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Genomic Mining of a Gut-Associated Pseudomonas aeruginosa Isolated from Invasive Suckermouth Catfish in Bangladesh Reveal Bioremediation Potential and Biosafety Challenges

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Genomic Mining of a Gut-Associated *Pseudomonas aeruginosa* Isolated from Invasive Suckermouth Catfish in Bangladesh Reveal Bioremediation Potential and Biosafety Challenges

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Abstract:

Bioremediation is the process of using living microorganisms to clean up polluted environments by converting harmful substances into safer ones. Microorganisms can act as bioremediating agents because of the stringent biodegradability nature, high specificity, low toxicity in nature and high effectiveness activity under extreme conditions. This study aimed to isolate and genomically characterize bioremediating bacteria *Pseudomonas aeruginosa* BCSIR-JnU-ISO2 from the gut of the invasive suckermouth catfish (*Hypostomus plecostomus*) collected from the heavily polluted Buriganga River, Bangladesh. Out of the three isolates, one specific isolate *Pseudomonas aeruginosa* BCSIR-JnU-ISO2 was selected and focused due to the highest oil bioremediation potential in specific conditions. This isolate could utilize waste cooking oil (WCO) as a sole carbon source enriched in Bushnell-Haas broth. WCO biodegradation was qualitatively evaluated using the 2,6-dichlorophenolindophenol (DCPIP) assay. *Pseudomonas aeruginosa* BCSIR-JnU-ISO2 can produce surface-active agents, and it was qualitatively confirmed using drop-collapse, Parafilm M, and oil-spreading assays, and Fourier Transform Infrared Spectroscopy (FTIR). Whole-genome sequencing (WGS) of *Pseudomonas aeruginosa* BCSIR-JnU-ISO2 was employed to identify the genetic basis for hydrocarbon degradation, predicted heavy metal tolerance, and rhamnolipid biosynthesis.

Genomic analysis using PubMLST substantiated this isolate as *Pseudomonas aeruginosa*. Further analysis revealed a genetic repertoire, including genes associated with hydrocarbon biodegradation and the *rhl*ABCR operon (surface-active agents' production). Additionally, genomic annotation possesses genetic determinants predicted to confer multi-metal resistance, including arsenic (*arsBCH*), copper (*copBDR*), and cobalt-zinc-cadmium (*czc*) efflux systems. The isolate also produced presumptive crude surface-active compounds (2.7 g/L) and exhibited WCO degradation under laboratory conditions.

According to the genomic findings, *Pseudomonas aeruginosa* BCSIR-JnU-ISO2 is a candidate for the bioremediation of hydrocarbon- and metal-contaminated environments. However, this study is limited by the absence of phenotypic validation for metal resistance and the potential biosafety risks associated with releasing a WHO priority pathogen into the environment. Future research should also investigate strategies such as enzyme-based bioremediation, immobilized-cell

systems, or the exploitation of non-pathogenic hydrocarbon-degrading microorganisms to minimize biosafety risks while maintaining remediation efficiency.

Keywords: Rhamnolipids; Whole-genome sequencing; Waste cooking oil degradation; Metal resistance genes; Buriganga River; Fish gut microbiota

Background

The suckermouth catfish (SMCF), *Hypostomus plecostomus*, is widely recognized for its ability to persist in environmentally contaminated aquatic systems (1, 2). This resilience is supported by several physiological and morphological characteristics, including a protective armored body, a ventrally positioned suction mouth, the capacity for aerial respiration, and a high tolerance to hypoxia, organic pollution, and toxic contaminants (1, 3). These traits allow the suckerfish to establish stable populations in habitats where many native fishes decline or disappear. In several regions like Bangladesh, SMCF has been reported to dominate heavily polluted environments and influence benthic conditions through its feeding activity (4, 5).

This ecological pattern is particularly evident in the Buriganga River of Bangladesh, one of the most polluted river systems in South Asia. The river is characterized by extremely low dissolved oxygen, high organic load, heavy metal contamination, and continuous discharge of untreated industrial effluents (6, 7). Under these conditions, native fish diversity has decreased substantially, whereas SMCF remains abundant throughout the most contaminated reaches (15). As many native or commonly occurring freshwater species are either absent or present at low abundance in these highly polluted zones, this scenario is limiting consistent sampling and reducing their suitability as sources of pollution-adapted microbiota (8). On the other hand, the long-term persistence and dominance of SMCF under extreme environmental stress suggest as sources of pollution-adapted microbiota involving adaptive mechanisms that may involve functional contributions from its intestinal microbial community (6, 9, 10).

Previous studies also demonstrated that host-associated microbiota play an important role in supporting survival under adverse environmental conditions by contributing to nutrient utilization, detoxification processes, and the transformation of environmental pollutants (11, 12). In contaminated ecosystems, gut microbial communities may be selectively enriched with metabolically versatile and stress-tolerant bacteria capable of degrading complex organic

compounds or tolerating toxic substances (13). The gut microbiome of *H. plecostomus* is dominated by Actinobacteria, Firmicutes, and Proteobacteria, with core genera including *Mycobacterium*, *Clostridium*, and *Romboutsia* (6). Previous studies have reported that gut microorganisms of SMCF can participate in heavy metal transformation and may contribute to host adaptation in polluted environments(14). In addition, the bacterial species has been associated with reductions in organic matter, suspended solids, nutrients, and biochemical oxygen demand (BOD) in wastewater systems (6, 15). However, the specific functional and genetic contributions of its intestinal microbiota to these processes remain insufficiently characterized. Therefore, SMCF represents a practical sentinel host for exploring indigenous gut microorganisms and their genomic traits, which are naturally adapted to chronic environmental stress.

Among pollution-associated bacterial groups, members of the genus *Pseudomonas* are of particular interest due to their broad metabolic versatility. Many species produce crude surface-active agents, like biosurfactants that enhance the degradation of hydrophobic substrates such as oils and hydrocarbons and support survival under chemically stressful conditions (13, 16, 17). These functional properties highlight the ecological and biotechnological relevance of gut-associated bioremediating *Pseudomonas* in environmental detoxification and waste treatment applications.

Numerous studies indicate that environmental *P. aeruginosa* isolates may degrade about 60–90% of waste cooking oil (WCO) under optimized laboratory settings, concurrently generating significant amounts of crude biosurfactants. These strains generally possess conserved genes associated with lipase production (*lipA, lipB*), rhamnolipid biosynthesis (*rhlA, rhlB, rhlC*), fatty acid metabolism, β-oxidation, and aromatic hydrocarbon degradation, suggesting that WCO utilization is facilitated by an integrated metabolic network rather than a singular catabolic pathway. Nonetheless, whereas several studies have documented the biodegradation of WCO by environment-specific *Pseudomonas* isolates, there is limited understanding of pollution-adapted gut-associated bacteria and their genetic changes that may facilitate oil degradation in chronically contaminated aquatic habitats.

Therefore, this study focuses on the isolation and genomic characterization of a pollution-adapted *Pseudomonas spp.* from the gut of invasive SMCF collected from the Buriganga River. Specifically, the objectives were to (i) isolate *Pseudomonas aeruginosa* capable of utilizing waste

cooking oil as the sole carbon source, (ii) evaluate its crude surface-active agent's production using qualitative screening assays, and (iii) perform whole-genome analysis to identify genetic determinants associated with hydrocarbon degradation, predicted heavy metal tolerance, and crude surface-active agents' biosynthesis. By providing strain-level functional and genomic insights, this work evaluates the potential of SMCF gut microbiota as a source of indigenous microorganisms adapted to extreme pollution conditions and relevant for sustainable bioremediation applications.

Results

Isolation, and Identification of WCO-Degrading *Pseudomonas aeruginosa*

Sequential enrichment of suckermouth catfish intestinal homogenates in Bushnell-Haas broth containing waste cooking oil (WCO) as the sole carbon source resulted in visible turbidity and oil emulsification within 48 h, indicating the enrichment of WCO-utilizing bacteria (Figure 1A-B). After three rounds of enrichment, distinct bacterial colonies were recovered on Bushnell-Haas agar with 1% WCO and subsequently purified on cetrimide agar. Several isolates produced characteristic greenish fluorescent colonies after 18-72 h of incubation (Figure 1C-D). Six fluorescent colonies were initially recovered from which three morphologically distinct isolates (BCSIR-JnU-ISO1, BCSIR-JnU-ISO2, and BCSIR-JnU-ISO3) were selected for further characterization.

All three isolates exhibited biochemical characteristics typical of *Pseudomonas aeruginosa*, including positive reactions for oxidase, catalase, citrate utilization, motility, hemolysis, and CTAB agar activity, while showing negative reactions on Kligler Iron Agar (Figure 1E). Species identity was further supported by PCR amplification of the *O*-antigen acetylase gene, which produced the expected 232-bp amplicon in all three isolates (Figure 1F), confirming their assignment to the *P. aeruginosa*.

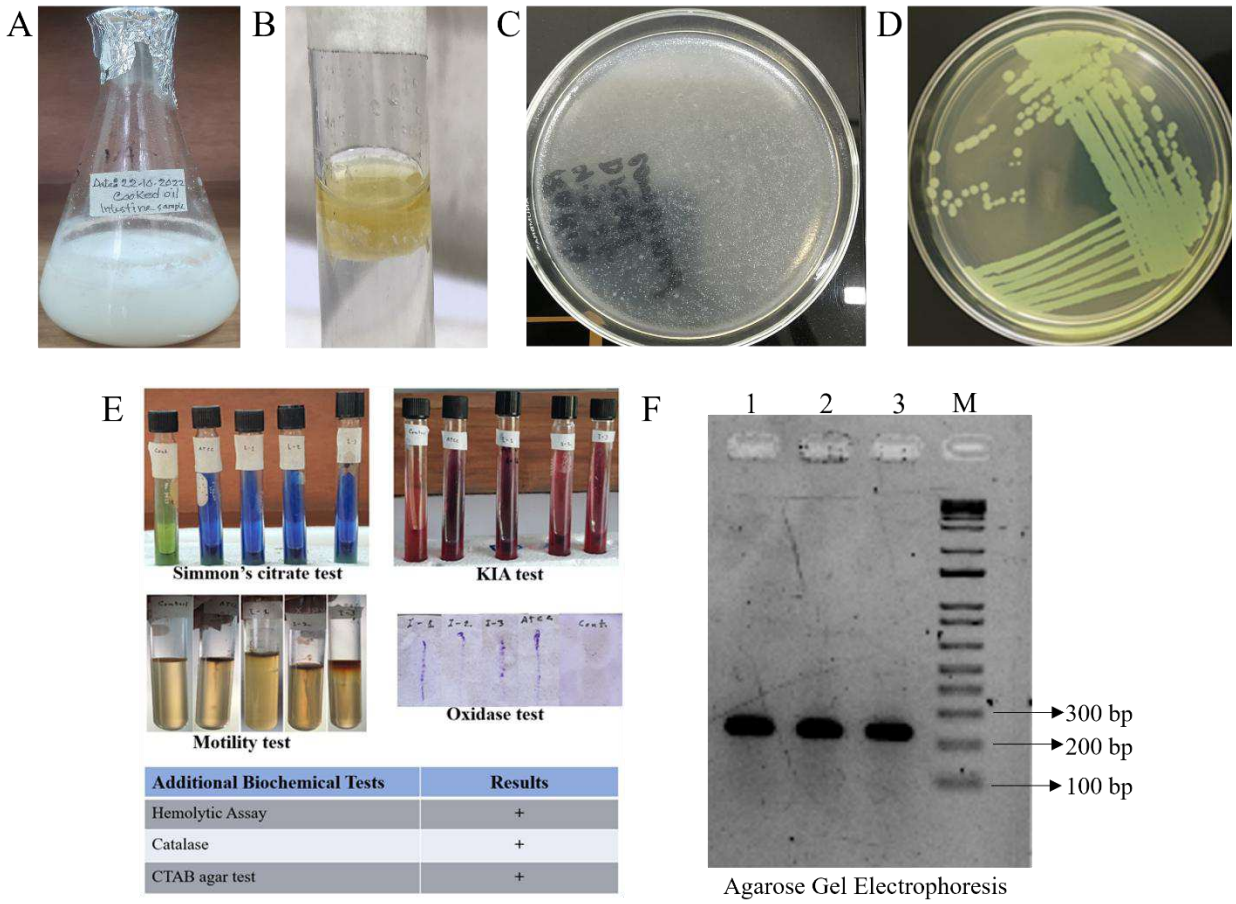


Figure 1: Isolation and identification of WCO-degrading *Pseudomonas aeruginosa*. (A, B) Sequential enrichment of gut homogenates in Bushnell-Haas broth supplemented with 5% waste cooking oil (WCO). (C, D) Representative colonies on selective media. (E) Biochemical characterization showing positive citrate, oxidase, motility, hemolysis, catalase, and CTAB agar tests, and a negative KIA reaction. (F) Species-specific PCR amplification of the *O*-antigen acetylase gene (232 bp) confirming isolates BCSIR-JnU-ISO1, BCSIR-JnU-ISO2, and BCSIR-JnU-ISO3; M, Invitrogen 1 kb Plus DNA ladder. The product size was 232 bp.

Waste Cooking Oil Degradation

All three isolates reduced DCPIP color intensity in WCO-containing medium. Gravimetric analysis after 72 hours, *Pseudomonas aeruginosa* BCSIR-JnU-ISO1 and BCSIR-JnU-ISO3 degraded 64.72% and 40.1% of WCO, respectively. BCSIR-JnU-ISO2, on the other hand, demonstrated a higher degradation efficiency, achieving 67.58% WCO degradation during the same period (Figure 3). These findings emphasize that BCSIR-JnU-ISO2 is the most promising candidate for oil bioremediation.



Figure 3: Oil Degradation Potential of *Pseudomonas aeruginosa* BCSIR-JnU-ISO2. Representative image of DCPIP test. Oil degradation percentage was measured using 0.01% DCPIP in BHB where WCO (5%) was used as carbon sources and gut isolated *Pseudomonas* pure culture were given to measure the percentage of degradation by each isolate. The color change from blue (Control) to colorless/light gray is indicative of WCO degradation after incubation. From left to right (control, isolate 1,2,3).

Screening for Presumptive Surface-active Agents Production

Preliminary screening tests such as oil spreading assay, drop-collapse assay and parafilm M-tests were employed to confirm presumptive biosurfactant produced by the identified *Pseudomonas aeruginosa* strains. All three isolates showed positive results in qualitative biosurfactant screening assays. Oil spreading assay produced clear displacement zones compared with the control (Figure 2A). Drop-collapse assay showed flattened droplets compared with the control (Figure 2B). Parafilm M test showed increased droplet diameter (9-11 mm) relative to the control (5 mm) (Figure 2C).

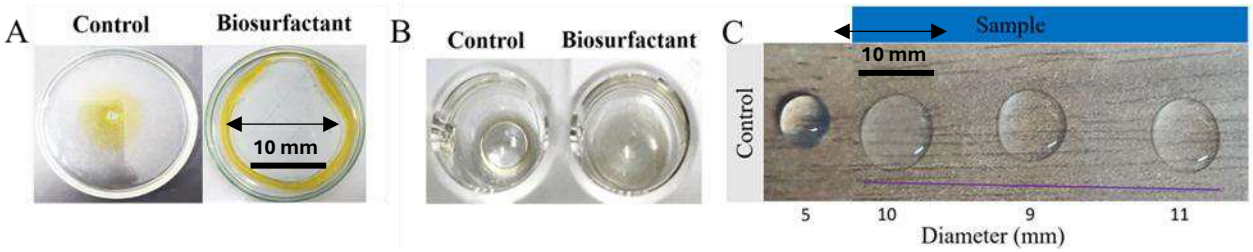


Figure 2: Presumptive Biosurfactant Production Potential test of *Pseudomonas aeruginosa* species by different screening assays. (2A) A clear zone is visible (right) compared to the control (left) in oil spreading assay. (2B) Drop collapse assay shows the culture supernatant (right) causing the droplet to flatten or collapse in the well plate compared to the water control (left), confirming the presence of surface-active agents. (2C) Droplets of the culture supernatant (sample) maintain a significantly larger diameter (9-11 mm) compared to the Control droplet (5 mm) on the hydrophobic Parafilm M surface. ⟷ indicate scale bars. Every experiment was replicated three times.

Quantification of the Extracted Surface-active Agents

The presumptive surface-active agents obtained from *Pseudomonas aeruginosa* BCSIR-JnU-ISO2 after drying represented a recovery yield of 2.7 g/L from 1L culture after 72 hours grown on WCO as the sole carbon source under the tested laboratory conditions. The product was stable, non-oily, and readily soluble in organic solvents, consistent with the characteristics of lipid-based biosurfactants (Figure 4).

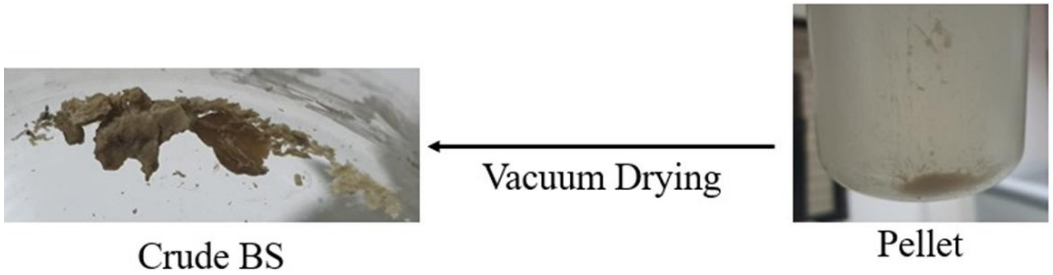
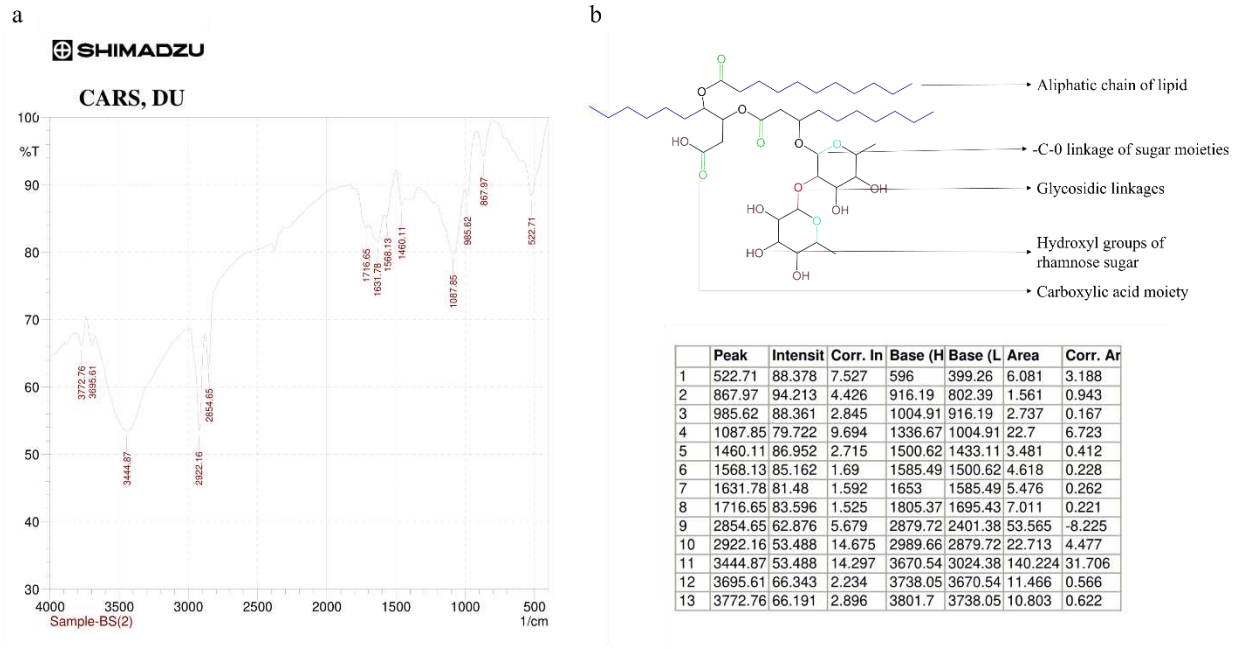


Figure 4: Quantification of the extracted crude biosurfactant produced by *Pseudomonas aeruginosa* BCSIR-JnU-ISO2. The representative image shows the recovered crude surface-active agents, yielding approximately 2.71 g/L after 72 hours of fermentation.

FTIR Characterization of the Presumptive Surface-active agents (biosurfactants)

FTIR analysis of the extracted biosurfactant product from isolate BCSIR-JnU-ISO2 showed characteristic absorption peaks at 3444, 2922, 2854, 1716, and 1087 cm^{-1} (Figure 5). The spectral profile corresponded to hydroxyl, aliphatic, and ester functional groups typical of glycolipid biosurfactants such as rhamnolipid.



	Peak	Intensit	Corr. In	Base (H	Base (L	Area	Corr. Ar
1	522.71	88.378	7.527	596	399.26	6.081	3.188
2	867.97	94.213	4.426	916.19	802.39	1.561	0.943
3	985.62	88.361	2.845	1004.91	916.19	2.737	0.167
4	1087.85	79.722	9.694	1336.67	1004.91	22.7	6.723
5	1460.11	86.952	2.715	1500.62	1433.11	3.481	0.412
6	1568.13	85.162	1.69	1585.49	1500.62	4.618	0.228
7	1631.78	81.48	1.592	1653	1585.49	5.476	0.262
8	1716.65	83.596	1.525	1805.37	1695.43	7.011	0.221
9	2854.65	62.876	5.679	2879.72	2401.38	53.565	-8.225
10	2922.16	53.488	14.675	2989.66	2879.72	22.713	4.477
11	3444.87	53.488	14.297	3670.54	3024.38	140.224	31.706
12	3695.61	66.343	2.234	3738.05	3670.54	11.466	0.566
13	3772.76	66.191	2.896	3801.7	3738.05	10.803	0.622

Figure 5: Presumptive rhamnolipid secreted from *Pseudomonas aeruginosa* BCSIR-JnU-ISO2. (5A) FTIR Spectroscopy analysis of the produced surface-active agents. The spectrum displays characteristic peaks indicative of a rhamnolipid structure **(5B)** According to the peaks; functional groups were matched with presumptive rhamnolipid structure. The structure represents a di-rhamnolipid molecule, where the color-coded lines on the structure correspond to the functional groups identified in the FTIR spectrum.

Genomic Insights of *Pseudomonas aeruginosa* BCSIR-JnU-ISO2

The whole-genome sequencing of the *Pseudomonas aeruginosa* strain isolated revealed a complex genome harboring significant bioremediation potential. Genomic analysis identified specialized operons involved in surface active agents' production, specifically for presumptive rhamnolipid biosynthesis, as well as pathways for hydrocarbon biodegradation and heavy-metal tolerance. To ensure data accessibility and reproducibility, the raw sequencing reads generated in this study were deposited in the NCBI Sequence Read Archive (SRA) database under the accession number

SRR36346617. The assembled genome consisted of 422 contigs with a total size of 6,414,891 bp and an average G+C content of 66.05%. Genome annotation identified 6,493 protein-coding sequences, 53 tRNA genes, and 3 rRNA genes. PubMLST analysis classified the isolate as *Pseudomonas aeruginosa* (Figure 6A). The genome was further annotated using the RAST toolkit (RASTtk) and assigned the identifier 286.10436 under bacterial genetic code 11 (Figure 6B). Phylogenetic analysis placed strain BCSIR-JnU-ISO2 within a cluster containing *P. aeruginosa* reference strains, including *Pseudomonas aeruginosa* DSM 50071 (replicated thrice), *Pseudomonas paraeruginosa* ATCC 9027T, *Pseudomonas delhiensis* CCM 7361, along with closely related species such as *P. paraeruginosa*, *P. delhiensis*, *P. humi*, *P. citronellolis*, and *P. nitroreducens* (Figure 6C). This clustering reflects its evolutionary relationship within the *Pseudomonas* genus and suggests close genetic affiliation with the *P. aeruginosa* lineage. Average Nucleotide Identity (ANI) analysis showed 99.1% similarity with *P. aeruginosa* PAO1 (GCF_000006765.1), with 5,354 shared gene clusters between the two genomes (Figure 6D). These results confirm the species-level identity of strain BCSIR-JnU-ISO2 and indicate a high degree of genomic similarity to the reference strain.

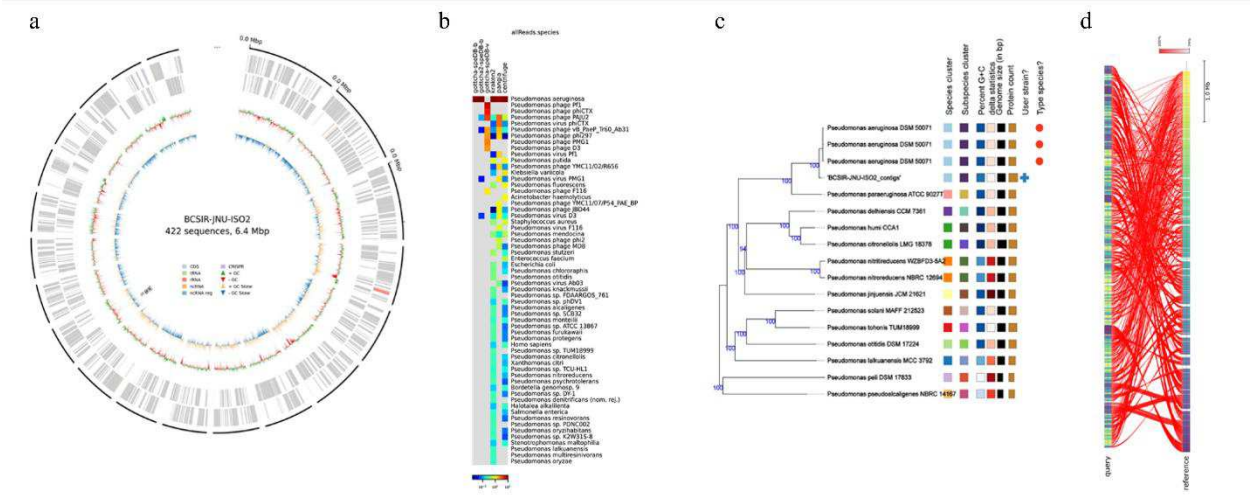


Figure 6: Genomic Features, Taxonomic Classification, Phylogenetic Analysis, and Average Nucleotide Identity of *Pseudomonas* BCSIR-JNU-ISO2. (6A) Genome visualization were done using the Bakta web tool (42) highlights the key genomic features, including annotated coding sequences (CDS) and tRNA genes. **(6B)** Taxonomic classification were done using multiple classifiers (PANGIA, Kraken2, Centrifuge, and GOTTCHA) through the EDGE Bioinformatics platform (36) **(6C)** Phylogenetic tree were constructed using the TYGS web tool that illustrates its

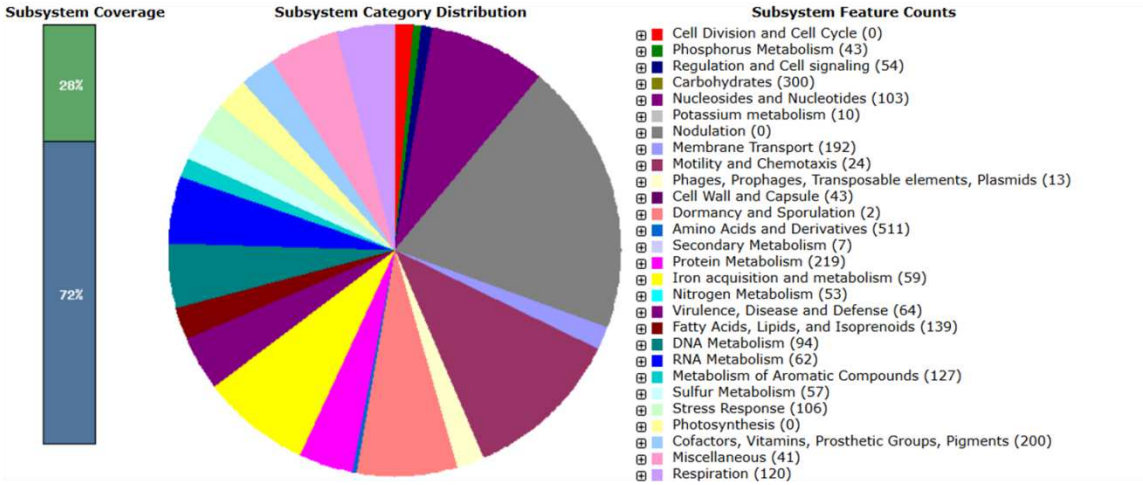
evolutionary relationship with other *Pseudomonas* strains. (**6D**) ANI of *Pseudomonas aeruginosa* BCSIR-JnU-ISO2 with one reference strain of *Pseudomonas* were done using the PROKSEE tool.

RAST, COG, and KEGG Pathway Analysis

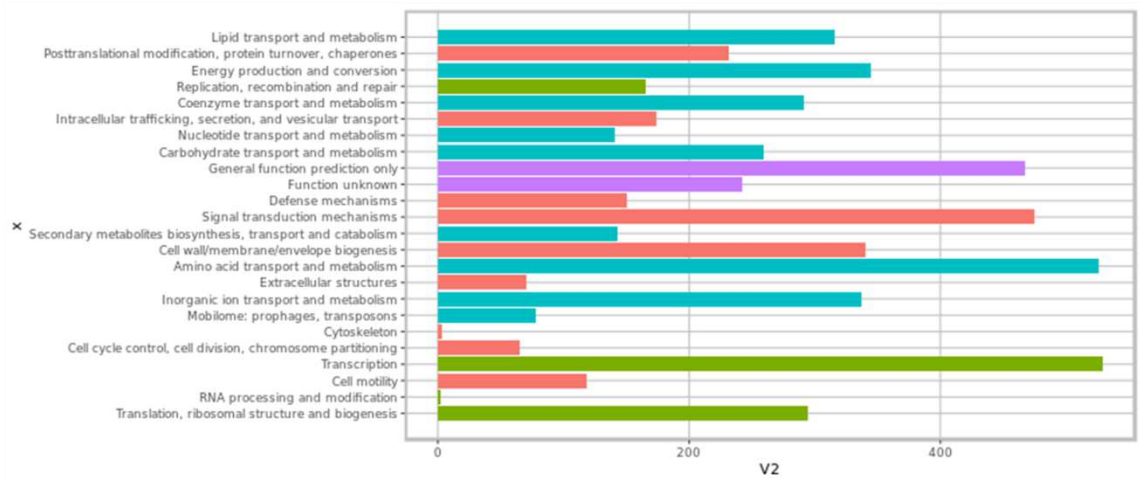
Pseudomonas aeruginosa BCSIR-JnU-ISO2 genome identified genes distributed across multiple functional categories. A total of 127 features were assigned to the "Metabolism of Aromatic Compounds" subsystem, indicating the presence of genes associated with the degradation of aromatic hydrocarbons. Genes were also abundant in core metabolic categories, such as, "Amino Acids and Derivatives" (511 features) and "Fatty Acids, Lipids, and Isoprenoids" (139 features). In addition, 106 features were assigned to the "Stress Response" subsystem, while categories related to "Membrane Transport" (192 features) and "Iron acquisition and metabolism" (59 features) were also identified. The "Respiration" subsystem contained 120 features, including genes associated with aerobic and alternative respiratory processes (Figure 7A).

Functional classification using Cluster of orthologous (COG) analysis revealed genes involved in metabolism, cellular processes and signaling, and information storage and processing (Figure 7B). KEGG pathway mapping identified genes associated with xenobiotic degradation, energy metabolism, carbohydrate metabolism, and lipid metabolism (Figure 7C). Enzyme annotations included monooxygenases, dioxygenases, and dehydrogenases involved in the oxidative breakdown of hydrocarbons and aromatic compounds. Transporter and efflux-related genes were detected in both COG and KEGG categories. KEGG analysis also identified pathways related to fatty acid metabolism and glycolipid biosynthesis, including genes associated with rhamnolipid production and quorum-sensing regulatory systems. Genes related to oxidative stress response, membrane function, and cellular homeostasis were also identified.

a


Subsystem Coverage
28%
72%
Subsystem Category Distribution
Subsystem Feature Counts
Cell Division and Cell Cycle (0)
Phosphorus Metabolism (43)
Regulation and Cell signaling (54)
Carbohydrates (300)
Nucleosides and Nucleotides (103)
Potassium metabolism (10)
Nodulation (0)
Membrane Transport (192)
Motility and Chemotaxis (24)
Phages, Prophages, Transposable elements, Plasmids (13)
Cell Wall and Capsule (43)
Dormancy and Sporulation (2)
Amino Acids and Derivatives (511)
Secondary Metabolism (7)
Protein Metabolism (219)
Iron acquisition and metabolism (59)
Nitrogen Metabolism (53)
Virulence, Disease and Defense (64)
Fatty Acids, Lipids, and Isoprenoids (139)
DNA Metabolism (94)
RNA Metabolism (62)
Metabolism of Aromatic Compounds (127)
Sulfur Metabolism (57)
Stress Response (106)
Photosynthesis (0)
Cofactors, Vitamins, Prosthetic Groups, Pigments (200)
Miscellaneous (41)
Respiration (120)

b


Lipid transport and metabolism
Posttranslational modification, protein turnover, chaperones
Energy production and conversion
Replication, recombination and repair
Coenzyme transport and metabolism
Intracellular trafficking, secretion, and vesicular transport
Nucleotide transport and metabolism
Carbohydrate transport and metabolism
General function prediction only
Function unknown
Defense mechanisms
Signal transduction mechanisms
Secondary metabolites biosynthesis, transport and catabolism
Cell wall/membrane/envelope biogenesis
Amino acid transport and metabolism
Extracellular structures
Inorganic ion transport and metabolism
Mobilome: prophages, transposons
Cytoskeleton
Cell cycle control, cell division, chromosome partitioning
Transcription
Cell motility
RNA processing and modification
Translation, ribosomal structure and biogenesis
0
200
400
V2

c

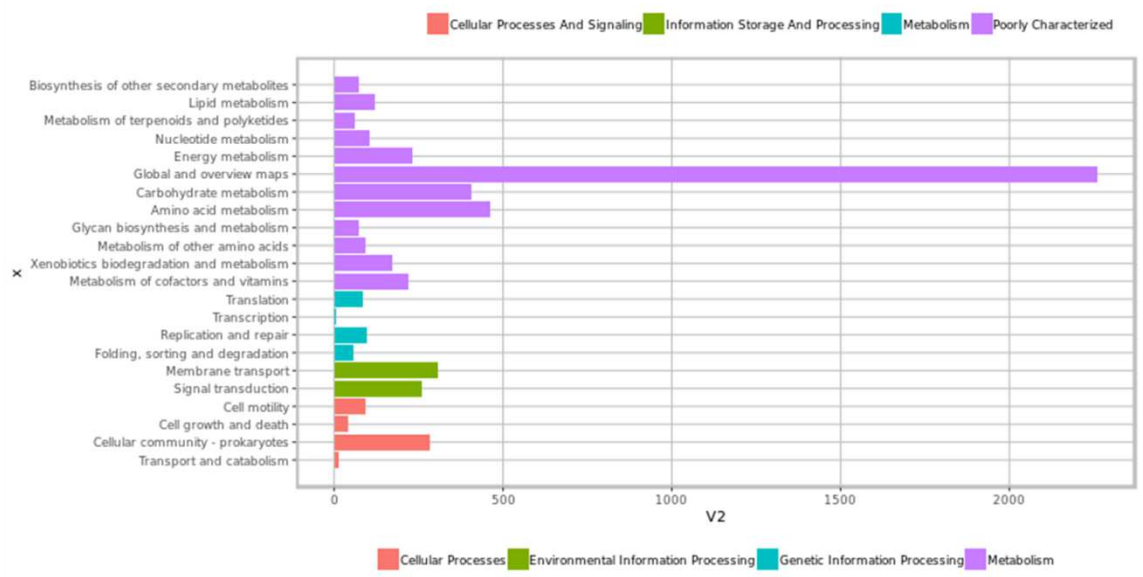

Cellular Processes And Signaling
Information Storage And Processing
Metabolism
Poorly Characterized
Biosynthesis of other secondary metabolites
Lipid metabolism
Metabolism of terpenoids and polyketides
Nucleotide metabolism
Energy metabolism
Global and overview maps
Carbohydrate metabolism
Amino acid metabolism
Glycan biosynthesis and metabolism
Metabolism of other amino acids
Xenobiotics biodegradation and metabolism
Metabolism of cofactors and vitamins
Translation
Transcription
Replication and repair
Folding, sorting and degradation
Membrane transport
Signal transduction
Cell motility
Cell growth and death
Cellular community - prokaryotes
Transport and catabolism
0
500
1000
1500
2000
V2
Cellular Processes
Environmental Information Processing
Genetic Information Processing
Metabolism

Figure 7: Metabolic Capabilities of *Pseudomonas aeruginosa* BCSIR-JnU-ISO2. (**7A**) Subsystem-based functional annotation of *Pseudomonas aeruginosa* BCSIR-JnU-ISO2. **(7B)** COG-based analysis of functional genes in *Pseudomonas aeruginosa* BCSIR-JnU-ISO2. **(7C)** KEGG-based analysis showing the metabolic adaptability of *Pseudomonas aeruginosa* BCSIR-JnU-ISO2.

Hydrocarbon Degradation and Heavy Metal Resistance Potential

Genome analysis of *Pseudomonas aeruginosa* BCSIR-JnU-ISO2 identified multiple gene clusters associated with surface-active agents' production, hydrocarbon metabolism, stress tolerance and heavy metal resistance. The rhamnolipid biosynthesis and regulatory genes (*rhlA, rhlB, rhlC*, and *rhlR*) were detected, along with lipid metabolism genes (*lip1, lipA*, and *lipB*) involved in fatty acid hydrolysis and utilization. Several genes related to aromatic compound degradation were also identified, including *benC*, *catA*, *catB*, *catC*, *pcaG*, *pcaH*, and *pobA*, which are components of the catechol and protocatechuate pathways. The genome contained *bphD*, associated with biphenyl degradation, and *roxA*, encoding rubber oxygenase involved in the oxidative cleavage of poly (cis-1,4-isoprene) (Figure 8A). Multiple genes associated with multidrug and biocide resistance were identified. These included several Resistance-Nodulation-Division (RND) family efflux pump components, such as *mexV*, *mexW*, *mexD*, and *mexY*, located on both forward and reverse genomic strands. Regulatory genes involved in the control of efflux system expression, including *soxR* and *mexT*, were also detected (Figure 8B). Genes associated with resistance to heavy metals are present, including the arsenic resistance operon (*arsB, arsC, and arsH*), copper homeostasis genes (*copB, copD*, and *copR*), and the cobalt-zinc-cadmium efflux system (*czcA, czcB, czcC*, and *czcD*). Zinc uptake and regulatory components (*znuA, znuB, znuC, zntR*, and *smtB*) and the mercury resistance regulator gene (*merR*) were also detected (Figure 8C).

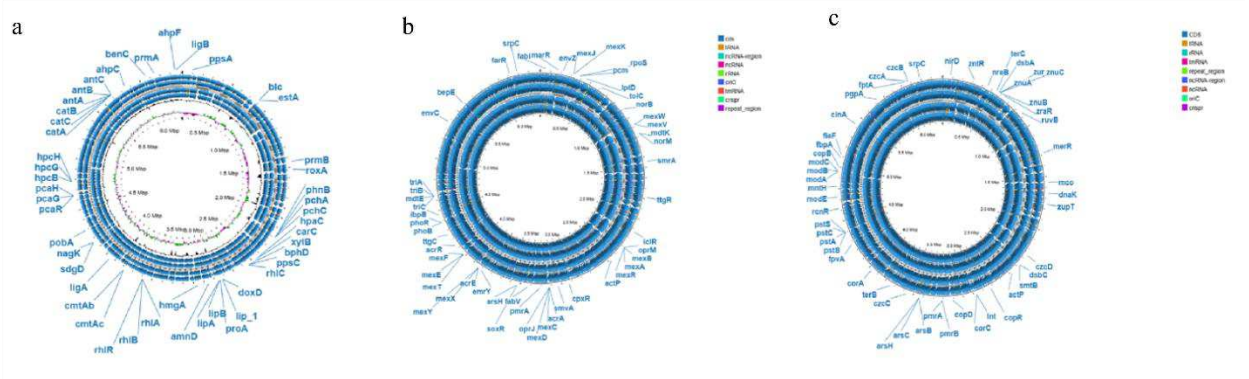


Figure 8: Gene Mapping of *Pseudomonas* aeruginosa BCSIR-JnU-ISO2. **(8A)** Hydrocarbon aerobic degradation genes. **(8B)** Biocide resistance genes. **(8C)** Metal resistance genes.

Materials and Methods

Ethical approval and consent to participate

This study involves Suckermouth catfish (*Hypostomus plecostomus*) which is not endangered or protected species in Bangladesh (18, 19). Ethical review committee, Faculty of life and Earth Sciences, Jagannath University, Bangladesh granted an exemption from ethical approval for this study. All experimental procedures were conducted according to the principles of the ethical committee, Jagannath University, Bangladesh and the National Research Ethics Committee (NREC) of Bangladesh following previous report (19).

Sample Collection & Processing

Suckermouth catfish (*Hypostomus plecostomus*) were collected from the heavily polluted Buriganga, Dhaka, Bangladesh, between July 2023 and June 2025. Fish samples were collected by professional fishermen targeting indigenous fish species, where invasive suckermouth catfish were obtained as bycatch. The collected fish were already dead or moribund at the time of sampling; therefore, no euthanasia or experimental sacrifice was performed as part of this study. Sampling was conducted at multiple representative sites along the river stretch encompassing Sadar Ghat and Lalkuti Ghat, two major riverine zones characterized by high anthropogenic pressure and fishing activity. The geographic coordinates of the sampling area ranged between 23°42′00″–23°47′20″ N latitude and 90°22′00″–90°27′30″ E longitude (Figure 9A). Immediately after capture, fish samples were transported to the microbiology laboratory within 2-3 hours in insulated refrigerated containers. Under aseptic conditions, the intestinal tract was excised using sterile instruments such as sharp knife and forceps (Figure 9B and C), homogenized using a sterile blender, and serially diluted in sterile 0.85% (w/v) NaCl solution following standard microbiological standard (Figure 9D).

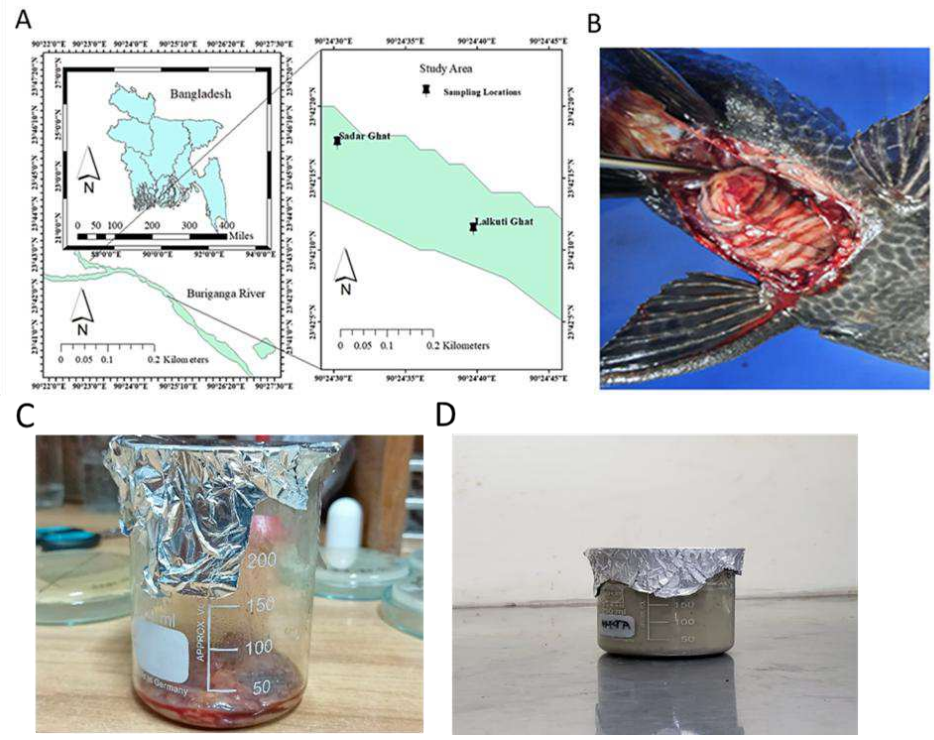


Figure 9: Sample collection and processing for isolating *Pseudomonas aeruginosa* from suckermouth catfish. **(9A)** Represents the location of the sampling site. **(9B)** Fish stomach was open with sterile forceps. **(9C)** 10 g intestinal contents of SMCF were taken into a sterile beaker. **(9D)** Homogenized intestinal contents.

Enrichment of Bioremediating Bacteria

An enrichment technique (20-22) with some modifications was used to selectively isolate bioremediating bacteria by providing waste cook oil (WCO) as the sole carbon and energy source in media. Successive enrichment steps with oil concentration were employed to enhance selective pressure, allowing microorganisms capable of utilizing hydrophobic substrates to proliferate while suppressing non-adapted microbial populations. Bushnell-Haas broth (MBHB) was used to isolate oil-degrading bacteria. First, enrichment cultures were conducted in 250 mL Erlenmeyer flasks, with 50 mL of BHB, 5% filtered WCO and 1 mL of homogenized sample. The flasks were incubated for three days at 37 °C in a rotary shaker (Orbitek Shaker, Scigenics Pvt. Ltd., Chennai, India) set to 180 rpm. The BHB inoculum was serially diluted (10^{-1} to 10^{-10}) and then 100 µL of inoculum from each dilution was spread on Modified Bushnell-Hass Agar (MBHA) plate. The Modified Bushnell-Haas agar (MBHA) was prepared by supplementing BHB with 1.2% (w/v)

agar and 1% (v/v) waste cooking oil as the sole carbon source. Some single colonies grown were targeted for further study.

Isolation and Identification of Biosurfactant-Producing *Pseudomonas aeruginosa*

Single colonies from the enrichment culture were streaked onto Cetrimide agar, a selective medium for *Pseudomonas aeruginosa*, using sterile loops and incubated at 37 °C for 18–72 hours. Colonies exhibiting characteristic green pigmentation and fluorescence were presumptively identified as *P. aeruginosa* and subjected to standard biochemical tests, including Kligler Iron Agar (KIA), Sulfur Indole Motility (SIM), oxidase, and catalase assays (23). *Pseudomonas aeruginosa* ATCC 27853 was included as a positive control strain to validate the biochemical test results.

Three single isolates were selected for further molecular identification, among which one isolate demonstrated superior bioremediation potential. Genomic DNA was extracted from overnight cultures grown in Luria-Bertani (LB) broth using the Wizard Genomic DNA Purification Kit (Promega, USA). Species-specific confirmation was performed using PCR assay targeting the O-antigen acetylase gene, a marker for *P. aeruginosa*. PCR amplification employed the primer set PA431CF (5′-CTGGGTCGAAAGGTGGTTGTTATC-3′) and PA431CR (5′-GCGGCTGGTGCGGCTGAGTC-3′), producing a 232-bp amplicon. The cycling conditions consisted of an initial denaturation at 98 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 10 min (24).

Oil Degradation Potential

The oil degradation potential of the three *P. aeruginosa* isolates was assessed in batch culture experiments using 100-mL screw-capped flasks containing 25 mL of BHB supplemented with 5% (w/v) WCO and 0.01% DCPIP. Flasks were inoculated with 0.5 mL of actively growing culture ($OD_{600} \geq 0.6$) of three isolates, while uninoculated media served as controls. Finally, DCPIP color degradation percentage is measured. All treatments were performed in triplicate and incubated at 30°C with shaking at 180 rpm for 4 days (32, 33).

Screening of Surface-active Agents' Production

For surface-active agents' production, isolates were cultured in BHB supplemented with WCO as the sole carbon source. Surface-active agents' production was evaluated using a number of assays, including the drop-collapse test, oil-spreading assay (25, 26). Positive isolates were further

validated using the Parafilm M test and microplate-based surface tension assays. Triton X-100 and sterile deionized water were used as positive and negative controls, respectively.

Extraction, Purification and FTIR Analysis of Surface-active Agents'

After 72 hours fermentation in BHB media including 5% WCO, solvent extraction was executed to extract crude surface-active agents. A 25 mL aliquot of the culture broth was centrifuged at 7,100×*g* for 20 minutes to separate biomass (27). Residual undegraded oil droplets and surface-associated biofilm-like polysaccharide material present at the supernatant interface were carefully removed by pipetting. The clarified supernatant was subsequently filtered through sterile Whatman No. 1 filter paper to obtain a cell-free filtrate that was used for further analysis. The pH of the supernatant was lowered to 2.0 using 2M HCl to facilitate acid precipitation of the biosurfactant. The resulting precipitate was collected by centrifugation at 7,100 × g for 20 minutes. The pellet was then redissolved in 25 mL of a chloroform: methanol mixture (2:1, v/v). The mixture was vigorously agitated to enhance the extraction of lipid-based surfactants into the organic phase. After phase separation, the lower organic layer containing the surface-active agents' was carefully collected and evaporated to dryness using a rotary evaporator at 40 °C (28). A dried whitish product was obtained. The yield of the isolated biosurfactant was expressed as g/L. Structural characterization of the purified biosurfactant was performed using Fourier Transform Infrared (FTIR) spectroscopy (29). Approximately 1 mg of dried biosurfactant was mixed with 100–200 mg of finely powdered potassium bromide (KBr) and compressed into a transparent pellet under high pressure (10-15 tons for 1-2 min) (30, 31). FTIR spectra were recorded using a Bruker Alpha II spectrometer over a range of 4000-400 cm^{-1} at a resolution of 4 cm^{-1}, with 32-64 scans per sample. Functional groups were identified based on characteristic absorption peaks.

Whole-Genome Sequencing and Assembly

Based on superior bioremediation, one isolate was selected for whole-genome sequencing. Genomic DNA was extracted using the QIAamp DNA Kit (Qiagen, USA), and DNA quality and concentration were verified prior to library preparation. Sequencing libraries were prepared using the Nextera DNA FLEX Library Preparation Kit (Illumina, USA) and sequenced on an Illumina MiniSeq platform, generating 2 × 150 bp paired end reads. Raw sequencing data were quality-checked using FastQC (v0.11) (34), and low-quality reads and adapter sequences were removed using Trimmomatic (v0.39) (35). High-quality reads were de novo assembled into contigs using

the assembly pipelines of the PATRIC (36, 37) and EDGE (38) bioinformatics platforms to generate a high-quality draft genome.

Genome Annotation, Phylogeny, Subsystem Analysis (RAST)

Species confirmation and strain typing were conducted using the PubMLST database (39) and EDGE (38) platform. Whole-genome-based phylogenetic analysis was performed using the Type Strain Genome Server (TYGS) (40), while genomic relatedness to closely related strains was assessed by Average Nucleotide Identity (ANI) analysis using FastANI (41). Genome annotation was performed using the PATRIC (35). Subsystem-based functional categorization was carried out using the RAST server (https://rast.nmpdr.org/). Genes associated with heavy-metal resistance and bioremediation were identified using tools from the Center for Genomic Epidemiology (CGE) (42) and visualized using the PROKSEE platform (43).

COG and KEGG Functional Analysis

Predicted protein-coding sequences were mapped to COG functional categories and KEGG Orthology identifiers using the Type Strains Genome Database (GTyPe) (https://gctype.wdcm.org/) (44). Metabolic pathways were reconstructed to identify genes involved in hydrocarbon degradation, biosurfactant synthesis, energy metabolism, and environmental adaptation.

Discussion

The present study describes the isolation and genome-mining of a gut-associated *Pseudomonas aeruginosa* strain (BCSIR-JnU-ISO2) from invasive suckermouth catfish inhabiting the heavily polluted Buriganga River. The isolate demonstrated the ability to utilize waste cooking oil (WCO) as the sole carbon source and produced surface-active compounds consistent with rhamnolipid-type biosurfactants. Genomic analysis further revealed genes associated with biosurfactant synthesis, hydrocarbon metabolism, stress response, and predicted metal resistance. Collectively, these findings indicate that the strain possesses genetic and functional attributes consistent with adaptation to contaminated environments and potential relevance for environmental applications.

The enrichment strategy using Bushnell-Haas medium supplemented with WCO, followed by selective isolation on cetrimide agar, is consistent with established approaches for recovering hydrocarbon-degrading *Pseudomonas spp* (45, 46). Fluorescent colonies indicative of pyoverdine production suggest ecological fitness under iron-limited conditions commonly observed in polluted aquatic systems (47). Biochemical profiling and species-specific PCR targeting the O-

antigen acetylase gene confirmed the isolates as *P. aeruginosa* (48-50). The recovery of *P. aeruginosa* from intestines suggests that this species may represent a persistent component of the gut microbiota of bottom-dwelling fish exposed to chronic environmental contamination, consistent with previous reports of pollution-associated *Pseudomonas* in aquatic organisms (17, 51, 52).

Surface-active agents' production was supported by complementary qualitative assays, including oil spreading, drop-collapse, and Parafilm M tests. Although these methods are preliminary, their combined application reduces false-positive interpretation and is widely accepted for initial screening (25, 53-55). FTIR analysis indicated functional groups consistent with a rhamnolipid-type glycolipid (43-45), and the detection of the complete *rhlABCR* cluster provides genomic support for rhamnolipid biosynthesis (56, 57). However, detailed structural characterization using mass spectrometric techniques would be required to confirm the predicted surface-active agents' composition and purity.

BCSIR-JnU-ISO2 isolate demonstrated substantial WCO degradation under laboratory conditions, as indicated by DCPIP reduction (20). These experiments were conducted under aerobic shaking conditions to evaluate environmental biodegradation potential. It should be noted, however, that the intestinal environment of fish is typically microaerophilic to anaerobic (58). Therefore, the experimental conditions used in this study do not fully reflect the physicochemical conditions of the gut. Genome annotation identified genes within the respiration subsystem that are associated with facultative metabolic flexibility and alternative respiratory processes, suggesting the capacity to tolerate reduced oxygen levels (59). However, any proposed role of this strain in host-microbe functional synergy within the fish gut should be interpreted cautiously, and future studies should evaluate metabolic activity under gut-mimicking oxygen conditions to better assess in vivo relevance.

Whole-genome sequencing of BCSIR-JnU-ISO2 revealed a genome structure comparable to environmental *P. aeruginosa* strains, with high similarity to reference genomes and the presence of conserved core functions (60-62). Subsystem annotation assigned functions to approximately 34% of predicted coding sequences, indicating that a substantial portion of the genome remains uncharacterized. Within the annotated fraction, genes related to central metabolism, membrane transport, stress response, and xenobiotic transformation were identified, suggesting metabolic

versatility. The annotated genome contained multiple genes associated with the degradation of aromatic and hydrocarbon compounds, including pathways for catechol and protocatechuate metabolism (*catABC*, *pcaGH*, *pobA*), as well as *benC* and *bphD* that are linked to the transformation of structurally complex aromatic substrates (63, 64). Genes involved in lipid metabolism (*lipA*, *lipB*) further support the ability to utilize oil-derived substrates, consistent with the observed growth on WCO. KEGG and COG analyses also identified functional categories related to energy metabolism, transport systems, and oxidative enzymes (monooxygenases and dioxygenases), which are commonly involved in hydrocarbon oxidation and integration into central metabolism (65, 66).

The genome also harbours multiple genetic determinants associated with metal homeostasis and resistance, including arsenic (*ars*), copper (*cop*), cobalt-zinc-cadmium (*czc*), zinc transport (*znuABC*), and mercury regulation (*merR*). Such gene clusters are frequently reported in bacteria inhabiting contaminated environments and may contribute to survival under combined chemical stress (67, 68). For example, gut bacteria in SMCF contribute to heavy metal resistance by transforming toxic metals into less bioavailable or less toxic forms through enzymatic detoxification, efflux, and biotransformation processes, thereby reducing metal accumulation and enhancing host survival in contaminated environments (69, 70). However, these traits were inferred solely from genomic annotation, and no phenotypic validation of heavy metal tolerance was performed. Therefore, metal resistance and biodegradation capacity should be regarded as predicted potential rather than experimentally confirmed functions.

Additional features related to oxidative stress defense, iron acquisition, membrane transport, and alternative respiration suggest adaptation to fluctuating redox conditions typical of polluted sediments and wastewater environments (69, 70). The detection of the *roxA* gene further indicates potential capacity for degradation of polymeric substrates such as natural rubber, supporting the broad catabolic profile commonly observed in environmental *Pseudomonas* strains (71-73). Despite these findings, several limitations should be considered. Gene presence does not necessarily indicate expression or activity under environmental conditions. Phenotypic validation of heavy metal tolerance, assessment of anaerobic or microaerophilic degradation, and transcriptomic or proteomic analyses would be required to confirm the ecological functionality of this strain.

Overall, the genomic and functional profile of *P. aeruginosa* BCSIR-JnU-ISO2 indicates a metabolically flexible organism with capabilities for surface-active agents' production, predicted hydrocarbon transformation, and tolerance to multiple environmental stressors. These features are consistent with adaptation to polluted habitats such as the Buriganga River and suggest that the strain may represent a promising candidate for sustainable bioremediation strategies, pending comprehensive functional validation and biosafety assessment.

Conclusion

This study focused on genome-mining of a gut-associated *Pseudomonas aeruginosa* BCSIR-JnU-ISO2 isolated from suckermouth catfish inhabiting the polluted Buriganga River, demonstrating aerobic waste cooking oil utilization, surface-active (rhamnolipid-type) production, and genomic features associated with hydrocarbon metabolism, stress response, and metal homeostasis. These findings indicate predicted metabolic potential consistent with adaptation to contaminated environments and relevance for future bioremediation research. However, functional interpretations are limited by the absence of phenotypic validation of heavy metal tolerance, the aerobic experimental conditions that do not fully reflect the microaerophilic fish gut environment, and the fact that only 34% of the genome received subsystem annotation. Therefore, further studies under low-oxygen conditions, with functional validation and biosafety assessment, are required before environmental application.

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Data Availability

The present whole-genome shotgun project has been deposited in NCBI. The associated BioSample and BioProject identifiers are SAMN53736840 and PRJNA1376520, respectively. Raw sequencing reads are available in the Sequence Read Archive under accession number SRR36346617.

Authors' contributions:

AKA, AAM, MMHS, MASS, SA conceived and designed the project. MMHS, NU, and STT performed genomic analyses with assistance from JF, MASS, and AS. MASS conducted enrichment, microbial isolation, purification, and quantification steps with help from AKA, SJB, and RH. MMHS and MASS generated the figure with assistance from SFC and SRN. AKA, MZM, MAI, and MSI discussed the results. MASS, AKA, NZ wrote the first draft of the manuscript; NZ and MMHS finalized the manuscript. All the authors contributed to manuscript revision and approved the final manuscript.

Declaration of interests

All the contributing 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. They declare that they have no conflict of interest regarding contribution, submission and publication.

Declarations

Ethical clearance for this study was formally sought from the Ethical Review Committee of the Faculty of Life and Earth Sciences, Jagannath University. The committee reviewed the project and confirmed that it is exempt from ethical review, as the study involved only non-human, non-mammalian organisms (fish) and did not include procedures causing pain, distress, or harm beyond standard handling practices. The exemption letter (dated June 01, 2024) issued by the Dean, Faculty of Life and Earth Sciences, Jagannath University, was submitted with the manuscript as supplementary file.

Figure Legends

Figure 1: Identification of WCO-utilizing bacteria by sequential enrichment, cultural morphology, biochemical test and PCR assay.

(A) Figure 1A represents the representative image of first enrichment. **(B)** Figure 1B represents the representative image of second enrichment. **(C)** Figure 1C represents the representative image of 3rd enrichment. **(D)** Figure 1D represents the representative image of subcultured fluorescent *Pseudomonas* spp. colonies. **(E)** Figure 1E represent the representative image of Simmon's citrate test (positive result indicated by blue color change), KIA test, motility test (diffuse growth) and oxidase test (positive result indicated by purple color). The table summarizes the positive (+) ve results for the hemolytic activity, catalase test, and CTAB agar test, which collectively confirm the presumptive identity of the isolates as *Pseudomonas aeruginosa*. **(F)** Figure 1F represent the representative images of lanes 1–3 which confirmed *Pseudomonas aeruginosa* BCSIR-JnU-ISO1, BCSIR-JnU-ISO2, and BCSIR-JnU-ISO3, respectively; M denotes the Invitrogen 1kb+ ladder. The product size was 232 bp. Primer sequences used were PA431CF (5′-CTGGGTCGAAAGGTGGTTGTTATC-3′) and PA431CR (5′-GCGGCTGGTGCGGCTGAGTC-3′).

Figure 2: Biosurfactant Production Potential test of *Pseudomonas aeruginosa* species by different screening assays. Different screening assays such as oil spreading assay, drop-collapse

assay and parafilm M-tests were employed to confirm biosurfactant produced by the identified *Pseudomonas aeruginosa*. (2A) A clear zone is visible (right) compared to the control (left) in oil spreading assay. (2B) Drop collapse assay shows the culture supernatant (right) causing the droplet to flatten or collapse in the well plate compared to the water control (left), confirming the presence of surface-active agents. (2C) Droplets of the culture supernatant (sample) maintain a significantly larger diameter (9–11 mm) compared to the Control droplet (5 mm) on the hydrophobic Parafilm M surface. indicate scale bars. Every experiment was replicated three times.

Figure 3: Oil Degradation Potential of *Pseudomonas aeruginosa* BCSIR-JnU-ISO2. Representative image of DCPIP test. Oil degradation percentage was measured using 0.01% DCPIP in BHB where WCO (1%, 3% and 5%) was used as carbon sources and gut isolated *Pseudomonas* pure culture were given to measure the percentage of degradation by each isolate. The color change from blue (Control) to colorless/light gray is indicative of WCO degradation after incubation.

Figure 4: Quantification of the extracted crude biosurfactant produced by *Pseudomonas aeruginosa* BCSIR-JnU-ISO2. The representative image shows the recovered crude biosurfactant, yielding approximately 2.71 g/L after 72 hours of fermentation.

Figure 5: Presumptive rhamnolipid secreted from *Pseudomonas aeruginosa* BCSIR-JnU-ISO2. (5A) FTIR Spectroscopy analysis of the produced biosurfactant. The spectrum displays characteristic peaks indicative of a rhamnolipid structure **(5B)** According to the peaks, functional groups were matched with presumptive rhamnolipid structure. The structure represents a di-rhamnolipid molecule, where the color-coded lines on the structure correspond to the functional groups identified in the FTIR spectrum.

Figure 6: Genomic Features, Taxonomic Classification, Phylogenetic Analysis, and Average Nucleotide Identity of *Pseudomonas* BCSIR-JNU-ISO2. (6A) Genome visualization were done using the Bakta web tool(42) highlights the key genomic features, including annotated coding sequences (CDS) and tRNA genes. **(6B)** Taxonomic classification were done using multiple classifiers (PANGIA, Kraken2, Centrifuge, and GOTTCHA) through the EDGE Bioinformatics platform (36) **(6C)** Phylogenetic tree were constructed using the TYGS web tool that illustrates its evolutionary relationship with other *Pseudomonas* strains. (**6D**) ANI of *Pseudomonas aeruginosa* BCSIR-JnU-ISO2 with one reference strain of *Pseudomonas* were done using the PROKSEE tool.

Figure 7: Metabolic Capabilities of *Pseudomonas aeruginosa* BCSIR-JnU-ISO2. (**7A**) Subsystem-based functional annotation of *Pseudomonas aeruginosa* BCSIR-JnU-ISO2. In SEED Viewer, 34% of the genome is covered by the subsystems. **(7B)** COG-based analysis of functional genes in *Pseudomonas aeruginosa* BCSIR-JnU-ISO2, revealing genes involved in cell biogenesis, signal transduction mechanisms, transcription, translation and ribosomal structure, and replication, recombination, and repair. **(7C)** KEGG-based analysis showing the metabolic adaptability of *Pseudomonas aeruginosa* BCSIR-JnU-ISO2.

Figure 8: Gene Mapping of *Pseudomonas* aeruginosa BCSIR-JnU-ISO2. **(8A)** Hydrocarbon aerobic degradation genes. **(8B)** Biocide resistance genes. **(8C)** Metal resistance genes using PROKSEE tool.

Figure 9: Sample collection and processing for isolating *Pseudomonas aeruginosa* from suckermouth catfish. (9A) Represents the location of the sampling site. SMCF samples were collected from Sadarghat and Lalkutighat, Buriganga, Dhaka city. **(9B)** Fish stomach was open with sterile forceps. **(91)** With the help of sterile forceps, 10 g intestinal contents of SMCF was taken into a sterile beaker. **(9D)** Homogenized intestinal contents of SMCF after homogenization.