at 97.67%, emphasizing their pivotal role as primary decomposers and contributors to sedimentary processes. The presence of Eukaryote at 1.09% suggests the coexistence of higher organisms in this ecosystem, potentially influencing nutrient cycling and community dynamics. The 1.16% unclassified sequences hint at undiscovered or less characterized taxa, underscoring the need for further exploration and taxonomic refinement. Archaea, comprising 0.80%, contribute to sediment biogeochemistry, participating in key metabolic processes. This diverse microbial community in Parangipettai sediment highlights the intricate relationships within the ecosystem and provides a foundation for ecological studies aimed at understanding the role of microorganisms in sedimentary environments (Figure 4).

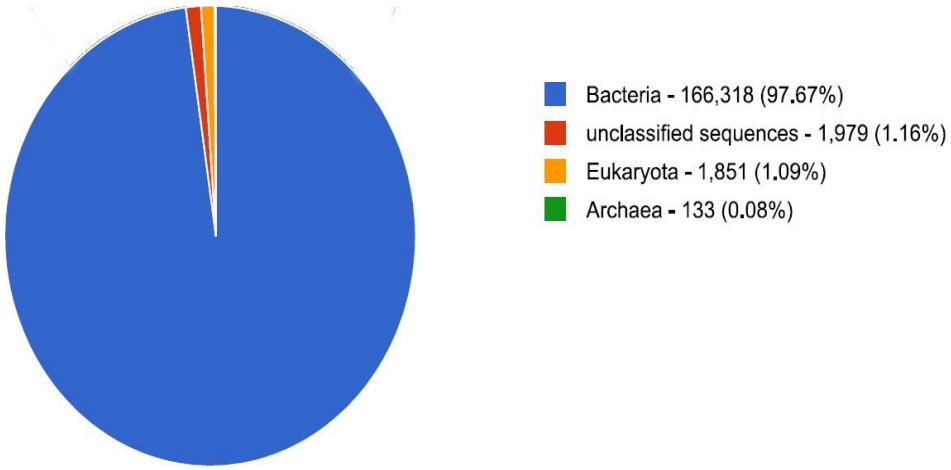

Figure 4: Distribution of taxa present in the Parangipettai sediment sample

Microbial community structure of sediment of the pichavaram sediment sample obtained from MG-RAST-phylogenetic distribution of metagenomic sequence.

In the microbial landscape of the analyzed sample, unclassified bacteria, derived from various sources, constitute a substantial proportion at 20.81%, suggesting the presence of diverse and potentially novel bacterial taxa. Notably, Planctomycetacia follows closely at 13.52%, showcasing their ecological significance. The diversity extends further with the presence of Gammaproteobacteria (11.36%), Clostridia (9.72%), Bacilli (8.97%), and Deltaproteobacteria (6.84%), each contributing to the intricate microbial community structure. Actinobacteria (5.95%) adds another layer of diversity, known for their versatile metabolic capabilities. Interestingly, the unexpected presence of Insecta (2.28%) highlights the potential influence of insect-associated microorganisms in this environment. Dehalococcoidetes (1.99%), Fusobacteria (1.96%), Alphaproteobacteria (1.75%), Nitrospira (1.58%), Chloroflexi (1.33%), and Flavobacteria (1.27%) further contribute to the richness of the microbial community (Figure 5). This comprehensive taxonomic profile underscores the complexity of the sampled ecosystem, providing a foundation for future studies elucidating the ecological roles of these diverse microbial groups and their potential implications for the surrounding environment.

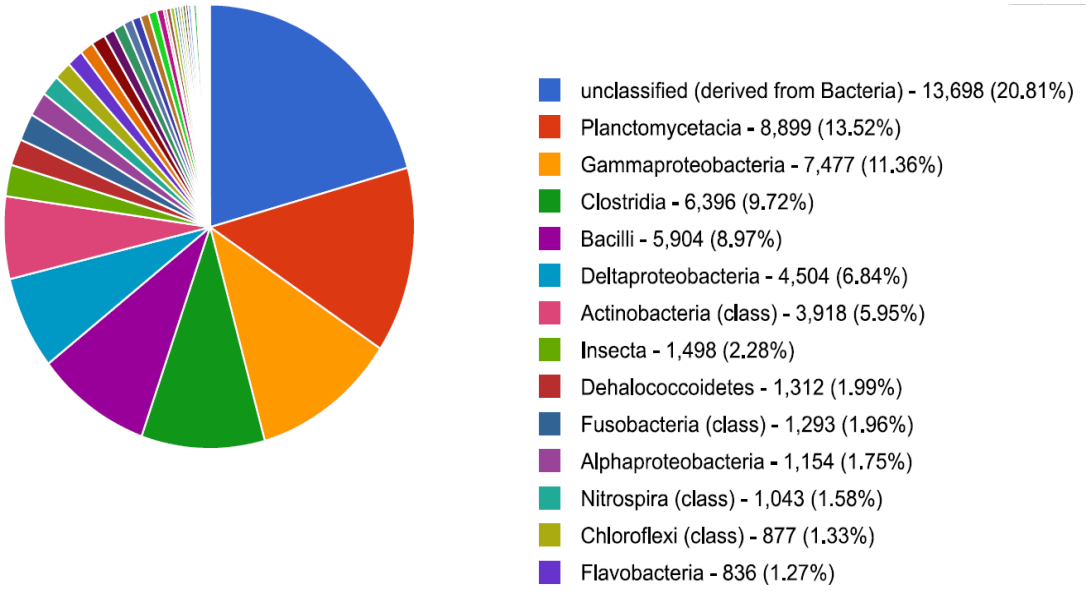

Figure 5: Microbial community structure of sediment of the pichavaram sediment sample obtained from MG-RAST-phylogenetic distribution of metagenomic sequence.

Microbial community structure of sediment of the Parangipettai sediment sample obtained from MG-RAST-phylogenetic distribution of metagenomic sequence.

Microbial community structure of sediment of the Parangipettai sediment sample obtained from MG-RAST-phylogenetic distribution of metagenomic sequence 22% present in the unclassified bacteria. Planctomycetales follow closely at 12.02%, contributing to the ecosystem's complexity. Noteworthy bacterial orders include Alteromonadales (10.29%), Vibionales (6.94%), and Clostridiales (5.78%), each playing distinct roles in the community dynamics. The presence of unclassified sequences, derived from Gammaproteobacteria (3.95%), indicates the potential for novel taxa within this class. Lactobacillales, Flavobacteriales, Oceanospirillales, and Bacillales further enrich the diversity at varying proportions. Intriguingly, unclassified sequences originating from an undefined source constitute 22.36%, underscoring the need for deeper taxonomic investigations (Figure 6). Additionally, unclassified sequences derived from Deltaproteobacteria, Actinomycetales, and Rhodobacterales add layers to the community structure. This comprehensive taxonomic overview unravels the intricate microbial relationships within the sampled environment, offering a foundation for future research aimed at understanding the ecological roles and potential biotechnological applications of these diverse microbial groups.

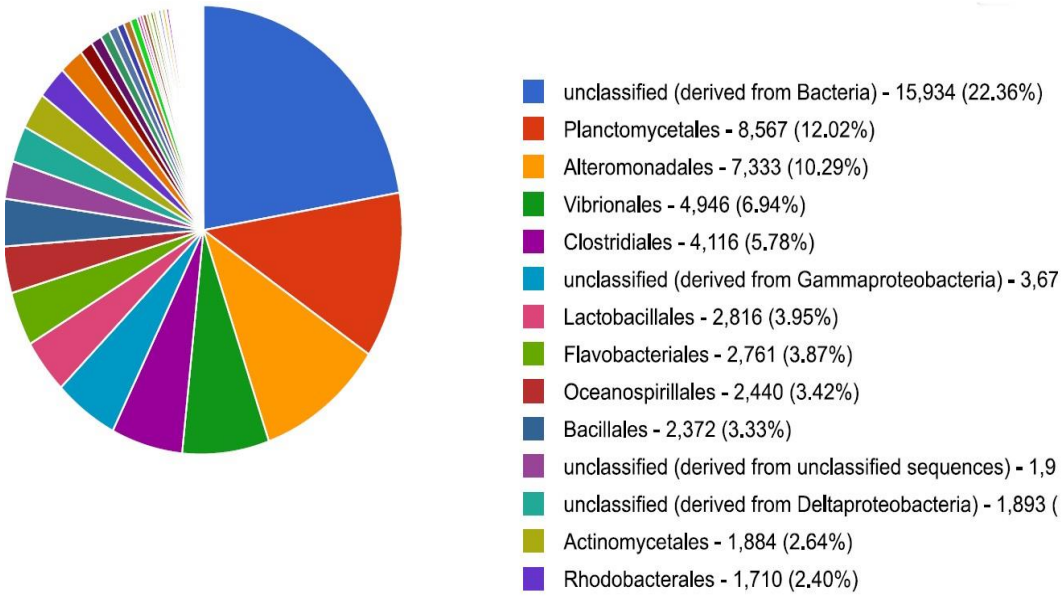

Figure 6: Microbial community structure of sediment of the Parangipettai sediment sample obtained from MG-RAST-phylogenetic distribution of metagenomic sequence.

Genes Involved in Biosynthesis of Secondary Metabolites and Degradation and Metabolism of Xenobiotics

In the sediment soils of Pichavaram and Parangipettai, the presence of key functional genes in bacteria reveals a diverse array of enzymatic activities crucial for various metabolic processes. Enzymes such as rfbB, rmlB, and rffG play a pivotal role in the biosynthesis of secondary metabolites, contributing to the microbial community's ability to produce bioactive compounds with ecological and potentially industrial significance. The Pgl gene involved in carbon metabolism is essential for energy production and carbon utilization, influencing the overall metabolic landscape of the bacterial community. Additionally, araB and manB, associated with pentose and glucuronate interconversion, and amino sugar and nucleotide sugar metabolism, respectively, underscore the microbial capacity to manipulate sugar derivatives for various cellular functions. The presence of tktA, implicated in microbial metabolism in diverse environments, suggests adaptability to varied ecological niches.

Enzymes glsA and GLS, participating in alanine, aspartate, and glutamate metabolism, are crucial for amino acid biosynthesis and nitrogen cycling, essential processes in microbial growth and ecosystem functioning. The cysJ gene involved in sulfur metabolism contributes to the microbial community's ability to utilize and cycle sulfur compounds, impacting both nutrient cycling and community interactions. Lastly, ndhE, associated with oxidative phosphorylation, plays a central role in energy production, highlighting the importance of efficient energy generation within the bacterial community.

Collectively, these enzymes not only contribute to fundamental metabolic processes but also play a significant role in the production of secondary metabolites, influencing the ecological dynamics and potential biotechnological applications of the microbial communities in Pichavaram and Parangipettai sediment soils. Understanding the functional gene repertoire provides insights into the adaptive strategies and ecological roles of these bacteria in their respective environments.

DISCUSSION

The metagenomic data analyses of Pichavaram and Parangipettai mangrove sediment samples provide detailed insights into the characteristics and complexity of microbial communities in these ecosystems. In Pichavaram, the dominant presence of bacteria at 97.13% emphasizes their crucial role in sedimentary processes, while the presence of Eukaryota at 2.19% adds to the overall biodiversity. The identification of unclassified sequences at 0.37% suggests potential novel taxa, highlighting the need for continued exploration and taxonomic refinement. The minimal presence of Archaea (0.30%) raises questions about their specific roles in sediment biogeochemistry, while the absence of viruses (0.00%) prompts further investigation into viral dynamics and sample processing methods. In Parangipettai, bacteria similarly dominate at 97.67%, emphasizing their significance as primary decomposers in sedimentary processes. The presence of Eukaryota at 1.09% suggests a coexistence of higher organisms, influencing nutrient cycling and community dynamics. Unclassified sequences at

1.16% further underscore the potential for undiscovered or less characterized taxa, emphasizing the need for taxonomic exploration. Archaea at 0.80% contribute to sediment biogeochemistry, and the overall diversity highlights the intricate relationships within the ecosystem.

The taxonomic analyses reveal a diverse microbial community structure in both sediment samples, with unclassified bacteria, Planctomycetales, Gammaproteobacteria, Clostridia, Bacilli, and Deltaproteobacteria playing significant roles. Actinobacteria, Insecta, Dehalococcoidetes, Fusobacteria, Alphaproteobacteria, Nitrospira, Chloroflexi, and Flavobacteria contribute to the richness and complexity of the microbial communities [16]. Furthermore, the metagenomic data analyses provide valuable details about the size, quality, and functional potential of the datasets. The identification and removal of artificial duplicate reads during quality control underscore the importance of rigorous procedures in ensuring accurate subsequent analyses. Predicted protein and rRNA features indicate the genomic complexity of the microbial communities, offering a foundation for exploring functional pathways and taxonomic composition.

The presence of key functional genes involved in biosynthesis of secondary metabolites and degradation/metabolism of xenobiotics in both sediment samples highlights the microbial community's metabolic versatility. Enzymes such as rfbB, rmlB, rffG, Pgl, araB, manB, tktA, glsA, GLS, cysJ, and ndhE play pivotal roles in various metabolic processes, including the production of bioactive compounds and energy generation [17]. The integrated analysis of metagenomic data, taxonomic distribution, and functional gene presence provides a comprehensive understanding of microbial communities in Pichavaram and Parangipettai mangrove sediments. These findings contribute valuable insights for ecological assessments, further taxonomic exploration, and potential biotechnological applications of the diverse microbial groups present in these ecosystems.

Bacteria can produce anticancer agents through intricate metabolic pathways that involve the biosynthesis of bioactive compounds with cytotoxic properties. One notable class of such compounds is the polyketides, which are synthesized by polyketide synthases (PKS) and are known for their diverse biological activities, including anticancer properties. Polyketides are formed by the sequential condensation of simple carboxylic acid building blocks, typically acetyl and propionyl coenzyme A (CoA) derivatives. These reactions are catalyzed by polyketide synthase enzymes, which are large, multifunctional protein complexes. The diversity in polyketide structures arises from variations in the number and types of building blocks, as well as the modifications introduced during the biosynthetic process. Streptomyces, a genus of bacteria known for its prolific production of bioactive compounds, is a well-studied example of bacteria that produce anticancer agents. Streptomyces species often harbor gene clusters responsible for the biosynthesis of polyketide-derived compounds, including anthracyclines like doxorubicin, which is a widely used anticancer drug. The biosynthetic pathways leading to anticancer polyketides involve a series of enzymatic reactions, including chain initiation, elongation, cyclization, and modification steps. These pathways are orchestrated by various enzymes within the polyketide synthase assembly line, each responsible for a specific transformation [18]. For instance, in the case of doxorubicin biosynthesis, the polyketide synthase assembly line incorporates malonyl-CoA and 4-methyl-3-hydroxyanthranilic acid as building blocks. Elongation and modification steps result in the formation of the anthracycline ring system, which imparts the anticancer activity.

Understanding these intricate metabolic pathways allows researchers to manipulate and optimize the production of specific anticancer agents. Advances in synthetic biology and genetic engineering further enable the design of engineered bacterial strains with enhanced yields and novel bioactive compounds. The exploration of bacterial metabolic pathways for anticancer agent production not only contributes to drug discovery but also showcases the potential of microbial resources in developing novel therapeutic strategies against cancer.

4. Conclusion

The metagenomic analyses of Pichavaram and Parangipettai mangrove sediment samples unveil a rich and diverse microbial tapestry, shedding light on the intricate taxonomic distribution and functional potential within these ecosystems. The dominance of bacteria, coupled with the presence of unclassified sequences and key functional genes, underscores the complexity and adaptability of microbial communities in these environments. These findings not only enhance our understanding of the ecological roles played by microorganisms in sedimentary processes but also lay the groundwork for future investigations into the untapped potential of these microbial communities for biotechnological applications. The comprehensive insights provided by this study emphasize the need for continued research to unravel the nuances of microbial interactions and their broader ecological significance in mangrove sediment ecosystems.

Conflict of interest: Nil

Funding: Nil.

Acknowledgement: The authors would like to express their gratitude to Annamalai University for allowing them to conduct this research study.

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