

Pathogen Detection and Functional Gene Analysis of Microbial Communities in Waste Impacted River System

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INTRODUCTION & AIM

- ❑ Rapid population growth → rising solid & liquid waste in ecosystems.
- ❑ Poor waste management → dumping sites & polluted rivers.
- ❑ Contaminated environments act as reservoirs for pathogenic bacteria and antibiotic resistance (AR) genes.
- ❑ Knowledge of pathogens in natural ecosystems remains limited due to cultivation challenges.
- ❑ High-throughput sequencing (HTS) enables detection of rare taxa, virulence genes, and resistance elements.
- ❑ The present study use Illumina sequencing to analyze microbial diversity in waste impacted river systems

METHOD

1. Sampling

- ❑ Samples collected from polluted environments
- ❑ Composite samples prepared.
- ❑ Stored at $-80\text{ }^{\circ}\text{C}$

2. DNA Extraction & Sequencing

- ❑ DNA isolated using FastDNA Kit.
- ❑ Quality checked with Nanodrop.
- ❑ 16S rRNA V3–V4 region amplified.
- ❑ Illumina MiSeq sequencing.

3. Bioinformatics Analysis

- ❑ Reads trimmed and filtered
- ❑ OTUs clustered
- ❑ Taxonomic assignment
- ❑ Shannon index

4. Pathogen Identification

- ❑ Reads compared with Human Bacterial Pathogen Database
- ❑ BLAST hits $\geq 97\%$ identity considered putative pathogens.

5. Functional Prediction

- ❑ Functional metagenome analysis with PICRUSt.
- ❑ KEGG pathway annotation.

6. Statistics

- ❑ Richness/evenness.
- ❑ Heatmap of top genera
- ❑ Functional differences analysis

CONCLUSION

All sites harbored pathogenic bacteria, highlighting polluted ecosystems as reservoirs of infection. Functional prediction revealed genes linked to amino acid and carbohydrate metabolism, antibiotic resistance, and xenobiotic degradation. Findings demonstrate that contaminated ecosystems are metabolically active and pose significant risks to public and environmental health.

RESULTS

Microbial Diversity

- ❑ Proteobacteria and Bacteroidetes were dominant phyla across all contaminated samples.
- ❑ Site-specific variation included enrichment of Actinobacteria, Acidobacteria, Verrucomicrobia, Firmicutes
- ❑ Genera detected: *Acinetobacter*, *Flavobacterium*, *Prevotella*, *Corynebacterium*, *Pseudomonas* etc.

Pathogen Detection

- ❑ Several putative pathogens identified:
- ❑ *Salmonella enterica* → waterborne disease risk
- ❑ *Escherichia coli* → fecal contamination indicator
- ❑ *Staphylococcus aureus* → antibiotic resistance concern
- ❑ *Pseudomonas aeruginosa* → opportunistic infections

Functional Gene Analysis

- ❑ Functional prediction revealed enrichment of genes for:
 - Amino acid and carbohydrate metabolism
 - Xenobiotic degradation pathways
 - Antibiotic resistance mechanisms
- ❑ KEGG pathway analysis confirmed metabolically active communities adapted to polluted environments.

DISCUSSION

Polluted sites showed microbial communities dominated by Proteobacteria and Bacteroidetes, with variation among other phyla. Several clinically relevant pathogens, including *Salmonella enterica*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, were detected, confirming that waste-impacted environments act as reservoirs of infection. Functional predictions revealed genes linked to metabolism, xenobiotic degradation, and antibiotic resistance, indicating that these ecosystems are metabolically active but also pose significant risks to environmental and public health.

FUTURE WORK / REFERENCES

Future studies will focus on shotgun metagenomic approaches to track pathogens, resistance genes, and pollutant degradation in contaminated ecosystems.