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Whole-genome sequences of four *Vibrio* species isolated from shrimp in local markets of Bangladesh

Mohammad Sharif Uddin,[1] Kazi Chamonara,[2] Sumaia Sultana,[1] Sajedul Islam,[1] Afifa Siddiqua,[3] Md. Habib Ullah Masum[4]

AUTHOR AFFILIATIONS See affiliation list on p. 2.

ABSTRACT Bangladesh's aquaculture is currently faced with *Vibrio* contamination, which poses a threat to public health, exports, and product quality. This research provides a detailed molecular characterization of *Vibrio* sp. found in shrimp through the use of Oxford Nanopore sequencing, ensuring precise identification and enhancing shrimp quality and food security.

KEYWORDS zoonotic infections, fish microbiology

The aquaculture sector in Bangladesh substantially contributes to the national economy and food security, which accounts for 3.52% of GDP and 26.37% of agricultural GDP, ranking Bangladesh third in inland capture and fifth in aquaculture globally (1). Among the fish species, shrimp are significant contributors to export revenue. Nevertheless, the consumption of contaminated shrimp poses a significant risk of foodborne infections, thereby threatening both public health and the sustainability of this sector (2). The contamination frequently occurs due to inadequate practices in processing, preservation, and storage, which provide favorable growth conditions for food spoilage bacteria, like *Vibrio* spp. (3–5). Twelve species of *Vibrio* are pathogenic to humans, with *V. parahaemolyticus* and *V. cholerae* being the most prevalent (6). These pathogens also pose significant zoonotic and public health risks (4). Additionally, the shrimp export sector in Bangladesh encounters significant challenges due to quality decline stemming from *Vibrio* contamination (7). However, prompt identification of pathogenic *Vibrio* species can enhance shrimp quality, export prospects, and customer safety (7, 8). Using Oxford Nanopore sequencing, this study reveals the molecular profiles of *V. cholerae* and *V. parahaemolyticus* from shrimp samples of Noakhali district, filling gaps in *Vibrio* molecular characterization.

The shrimp samples were enriched in alkaline peptone water (at 37°C), then incubated onto thiosulfate–citrate–bile salts–sucrose (TCBS) agar plates (for 18–24 h at 37°C) (9). Presumptive *Vibrio* isolates were initially identified based on morphological traits, followed by confirmation through polymerase chain reaction (PCR) aimed at the genus-specific groEL gene. Subsequent culture of the isolates in nutrient agar was applied for the total DNA extraction by using QIAamp DNA Mini Kit following the manufacturer's instructions and quantified using a Qubit four fluorometer. In advance of DNA sequencing, a native barcoding kit (SQK.NBD.114.24) was employed for library preparation (full length 16S rRNA, V1-V9), and the sequencing run was executed on the Oxford Nanopore MinION Mk1C platform utilizing the FLO-MIN114 (R10.4.1) flow cell. The MINKNOW software (v.1.11.5) was utilized for gathering information. The Guppy (v.6.3.2) was employed for basecalling the MinION Mk1C sequencing data (FAST5 files) to provide pass reads (FASTQ format) with a mean quality score above 9. The adapter and barcode sequences were trimmed using Porechop (v.0.2.4) (10), and low-quality bases ($q < 10$) were removed with Nanofilt (11). The quality-trimmed reads were assembled into contigs using Flye (v.2.9.1-b1780) through the *de novo* assembly workflow (12).

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Address correspondence to Md. Habib Ullah Masum, habibullahmasummbg5005@gmail.com.

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TABLE 1 Genome features of four *Vibrio* isolates from shrimp in local markets of Noakhali, Bangladesh

Feature	Strain			
	SU37A	**SU91A**	**SU109**	**SU129B**
Species	*Vibrio parahaemolyticus*	*Vibrio parahaemolyticus*	*Vibrio cholerae*	*Vibrio cholerae*
GenBank accession no.	JBQRUG000000000.1	JBQWLY000000000.1	JBQWLZ000000000.1	JBQWMA000000000.1
SRA accession no.	SRR34709126	SRR34709125	SRR34709124	SRR34709123
Genome size (Mbp)	5037599	5156510	4042637	4053857
No. of reads	820864	1195744	385028	819028
Genome coverage (x)	161.5	209.7	109.1	247.4
Coarse consistency (%)	99.9	99.7	99.8	99.7
Fine consistency (%)	98.7	91.9	98.5	96.7
Completeness (%)	97.04	86.55	97.63	93.08
No. of contigs	3	3	2	3
N50 (bp)	3252908	3376922	2870474	2888403
No. of L50	1	1	1	1
GC content (%)	45.43	45.34	47.67	47.63
ANI score (%)	98.49	98.44	98.35	98.34
dDDH (%)	86.60	86.10	85.10	85.60
No. of Genes (Prokka, RAST, PGAP)	4696, 4850, 4632	5155, 5383, 4740	3695, 3878, 3743	3742, 3938, 3707
CDS (Prokka, RAST, PGAP)	4523, 4681, 4459	4989, 5216, 4571	3558, 3743, 3602	3606, 3804, 3568
tRNA (Prokka, RAST, PGAP)	135, 132, 132	128, 130, 128	105, 106, 106	104, 104, 104
rRNA (Prokka, RAST, PGAP)	37, 37, 37	37, 37, 37	31, 29, 31	31, 30, 31
Proteins with functional assignments	3746	4157	3030	3106
Antibiotic resistance gene (CARD, PATRIC)	8, 36	11, 44	6, 33	6, 32

The Type (Strain) Genome Server (TYGS) was utilized to identify the species (13). The potential contamination within the genome and its completeness was assessed using ConEst16S (14), CheckM (v.1.2.4) (15), BUSCO (v.6.0.0) (16), and QUAST (v.5.2.0) (17). The average nucleotide identity (ANI) and digital DNA:DNA hybridization (dDDH) were determined using the OrthoANI (v. 0.93.1) tool available on the Ezbiocloud server (18) and the Genome-to-Genome Distance Calculator tool (v.3.0), respectively (19). The genome was annotated using three distinct tools: Prokka (1.14.6) (20), Rapid Annotations using Subsystems Technology (RAST) Tool Kit (v.1.3) (21), and NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (v.6.10) (22, 23) (Table 1).

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AUTHOR AFFILIATIONS

[1]Department of Microbiology, Noakhali Science and Technology University (NSTU), Noakhali, Bangladesh
[2]Department of Environmental Biotechnology, Faculty of Biotechnology and Genetic Engineering, Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram, Bangladesh
[3]Department of Physiology, Biochemistry and Pharmacology, Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram, Bangladesh
[4]Department of Genomics and Bioinformatics, Faculty of Biotechnology and Genetic Engineering, Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram, Bangladesh

AUTHOR ORCIDs

Md. Habib Ullah Masum http://orcid.org/0000-0002-0055-1895

AUTHOR CONTRIBUTIONS

Mohammad Sharif Uddin, Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – review and editing | Kazi Chamonara, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – review and editing | Sumaia Sultana, Data curation, Formal analysis, Methodology | Sajedul Islam, Data curation, Formal analysis, Methodology | Afifa Siddiqua, Data curation, Formal analysis, Methodology | Md. Habib Ullah Masum, Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review and editing

DATA AVAILABILITY

All sequencing data generated in this study have been deposited in the NCBI BioProject database under accession number PRJNA1294324, and are publicly available through the Sequence Read Archive (SRA) (SRR34709126, SRR34709125, SRR34709124, and SRR34709123). The complete genome data have been submitted to NCBI GenBank with the accession numbers JBQRUG000000000.1, JBQWLY000000000.1, JBQWLZ000000000.1, and JBQWMA000000000.1. The RAST annotation files have been deposited in the figshare as File S1 (https://doi.org/10.6084/m9.figshare.30390940.v1).

ETHICS APPROVAL

The Ethics Approval Committee of Noakhali Science and Technology University (NSTUEC) reviewed and authorized the study protocol (Approval no. NSTU/SCI/EC/2025/451).

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