Metagenomic Analysis of Microbial Communities From Acidic Marshland Habitat of India

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Research Article

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Abstract

Understanding the diversity and functioning of microbial communities in acidic marsh land is extremely less investigated in contrast to soils and aquatic ecosystems. This study implemented Illumina high-throughput sequencing to explore the microbial communities and xenobiotic degrading enzymes in the acidic marshland. Taxonomic analysis using SILVA SSU database stated that Proteobacteria (66.74 %), Bacteroidetes (6.98%) and acidobacteria (2.35 %) were the most abundant phylum in the acidic marshland. Functional analysis by SEED subsystems showed that 1.62 % substitute to metabolism of aromatic compound while KO indicates 1.86% of metabolism are involved in xenobiotic biodegradation. KO analysis also indicated the benzoate degradation pathway (ko00362) are predominant while Chlorocyclohexane and chlorobenzene degradation pathway (ko00361), Polycyclic aromatic hydrocarbon degradation pathway (ko00624) Aminobenzoate degradation pathway (ko00627) is largely present in the acidic marshland.

Introduction

Wetlands microorganisms are able to remove some conventional and toxic pollutants from waterbodies by using biogeochemical processes [1]. These specialized microorganism helps in maintaining the variety of important activities- sieving the polluted water, recharging the ground water, controlling the flood [2], facilitating natural surroundings for native and migratory birds and maintains different germplasm of different and threatening species [3].

Pallikaranai marsh land are few and last remaining marshland in Chennai [4] [5]. These wetlands are also called as bogs which is unusually acidic and low in nutrients because of accumulation of peats and deposition of dead plant materials [6]. According to National Wetland Conservation and Management Programme (NWCMP) of the Government of India, Pallikaranai is identified as wetland. It turns into ‘birdwatchers’ paradise’ in winter as more than 15,000 birds-101, plants-61, fish-50, butterflies-7, reptiles-21 species are spotted [7].

The focus of the research was to unravel the important role of bacteria present in acidic marsh land soil. For degradation of xenobiotic compounds, novel genes related to pathways can be identified. These potential microbes and genes could be used for the purpose of bioremediation, biodegradation and isolation of potential enzymes and their use at an industrial scale[8].The soil sample were obtained from the Pallikaranai wetland which is a freshwater marsh in the city of Chennai, India (12°56'15.72"N 80°12'55.08"E). Metagenomic DNA was extracted from the soil sample by DNeasy PowerMax Soil Kit (Qiagen, Germany) according to the manual instructions [10]. The paired end assembled contigs of the sample was annotated by Metagenome Rapid Annotation using Subsystem Technology (MG-RAST) server for taxonomic classification and function annotation [11]. Microbial taxonomy was analyzed by comparing the reads of MG-RAST best hit classification tool and SSU-SILVA (non-redundant) database. Functional classification was performed using SEED subsystems and Kyoto Encyclopedia of Genes and Genomes Orthologs (KO) [12] to recognize the importance of bacteria for xenobiotic metabolism in acidic soil, xenobiotic degradation related genes were taken from KO [13].

In the present study using a metagenomic application which applies high-throughput sequencing, to explore microbes and its function present in the acidic marsh land. Acidic marshland soil metagenome constitutes of 2.3 GB data with 4,422,356 sequences totaling 1,070,563,433 base pairs with an average length of 242 bps. The analysis of the taxonomic microbial community indicates that bacteria are majorly present with a total of 1,456,270 (97.62%) assigned reads followed by Eukaryota 16,894 (1.13%), Archaea 15,105 (1.01%), unclassified

sequences 2,805 (0.19%), Viruses 701 (0.05%) and other sequences 29 (0.00%) respectively. The alpha diversity species was considered to be 657. The taxonomic characterization estimated that the metagenomic community was composed of 83 orders from 51 classes within 32 phyla where most communities were Proteobacteria 158 (17.89%) and Chordata 132 (14.95%) that constitutes of approximately 33% reads. Additionally, bacterial phyla included Ascomyta (14.04%), Bacteroidetes (6.8%), Firmicutes (2.94%), Actinobacteria (2.49%), Planctomycetes (2.27%), Acidobacteria (0.17%). The remaining phyla constitute of almost 2% of total reads (Figure 2) Among bacteria, Proteobacteria was the most abundant phylum which was followed by Chordata, Ascomyta, Bacteroidetes, Firmicutes, Actinobacteria, Planctomycetes and Acidobacteria. Among the 126 families identified, Cytophagaceae (2.72%) and Planctomycetace (2.15%) was the most abundant in this community, followed by Solibacteraceae (13.6%), Flavobacteriaceae (1.13%), Burkholderiaceae (1.02%), Flammeovirgaceae (1.02%), Rhodospirillaceae (0.8%), Nitrospiraceae (0.7%), Verrucomicrobiaceae (0.6%), Clostridiaceae (0.45%) and Xanthomonadaceae (0.45%). Among 176 genera identified (Figure 1) demonstrated the primary genera, Candidatus Solibacter (1.36%) and Cytophaga (1.36%) was the most abundant genus in this community, followed by Burkholderia (0.91%), Rhodopirellula (0.76%), Planctomyces (0.068%), Dehalococcoides (0.057%), Flexithrix (0.057%), Hymenobacter (0.057%), Nitrospira (0.45%), Bacillus (0.34%), Desulfuromonas (0.34%), Flexibacter (0.34%), Frankia (0.34%), Gemmatimonas (0 .34%), Halomonas (0.34%), Isosphaera (0.34%), Pseudomonas (0.34%), Saprospira (0.34%), Sorangium (0.34%), Xanthomonas (0.34%).The remaining genera accounted for less than 1%. Thaumarchaeota (100%) occupied the full proportion in the archaeal domain. Chordata (14.95%) was the second most dominant phylum, followed by Ascomycota (4.38%), Streptophyta (4.24%), Arthropoda (3.53%), Chlorophyta (0.71%), Bacillariophyta (0.35%), Brachiopoda (0.35%) in the eukaryotic organisms, where kingdom fungi were mostly present in this community.

In wetland, biogeochemical activities are important that are led by microbes in the soil [14]. To understand the microbe's activity, we analyzed the identified protein sequences from acidic soil. SEED Subsystems and KEGG Orthologs (KO) databases helps in functional profiling of genes in the data. 11089 annotations hits were obtained against the metabolism of xenobiotics compound. For aromatic compounds metabolism, 210 annotations were obtained from SEED Subsystem. The aromatic compound metabolism pathways for the sample data are described (Fig. 2). Genes identified related to xenobiotic degradation with the specific organism shown in Table 1. The genes were found to be related with the phenyl alkanoic acid degradation (20.6 %), benzoate metabolism (11.1 %), Phenyl propanoid metabolism (7.9 %), N-heterocyclic compounds aromatic compound degradation (7.57%), phenylacetyl-CoA degradation (5.98 %), beta-ketoadipate pathway- Catechol branch (4.35%), beta-ketoadipate pathway- procatechuate branch (4.33 %), anthracene and naphthalene degradation (1.93 %), Gentisare degradation (1.86%), Biphenyl Degradation (1.68%), salicylate and gentisate metabolism (1.2 %), Quinate degradation (0.63%), Toluene degradation (0.51%), Salicylate ester degradation Chlorobenzoate degradation (0.37%) Anaerobic toluene and ethylbenzene degradation (0.32%), Cresol degradation (0.30%) and p-cymene degradation (0.18%).

Microbes expanding in marsh land of take part in important role on extracting nutrients and redundant contaminants from sewer effluents. The existence of some microbes plays a critical role in degrading aromatic compounds. In this work we confirmed that Proteobacteria and Chordata were the most dominating communities and *Burkholderia cepacia* and *Pseudomonas putida* are mainly involved with xenobiotic degradation with other microorganisms. This study should constitute a basis for future investigations of metagenomic analysis of Pallikaranai marsh land.

Table 1. Functional annotation of genes associated with aromatic compounds degradation

Species	Associated compound or pathway PAHs	Gene identified
Acetobacter pomoruma	Steroid degradation	cholesterol oxidase
Actinosynnema mirum	Drug metabolism - other enzymes	carboxylesterase 2
Aeromonas veronii	Polycyclic aromatic hydrocarbon degradation	phthalate 4,5-dioxygenase
Anaerobaculum hydrogeniformans	Polycyclic aromatic hydrocarbon degradation	3,4-dihydroxyphthalate decarboxylase
Azospirillum brasilense	Homogentisate pathway of aromatic compound degradation	L-tryptophan aminotransferase
Bacillus subtilis	Benzoate degradation Quinate_degradation	2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase 3-dehydroquinate dehydratase
Burkholderia cepacia	Aminobenzoate degradation Chlorocyclohexane and chlorobenzene degradation Chlorocyclohexane and chlorobenzene degradation Xylene degradation Chlorobenzoate_degradation Benzoate_catabolism	anthranilate dioxygenase catechol 2,3-dioxygenase catechol 2,3-dioxygenase 2-hydroxy-6-oxohepta-2,4-dienoate hydrolase 2-chlorobenzoate 1,2-dioxygenase reductase 2-halobenzoate 1,2-dioxygenase
Clostridium kluyveri	Anaerobic benzoate metabolism	3-hydroxybutyryl-CoA dehydrogenase
Cupriavidus necator	Nitrotoluene degradation	hydrogenase
Methylobacterium sp	Toluene degradation	methanol dehydrogenase
Pseudomonas putida	Benzoate degradation Aminobenzoate degradation Toluene degradation Chlorocyclohexane and chlorobenzene degradation Xylene degradation	2-hydroxymuconate tautomerase mandelate racemase toluene dioxygenase reductase chlorocatechol 2,3-dioxygenase 2,3-dihydroxy-p-cumate 3,4-dioxygenase

	Gentisare_degradation	p-hydroxybenzoate hydroxylase
	Biphenyl Degradation	4-hydroxyphenylacetaldehyde dehydrogenase
	p-cymene_degradation	cumic aldehyde dehydrogenase
Rhodococcus jostii	Biphenyl_Degradation	2,3-dihydroxybiphenyl 1,2-dioxygenase
	Polycyclic aromatic hydrocarbon degradation	3,4-dihydroxyphthalate decarboxylase
Sterolibacterium denitrificans	Nitrotoluene degradation	1-testosterone hydratase
Vibrio sp.	Homogentisate pathway of aromatic compound degradation	Maleylacetoacetate isomerase
Thauera aromatica	Phenylalkanoic acid degradation	3-hydroxyacyl-CoA dehydrogenase
	Anaerobic benzoate metabolism	hydroxybenzoate-CoA ligase
	Anaerobic_toluene_and_ethylbenzene_degradation	Succinyl-CoA:(R)-benzylsuccinate CoA-transferase

Declarations

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Data Availability Data are available in MG-RAST public repository at https://www.mg-rast.org/. for sample id mgs822751

Author contributions Khairun Nisha: Data curation, formal analysis and writing–original draft. Jithin S. Sunny: Data curation. Anuradha Natarajan: Data curation. Lilly M. Saleena: Project administration, Conceptualization, Writing—Review & Editing

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Figures

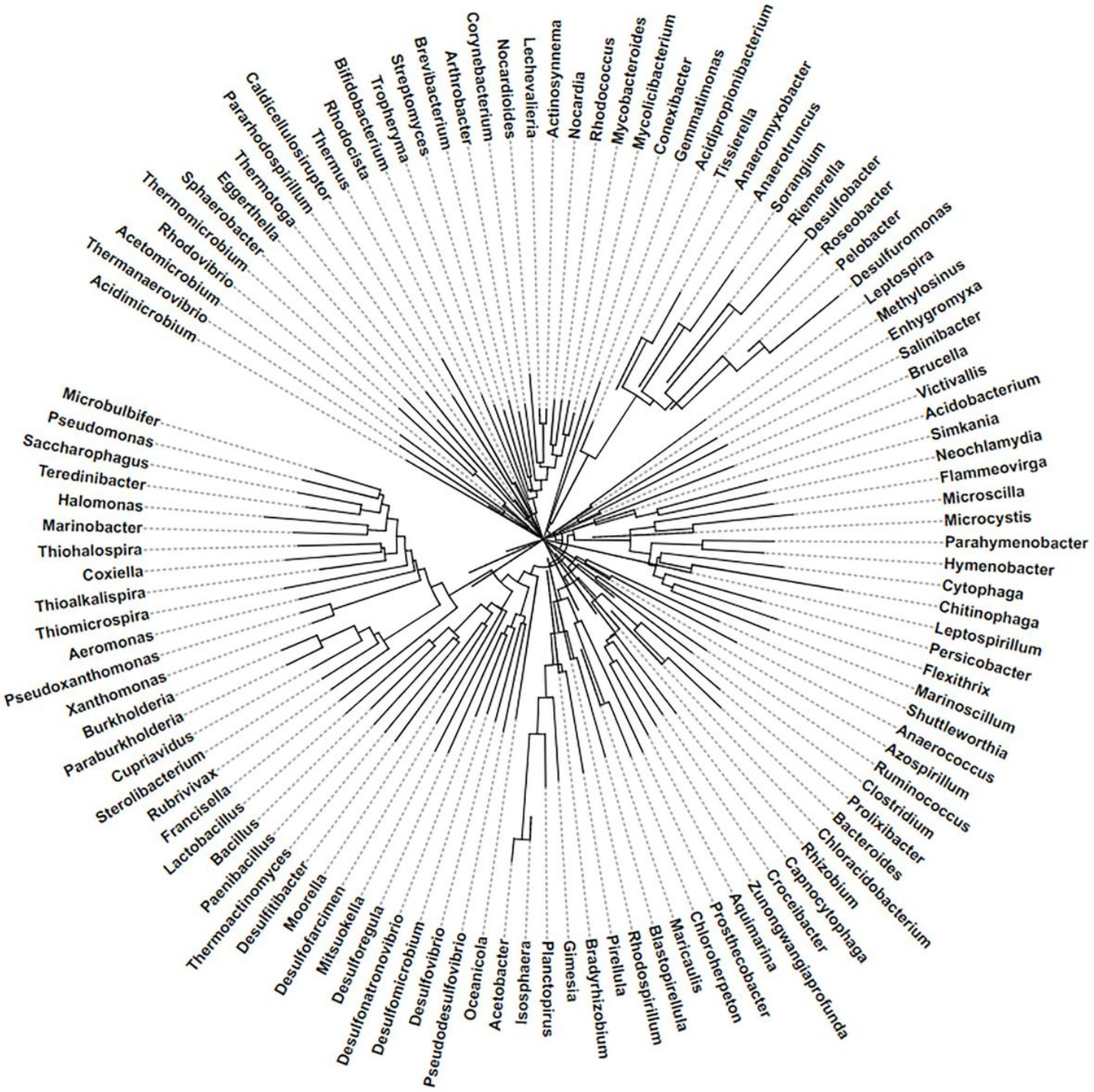

Figure 1

Phylogenetic tree of genus identified

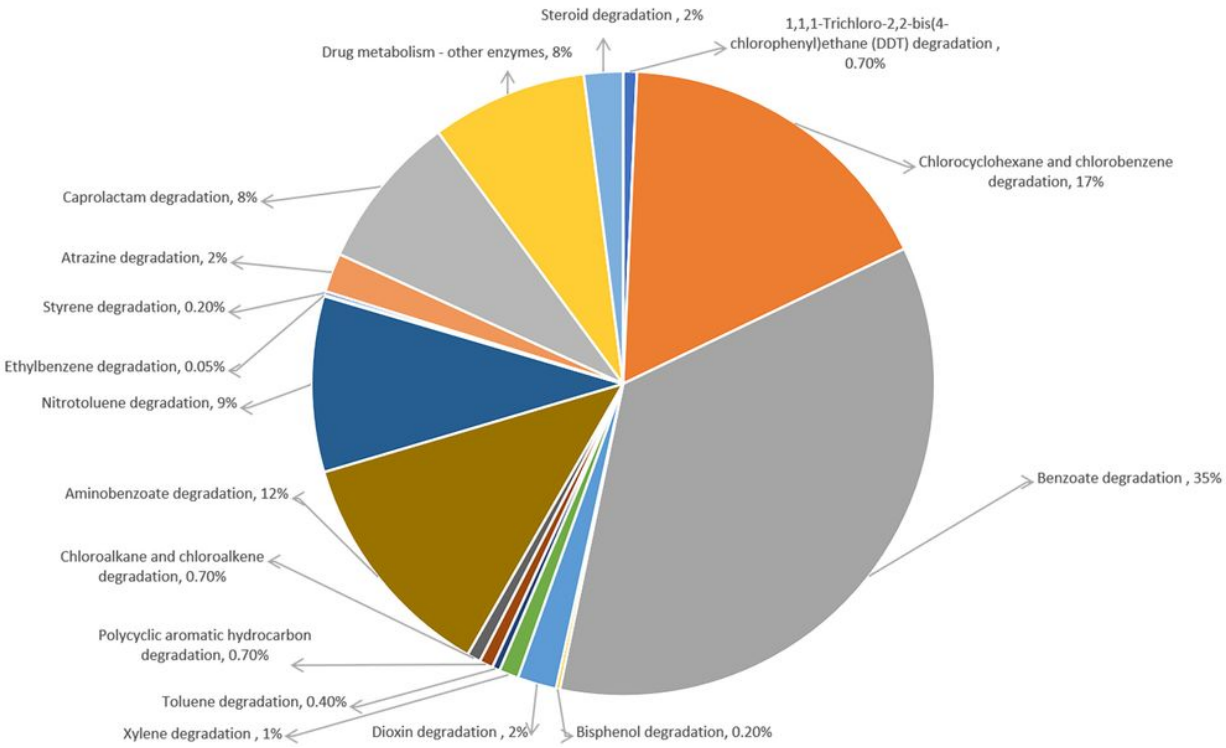

Figure 2

Xenobiotic Metabolism and Degradation identified in acidic marshland