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16S rRNA genes developed a baseline of the microbial community associated with soil, water, fish and shellfishes in the sundarbans of Bangladesh

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Abstract: The microbial environment of the Sundarbans mangrove forest contains sediments and water usually more diverse than the other aquatic environment, and pathogenic for finfish and shellfishes will be revealed by phylogenetic tree analysis of the positive controls PCR product. Approximately, 21 samples of the soil, 21 samples of water, 21 samples of fish, and shellfishes are dominated by typical mangrove habitats surrounded by Sathkhira, Khulna, and Bagherhat have been covered. The bio-physiochemical test was carried out after isolation and culture of the microorganisms from soil, water, fish & shellfishes. Initially, the bio-physiochemical and molecular survey tools were used to finally screen 63 samples, which were used for the genomic DNA extraction, and the accurate quantitative and qualitative estimation of the extracted DNA was confirmed by Nano-drop. Finally, 12 samples were confirmed using Nano-drop calibration for PCR amplification and UV-translumination. The allelic and loci variation, genetic diversity, heterozygosity, PIC, genetic distance, and similarity within the genotypes (samples) were examined. This research technique is a rapid and effective tool for diversity assessment; this result might be helpful to the development of a genetic baseline of the pathogenic bacterial and viral community in the Sundarbans of Bangladesh to control the diseases of fish and shellfishes in mangrove regions.

Keywords: DNA extraction, Sundarban mangrove forests, Bacterial diversity, 16S rRNA, Molecular techniques, Fish diseases

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1 Introduction

The Sundarbans is a huge forest and saltwater marsh in southeastern West Bengal state, northeastern India, and southern Bangladesh that forms the bottom half of the Padma Ganga-Brahmaputra River delta. It surrounds flat, densely forested and marshy islands with a network of estuaries, tidal rivers, and creeks linked by numerous channels. The track covers 160 miles (260 kilometers) west-east along the Bay of Bengal from the Hugli River estuary in India to the western part of the Meghna River estuary in Bangladesh. It reaches inland for roughly 50 miles (80 kilometers) at its widest point.

The Sundarbans is a deltaic mangrove forest located south-west of Bangladesh and south of West Bengal, India, created around 7000 years ago by the deposition of sediments from the Himalayan foothills through the Ganges river system. However, due to a withdrawal of water during the dry season from the Farakka Barrage in India, the discharge of sediment-laden fresh water into the Bay of Bengal through the Bangladesh part of the Sundarbans Mangrove Forests (BSMF) has been reduced for the previous 40 years (Aziz and Paul, 2015).

Sundarbans mangrove forests have great below-ground production and contribute significantly to above and below-ground carbon concentrations. As a result, mangrove ecosystems provide a unique and significant set of resources and functions. Therefore, a large number of birds, animals, fish, and invertebrates need Sundarban mangroves for at least part of their life cycle (Siddiqi et al., 1993).

Phytoplankton, zooplankton, microorganisms, benthic invertebrates, mollusks, amphibians, and mammals make up the Sundarban's rich biodiversity. The Sundarban region is home to 350 kinds of vascular plants, 250 species of fish, and 300 species of birds. The microbial diversity in the Sundarbans sediments has received little attention (Ghosh et al., 2010). Mangroves are well-known for being highly productive ecosystems with significant ecological significance. They safeguard and stabilize coastal zones, and nutrient-feeding and nurturing coastal water. They also serve as vital feeding and breeding grounds for a variety of creatures, including plants, animals, and microorganisms. The bio-geographical, anthropological and ecological factors impact the microbial community in mangrove sediment. The presence of organic and inorganic matters, the ecosystem's food web and nutrient cycling are among these features. Mangrove ecosystems are generally poor in nutrients, particularly nitrogen and phosphorus. Mangroves, despite this, are highly productive.

Climate, sea-level rise and temperature change, changes in precipitation and cyclone activity, population, and market growth are all causing significant changes in the Sundarban landscape. It is critical to identify the various factors contributing to mangrove degradation to formulate long-term management objectives. Because these microflorae are adapted to highly variable physicochemical conditions of flooding, salinity, light, and temperature, accounting for most of the diversity (Feller et al., 2010), mangrove ecosystems are considered hotspots of microbial diversity (Andreote et al., 2012). They play a key role in the sustenance, productivity, and recovery of mangroves (Ghosh et al., 2010; Roy et al., 2002; Santos et al., 2011).

The multiplex PCR assay is a quick, accurate, and sensitive technique for detecting and identifying common fish pathogenic bacteria in aquaculture. The genera *Aeromonas*, *Vibrio*,

Edwardsiella, and *Streptococcus* commonly cause severe disease outbreaks in cultured fish. The genus-specific multiplex PCR assay developed in this study can detect the bacteria of the four genera when present in the samples alone or together. For clinical diagnosis, the PCR technique is predicted to identify the causative agents more efficiently than conventional PCR, allowing control measures to be implemented sooner (Zhang et al., 2014). The current research aimed to identify and characterize metagenomic information about fish and shellfish diseases using molecular techniques of PCR-based amplification of 16S rRNA gene fragments followed by DNA sequence analysis.

2 Materials and Methods

2. 1. Sampling sites and size

In the present study, seven (07) areas such as Kainmari, Dhangmari, Harbaria, Koromjol, Chila, Joymoni, and Gagramari of the Sundarban south-west region of Bangladesh were selected for collection of samples from 2020 to 2021 for meta-genomic research. A total of 21 samples of 21 fish and shellfish species, 21 samples of water, and 21 samples of soil from 7 locations in the Sundarban were collected and kept in the icebox with zipper bags and stored at -26 °C in the Fish Molecular Lab of Fisheries and Marine Bioscience Department at Jashore University of Science and Technology until further research.

2. 2. Isolation and storage of bacteria isolates

Microorganisms were isolated from fish and shellfish from several areas of Bangladesh's Sundarbans mangrove forest. About 25 g of sediments, water, fish, and shellfish samples were carefully triturated in a sterile mortar and pestle with 225 ml of alkaline saline peptone water (ISO) (Thermo ScientificTM) and homogenized for 2 min in a stomacher (Life Technologies, California, USA) (Chonsin et al., 2016). Three duplicates of a ten-fold serial dilution of 1 ml of homogenates in alkaline saline peptone water (ASPW+) were prepared and shaken at 170 rpm at 37 °C for 18 h. The extract was plated onto thiosulfate-citrate-bile salts-sucrose (TCBS) plates for 24 hours (Remel Inc., Santa Fe Drive, Lenexa, USA). The plates were incubated at 37 °C for 24 h. The characteristic large colonies (3-4 mm) with light blue or green centers on TCBS and VPSA were regarded as presumptive microorganisms and further subjected to morphological, cultural, and biochemical characterization. A series of biochemical assays according to the BAM and USFDA techniques (Kaysner et al., 2004) was used to identify microorganism isolates. All colonies were collected and streaked on tryptone soya agar (TSA) supplemented with 3% (w/v) NaCl (Sigma-Aldrich, Germany). After a 24-hour incubation period, one colony from each plate was inoculated into TSB (Sigma-Aldrich, Germany) fortified with 3% (w/v) NaCl and cultured for 12 hours by shaking at 170 rpm at 37 °C. The suspension was incubated at 37 °C with constant shaking for 12 hours. The complete volume of the 12-hour culture (50 ml) was used as an inoculum for 500 ml of fresh media (500 ml), and the culture was maintained with shaking until the OD600 reached 0.6 (about 6–8 h) and was equivalent to approximately 2 x 108 cells per ml. Centrifugation at 8,500 rpm for 10 min at 4 °C, removed the cells and the supernatant was utilized to create crude protein fractions. After that, 1 ml of medium was combined with 1 ml of

Invitrogen TM ultrapure glycerol (50 percent (v/v)) and stored at 80 °C. The pellet was suspended in 400 l of ddH2O after centrifugation of 1 ml cells from an overnight culture. The suspension was centrifuged for 6 min at 11,000 rpm. The supernatant was employed as a DNA template for PCR and RT-PCR assays. The positive controls for a hypothetical microbial community in mangrove environments and microbial community in mangrove regions are enlisted in Table 1.

Table 1. Positive control to identify the microbial contents in the fish and shellfishes.

Diseases producing bacteria	**Finfish/shellfish**	**NCBI (BLAST) Ref:**
	Streptococcus agalactiae	CAACXY01000005.1
	S. iniae	MNAC01000005.1
	Vibrio sp.	D21202.1
	Aeromonas sp.	L31915.1
	Flavobacterium	LQ685220.1
	Edwardsiella tarda	S77842.1
	Edwardsiella ictaluri	FI274675.1
	Early Mortality Syndrome (AHPND); *Vibrio parahaemolyticus*	NZ_JPKS01000098.
Diseases producing virus		
	Tilapia Lake virus (TiLV)	MN939372.1
	Macrobrachium rosenbergii Nodavirus (MrNV)	MK113948.1
	White Spot Syndrome Virus (WSSV)	MZ327625.1

2. 3. Extraction of bacterial genomic DNA and plasmid DNA

Following the manufacturer's instructions, bacterial genomic DNA and plasmid DNA was isolated using DNAzolTM reagent (a huge spectrum, fast isolation, and large recovery of genomic DNA kits, InvitrogenTM, and PureLinkR Pro Quick96 Plasmid Kit, InvitrogenTM). The DNA of 21 isolates was extracted from fish and shellfish tissue using a standard DNA extraction procedure. In this study, the volume of template DNA in the 25 l PCR reaction was 0.01-1 ng of DNA isolated from a bacterial isolate or 10–100 ng from fish tissue.

Pure cultures of isolates were grown in tryptone soya broth (TSB) (Sigma-Aldrich, Germany) containing 1.5% NaCl and incubated at 37 °C for 24 h. Total RNA was isolated using MagMAX™-96 Total RNA Isolation Kit (Invitrogen™, Thermo Fisher Scientific Corporation, USA). The qualitative control was accessed using Thermo Scientific™ NanoDrop™ QC Software for the NanoDropOneC Spectrophotometer (Thermo Scientific™, USA). Then, the total RNA was reverse transcribed into cDNA with a SuperScript® III CellsDirectcDNA Synthesis Kit (Invitrogen™, Thermo Fisher Scientific Corporation, and USA). Ten-fold serial dilution of cDNA samples was used to generate a standard curve on the CFX Real-Time PCR Detection Systems, Bio-Rad Laboratories India Pvt. Ltd.). The reaction mixture and thermal profiles were performed as described in previous sections.

2. 4. Primers used for PCR amplification

The well-conserved regulatory genes of all positive control samples (Table 1) were analyzed

and examined for PCR amplification to produce the expression of isolates of different strains (Gutierrez West et al., 2013). All positive control samples' regulatory genes were applied to amplify the target sample's negative control (-V). The template DNA derived from suspected or positive 75 isolates was used for PCR amplification (Sirikharin et al., 2014) with a specific primer set for particular target genes (Table 2 and 3).

Table 2. The primers pair of the possible microorganisms initially found in mangrove areas used for PCR amplification.

Organisms	Primer Sequences	Length	Tm: °C	GC %	Product Size: bp
TLiV	F: GGCTGAGAGCTCAGGATGTT	20	59.6	55.0	221
	R: CCCTTCTGTTTTTGGGATTG	20	59.4	45.0	
Steaga	F: AAGGTACACCAGCTCTTCATCA	22	58.9	45.5	299
	R: CGCAAATCCTTTCTCAAACC	20	59.7	45.0	
Steini	F: CGAGCATCCAAGTGCTGATA	20	60.0	50.0	245
	R: CCTTTGCCACATCCATAACA	20	59.4	45.0	
Vibsp	F: CCGCAAGGTGTCTGATTTCT	20	60.3	50.0	189
	R: TCACCGGATTTCTTCTGGTC	20	60.0	50.0	
Ahydro	F: GAAGACCTGGGAAACCAACA	20	59.9	50.0	230
	R: CGACGATCTGCTGATACAGG	20	59.4	55.0	
Flavcol	F: TCTGTACGAGAGTATTCGCAGGT	23	60.3	47.8	220
	R: TGCCTAGTAGGTTCCCCAAA	20	59.6	50.0	
Pseflu	F: CATTACCCCGGTATTCGATG	20	60.0	50.0	240
	R: ATGGACTTGAAGCGATCCAG	20	60.2	50.0	
Edtard	F: GTGAGGATGGCTTGTCGATT	20	60.1	50.0	239
	R: ATTGATCACCGCATCTTCCT	20	59.5	45.0	
Edicta	F: GAGCGCACGGTAGAGCTG	18	60.9	66.7	246
	R: CTCTATCCGCGAGAAGTCCA	20	60.5	55.0	
WSSV	F: AAACGGCACATTACAAACCA	20	58.9	40.0	231
	R:GGAGACAGTGAAACAGAGTCCA	22	59.4	50.0	
AHPNV	F: TAAGCCCGCTTTCTTCAGAC	20	59.6	50.0	285
(Vibpara)	R: CACCAATCTGACGGAACTGA	20	59.7	50.0	
MrNV	F: AGGCAGGCTACGTCACAAGT	20	59.9	55.0	250
	R: GCATGGAAAATCCACAGACC	20	60.3	50.0	

Table 3. The primers pair of 16S rRNA Gene Sequences the possible microorganisms initially found in mangrove areas used for PCR amplification.

Primers	Amplifying Variable Regions of 16S rRNA Gene Sequences	Expected amplicon size	References
8f (27f)	AGAGTTTGATCMTGGCTCAG	8–27	(Lane, 1991)
341f	CCTACGGGRSGCAGCAG	341–357	(Baker et al., 2003)
519f	CAGCMGCCGCGGTAATWC	519–536	(Wang and Qian, 2009)
968f	AACGCGAAGAACCTTAC	968–984	(Nübel et al., 1996)
338r	TGCTGCCTCCCGTAGGAGT	337–355	(Fierer et al., 2008)
518r	ATTACCGCGGCTGCTGG	518–534	(Muyzer et al., 1993)
907r (926r)	CCGTCAATTCCTTTRAGTTT	907–926	(Liu et al., 1997)
1392r	ACGGGCGGTGTGTRC	1392–1406	(Lane, 1991)
1492r	TACGGYTACCTTGTTACGACTT	1492–1513	(Lane, 1991)

2. 5. PCR amplification of target bacterial genes

The template DNA derived from suspected or positive 75 isolates was used for PCR amplification (Sirikharin et al., 2014) with a specific primer set for particular target genes in Table 2. The final reaction volume was 10 µl, containing 5 µl of Green-Master mix (Go-TagG2), 1 µl of 10 µM primers, 1 µl of the template DNA sample, and 3µl nuclease-free water. In a thermal cycler (Professional standard gradient, Biometra, Germany), the thermal profile for DNA amplification was: 35 cycles of preheating at 95 °C for 2 min and 1 min denaturation at 94 °C. The annealing temperature was 58 °C (primer-specific) for 1 min, then 72 °C for 1 min, then 72 °C for 5 min. Moreover, 1% agarose gel was used to separate the amplified PCR product from each sample electrophoretically (NacalaiTesque, Inc., Kyoto, Japan) at a constant voltage of 120 for 30 min in the 1×TAE buffer. The gel was stained with 10 µl ethidium bromide (10 mg/ml in 100 ml of DW) for 10 min and visualized under ultraviolet (UV) light. The molecular weight marker was 1 Kb plus DNA ladder (InvitrogenTM, Thermo Fisher Scientific). The DNA bands were observed on a high-performance UV transilluminator and photographed with a gel documentation system (BioDoc-It TM Imaging system, Cambridge, UK).

2 .6. Data analysis

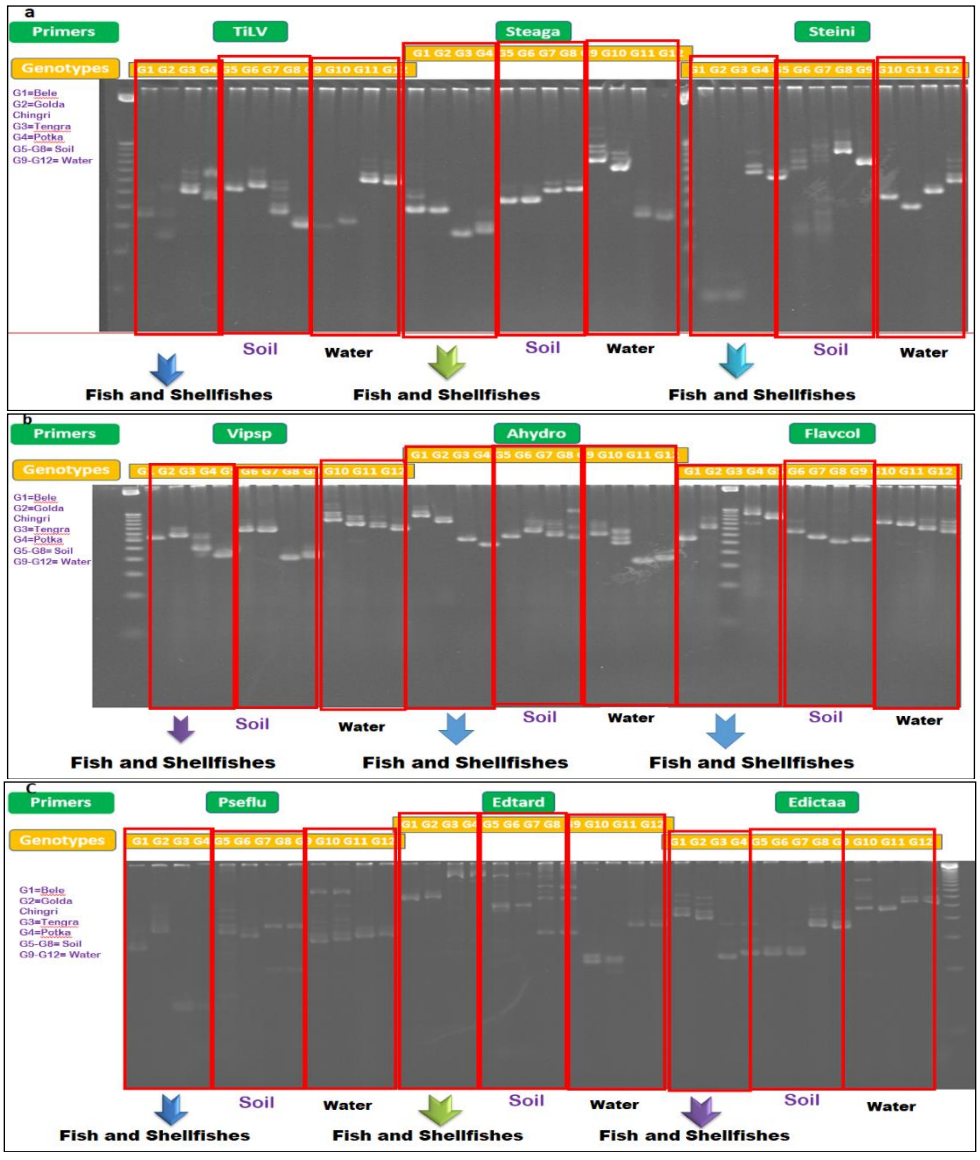
Farmer's responses were obtained using different questionnaire forms analyzed with IBM SPSS statistics. Allelic frequency, gene diversity, heterozygosity, and polymorphism in different locations were analyzed using power marker software. Clustering of the different populations using the unweighted Pair Group Method of Arithmetic Mean (UPGMA) algorithm, was constructed based on Nei's (1972) genetic distance summarizing differentiation according to microsatellite profiles (Excel to NTedit 1.1, checked coefficient SM (Similarity) by SimQual method and clustered (SAHN) UPGMA method using NTSYSpc 2.2 software). Genomic DNA sequences were analyzed using BLAST, which computed a significant pairwise alignment between a query and the database sequences based on partial DNA nucleotides sequences in NCBI.

3 Results and Discussions

The analysis of genetic diversity is a very important factor for the identification of microorganisms that can be obtained through DNA fingerprinting techniques, which are capable of exhibiting a large number of loci for extensive variability. Samples were collected from a different region of the Sundarbans and were analyzed using a highly repeatable PCR-based fingerprinting assay.

3. 1. Allelic size and loci variation, genetic diversity, heterozygosity, PIC within the genotypes

The DNA fingerprinting was constructed using the standard procedures. This study analyzed 12 genotypes of sediments, water, fish and shellfishes. The 12 primers were used in this study. The amplified microsatellite loci were analyzed for polymorphism using 1% Agarose Gel Electrophoresis (AGE). The result revealed amplicon size that all the primer pairs detected was monomorphic among the genotypes analyzed (Figure 1 a-d). The loci variation, genetic diversity, heterozygosity and PIC within the genotypes were shown in (Figure 2).



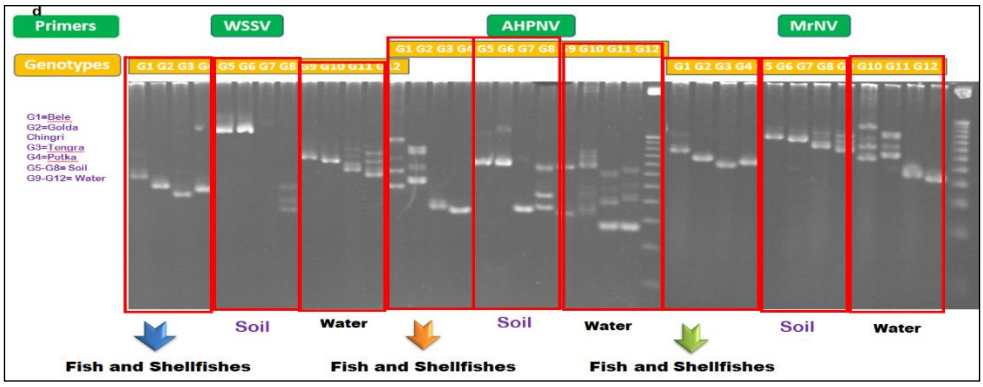


Figure 1 (a - d): The PCR amplicon from the different genotypes of the different regions of the Sundarbans. G1-G4 denotes fish and shellfishes, G5-G8 denotes soil, and G9-G12 denotes water.

Marker	Major Allele Frquency	Genotype No	Sample Size	No. of obs.	Allele No	Availability	Gene Diversity	Heterozygosity	PIC
G1	0.8750	2.0000	104.0000	104.0000	2.0000	1.0000	0.2188	0.0000	0.1948
G2	0.8654	2.0000	104.0000	104.0000	2.0000	1.0000	0.2330	0.0000	0.2058
G3	0.8846	2.0000	104.0000	104.0000	2.0000	1.0000	0.2041	0.0000	0.1833
G4	0.9038	2.0000	104.0000	104.0000	2.0000	1.0000	0.1738	0.0000	0.1587
G5	0.8654	2.0000	104.0000	104.0000	2.0000	1.0000	0.2330	0.0000	0.2058
G6	0.8846	2.0000	104.0000	104.0000	2.0000	1.0000	0.2041	0.0000	0.1833
G7	0.8846	2.0000	104.0000	104.0000	2.0000	1.0000	0.2041	0.0000	0.1833
G8	0.8173	2.0000	104.0000	104.0000	2.0000	1.0000	0.2986	0.0000	0.2540
G9	0.8365	2.0000	104.0000	104.0000	2.0000	1.0000	0.2735	0.0000	0.2361
G10	0.8558	2.0000	104.0000	104.0000	2.0000	1.0000	0.2469	0.0000	0.2164
G11	0.8654	2.0000	104.0000	104.0000	2.0000	1.0000	0.2330	0.0000	0.2058
G12	0.8654	2.0000	104.0000	104.0000	2.0000	1.0000	0.2330	0.0000	0.2058
Mean	0.8670	2.0000	104.0000	104.0000	2.0000	1.0000	0.2297	0.0000	0.2028

Figure 2: The allele frequency, genetic diversity, heterozygosity, and PIC of the different genotypes of the different regions of the Sundarbans. G1-G4 denotes fish and shellfishes, G5-G8 denotes soil, and G9-G12 denotes water.

3. 2. Genome sequences and distance tree analysis

The results of the DNA sequences of all genotypes were checked in NCBI using standard nucleotides BLAST (FASTA sequences) to produce significant alignment for query cover and identities between 12 genotypes sequences and other published template genome sequences of host species. Comparisons between genome sequences and pairwise alignment with similarity scores relative to the query sequence in NCBI (BLAST) were revealed.

3. 3. Similarity and genetic distance of the genotypes

The PCR fingerprinting data revealed the identity of the genotypes very quickly and protected intellectual property rights (IPR). The similarity and genetic distance within the 12 genotypes were initially computed by Nei (1972) using NTSYSpc 2.1e, are shown in Figure 3 and Figure 4. The similarity between fish samples G1 and G2 was about 79%, and the highest similarity between the G3 and G4 was about 82%. There was no distance observed between G1

and G3, but the highest difference was found between G2 and G4.

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Format: width=9 decimals=4
Page width: 120
Field width: 9
Decimal places: 4
Page width: 120

Comments:
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  by Cols
Matrix type = 3, size = 12 by 12, missing value code = "none"  (similarity)

        G1       G2       G3       G4       G5       G6       G7       G8       G9       G10      G11      G12
     ---------------------------------------------------------------------------------------------------------------
G1   |   1.0000
G2   |   0.7981   1.0000
G3   |   0.7788   0.7500   1.0000
G4   |   0.7788   0.7885   0.8269   1.0000
G5   |   0.7404   0.7308   0.7692   0.7885   1.0000
G6   |   0.7788   0.7692   0.7692   0.8077   0.8654   1.0000
G7   |   0.7788   0.7692   0.7692   0.8077   0.7692   0.7692   1.0000
G8   |   0.7308   0.7212   0.7019   0.7596   0.7019   0.7212   0.8173   1.0000
G9   |   0.7115   0.7212   0.7212   0.7596   0.7212   0.7212   0.7596   0.6923   1.0000
G10  |   0.7500   0.7404   0.7596   0.7788   0.7596   0.7404   0.7788   0.7308   0.8077   1.0000
G11  |   0.7596   0.7692   0.7692   0.7692   0.7308   0.7885   0.7500   0.6827   0.7019   0.7212   1.0000
G12  |   0.7596   0.7500   0.7500   0.7885   0.7692   0.8077   0.7500   0.6827   0.7212   0.7404   0.8269   1.0000

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Figure 3: The similarity within the 12 genotypes was originally computed by Nei (1972) using NTSYSpc 2.1e. G1-G4 denotes fish and shellfishes, G5-G8 denotes soil, and G9-G12 denotes water.

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Page width: 120
Field width: 9
Decimal places: 4
Page width: 120
Comments:
  SIMGEND: input=G:\My Drive\PROJECT and PROTOCOL and SPFTWARE\NEW PROJECT 2019\for analysis\a.NTS, coeff=NEI72, dir=Cols
Matrix type = 2, size = 12 by 12, missing value code = "none"  (dissimilarity) Genetic Distance:

        G1       G2       G3       G4       G5       G6       G7       G8       G9       G10      G11      G12
     ---------------------------------------------------------------------------------------------------------------
G1   |   0.0000
G2   |   1.5034   0.0000
G3   |   2.5249   0.0000   0.0000
G4   |   0.0000   2.4708   1.7006   0.0000
G5   |   0.0000   0.0000   2.5620   2.4708   0.0000
G6   |   2.5249   2.5620   0.0000   2.3937   0.7702   0.0000
G7   |   2.5249   2.5620   0.0000   2.3937   2.5620   0.0000   0.0000
G8   |   2.0615   2.0986   0.0000   1.9304   2.7917   2.7147   0.9229   0.0000
G9   |   0.0000   2.7361   0.0000   2.5679   2.7361   0.0000   1.9659   2.1957   0.0000
G10  |   2.6365   2.6736   2.5965   2.5053   1.9804   0.0000   1.9033   1.7276   0.9789   0.0000
G11  |   2.6020   1.9459   2.5620   0.0000   0.0000   1.8688   0.0000   0.0000   0.0000   0.0000   0.0000
G12  |   2.6020   2.6391   0.0000   2.4708   1.9459   1.4634   0.0000   0.0000   2.7361   2.6736   1.0296   0.0000

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Figure 4: The genetic distance within the 12 genotypes was originally computed by Nei (1972) using NTSYSpc 2.1e. G1-G4 denotes fish and shellfishes, G5-G8 denotes soil, and G9-G12 denotes water.

3. 4. UPGMA dendrogram

The unweighted pair Group Method of Average (UPGMA) dendrogram was constructed following the neighbor-joining method based on genetic distance computed by Nei (1972), resulting in two major clusters: the G1, G4, G2 and G5 populations were in one cluster, and the remaining eight (08) populations were in the other cluster (Figure 5).

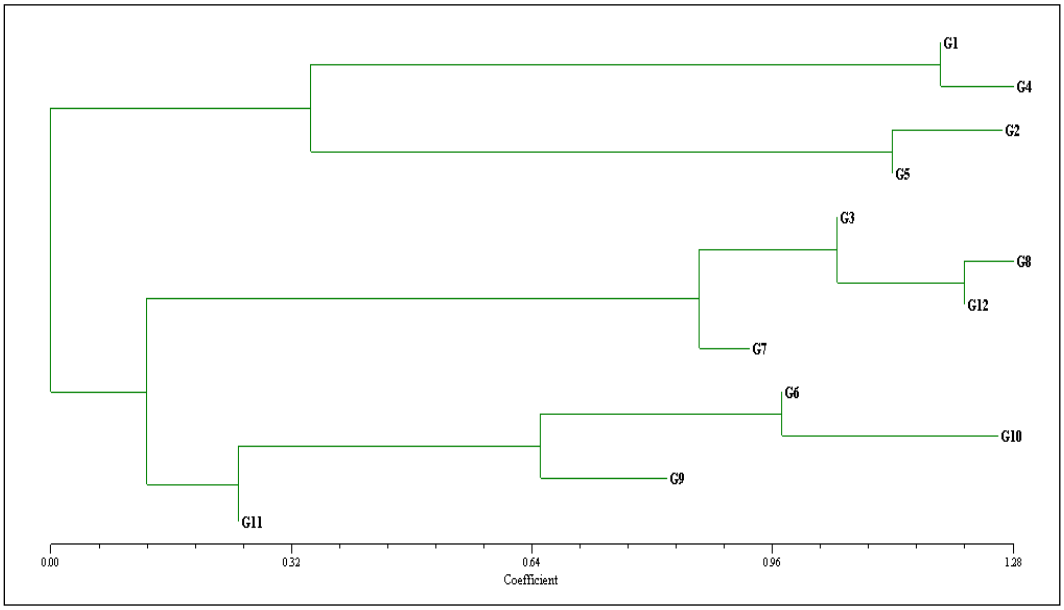

Figure 5: UPGMA dendrograms were constructed following the neighbor-joining method based on genetic distance computed by Nei (1972). G1-G4 denotes fish and shellfishes, G5-G8 denotes soil, and G9-G12 denotes water.

Metagenomic analysis of mangrove at Cardoso Island State Park, Brazil, through 16S rRNA pyrosequencing showed similar dominance of Proteobacteria (88% of overall sequence) irrespective of soil depth through 16S rRNA pyrosequence 25. Proteobacteria was the prominent phylum among the Bacteria, followed by Bacteroides, Firmicutes, and Actinobacteria. Similar bacterial phylum dominancy was found using PCR-Clone-based metagenomic library screening and 16S rRNA ribo-typing 27. The similarities within Kerala datasets seen across the higher taxonomic levels (Kingdom and Phylum) did not continue at the class level, where the composition of the bacteria varied at each location, especially among the classes within the *Proteobacteria* and *Bacteriodes* phyla (Imchen et al., 2017).

Partial sequencing of the 16S rRNA gene clones obtained in our study revealed a high prevalence of the Proteobacteria phylum, with Gammaproteobacteria being the most abundant group, followed by Betaproteobacteria, Deltaproteobacteria, and Alphaproteobacteria. Previous studies on Sundarbans sediment by (Ghosh et al., 2010) reported similar dominance of Proteobacteria.

In our study, the DNA sequences of all genotypes will be checked in NCBI using standard nucleotides BLAST (FASTA sequences) to produce significant alignment for query cover and identities between 12 genotypes sequences and other published templates genomes sequences of host species. Comparisons between genome sequences and pairwise alignment with

similarity scores relative to the query sequence in NCBI (BLAST) will be revealed. The similarity between G1 and G2 is about 79%, and the highest similarity between G3 and G4 is about 82%. There is no distance between G1 and G3 & the highest difference showed between G2 and G4.

Genetic diversity is a very important factor for the identification of microorganisms that can be obtained through DNA fingerprinting techniques, which are capable of exhibiting a large number of loci for extensive variability. The 12 primers were used in this study. Samples collected from a different region of the Sundarbans were analyzed using a highly repeatable PCR-based fingerprinting assay. The amplified microsatellite loci were analyzed for polymorphism using 1% Agarose Gel Electrophoresis (AGE). The result revealed amplicon size that all the primer pairs detected monomorphism among the genotypes analyzed.

To our knowledge, the present study is the first attempt to investigate the physical and chemical parameters with the spatial alteration in the microbial abundance and diversity in fish and shellfishes of the Sundarbans, and try to illuminate the loci variation, genetic diversity, heterozygosity within the genotypes of microbes which causes diseases.

4 Conclusion

The Sundarbans mangrove forest is full of different types of microbial organisms that play a vital role in the diversification of the nutrient cycling in the mangrove ecosystem. The bacterial communities of sediments and water in the Sundarbans mangrove forest were found to be more diverse than that of the other aquatic environment and were pathogenic for soil, water, finfish, and shellfishes, which was revealed by the phylogenetic tree analysis of the positive controls sequences. Initially, the bio-physiochemical and molecular survey tools were used to finally screen 21 soil samples, 21 water samples, and 21 samples of fish and shellfishes, which were used for the genomic DNA extracted as well as the accurate quantitative and qualitative estimation of the extracted DNA was confirmed by nano-drop, PCR and RT-qPCR. The PCR amplification of the 21 samples and all the positive controls has been confirmed in a thermal cycler. This research technique is a rapid and effective tool for diversity assessment; the result might be helpful to the development of a genetic baseline of the pathogenic bacterial and viral communities in the Sundarbans of Bangladesh to control the diseases of fish and shellfishes in mangrove regions.

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Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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