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Research Paper

Organochlorine tolerant microbial populations profiled from rivers yamuna and godavari using next generation sequencing

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Abstract

Residues of Organochlorine Pesticides (OCPs) such as DDT (dichlorodiphenyltrichloroethane), hexachlorocyclohexane (HCH), Heptachlor epoxide endosulfan, Aldrin, dieldrin, etc were primarily detected in aquatic habitats mainly rivers such as Ganges, Yamuna, Godavari etc. OCPs, due to recalcitrant nature degrade slowly and pose adverse health effects to the environment and community. It is beneficial to understand the bacterial community of these ecosystems which can be targeted for bioremediation of the OCPs. The current study profiled bacterial community from rivers Yamuna (North India) and Godavari (South India) that was found to be OCP tolerant. The samples collected were enriched until a Lindane and DDT tolerant population was established and subjected to environmental metagenomic analyses using Illumina platform. The amplicon sequencing profiled around 871 species in the water samples collected from rivers Yamuna and Godavari which also established a taxonomic biodiversity with a Shannon alpha-diversity of 3.0317.

Keywords: Yamuna, Godavari, Bacterial Community, Bioremediation, Dichlorodiphenyltrichloroethane (DDT), Hexachlorocyclohexane (HCH), Lindane, Metagenomics.

Introduction

One of the source sinks for persistent organic pollutants discharged into the environment is the aquatic habitats i.e. rivers and lake beds. A range of several organochlorine pesticides (OCPs) such as Endrin aldehyde, Endosulfansulfate and DDT were reported in River Yamuna[1] and similarly, residues such as DDT, Trans-chlordane and Endo- sulfansulfate were the dominant OCPs in soil sediments from River Godavari[2]. River Yamuna is the longest and one of the largest tributary rivers of the Ganges in northern India which travels a total length of 1,376 kilometres (855 Miles)[3] and river Godavari is the second longest river after the Ganga. It starts in Maharashtra and flows east for 1,465 kilometres (910 Miles) emptying into the Bay of Bengal through its extensive network of tributaries. In the current study, microbial consortium obtained from the water samples collected from both the rivers was enriched a Lindane and DDT tolerant population was established. The consortium subjected to environmental metagenomic analyses using next-generation sequencing (NGS) Illumina platform[4].

Materials and Methods

Chemicals

Lindane (γ-HCH) was of 97% purity and obtained from Sigma- Aldrich, USA. DDT, 99.4% pure, was donated by Hindustan Insecticides Ltd, India. All other chemicals and reagents used in the study were of analytical grade and were purchased from standard manufacturers.

Isolation and Enrichment of Microbial Consortium

Water samples from the rivers Yamuna (North India) and Godavari (South India) were collected in clean bottles and brought to the lab in sealed condition. These water samples were mixed and incubated with 1% (w/v) peptone in a rotary shaker maintained at 150 rpm until microbial growth was sufficient to make the broth highly turbid. This culture was continuously shaken only in presence of gradually increasing concentrations of Lindane and DDT for many months till a stable Lindane and DDT tolerant population were established in the flask[5].

Environmental Metagenomics-Bacterial Community Profiling

The microbial population was subjected to metagenomic analysis using the NGS HiSeq 2500System for16Ssequencing, the TruSeq® with Dual Index SequencingPrimer Box[6].The amplicon library was prepared using Nextera XT Index Kit (Illumina Inc.) as per the 16S Metagenomic Sequencing Library preparation and primers used for the amplification of the V3-V4 hypervariable region of 16S rDNA gene of bacteria and Archaea were synthesized (Table1) with following Oligo Sequence[7].

Table 1: Primers used for NGS

Oligo Name	Oligo Sequence (5' to 3')	Length of primer
Prokaryote V3-Forward	CCTACGGGNBGCASCAG	17
Prokaryote V4-Reverse	GACTACNVGGGTATCTAATCC	21

Results and Discussion

Rarefaction plot (Figure 1) was constructed that allowed calculation of species richness for a given number of individual samples, based on the construction of so-called rarefaction curves. This curve is a plot of the number of species as a function of the number of samples[8]. On the left, the steep slope indicates that a large fraction of the species diversity remains to be discovered. The vertical axis displays the diversity of the community, while the horizontal axis displays the number of sequences considered in the diversity calculation[9].

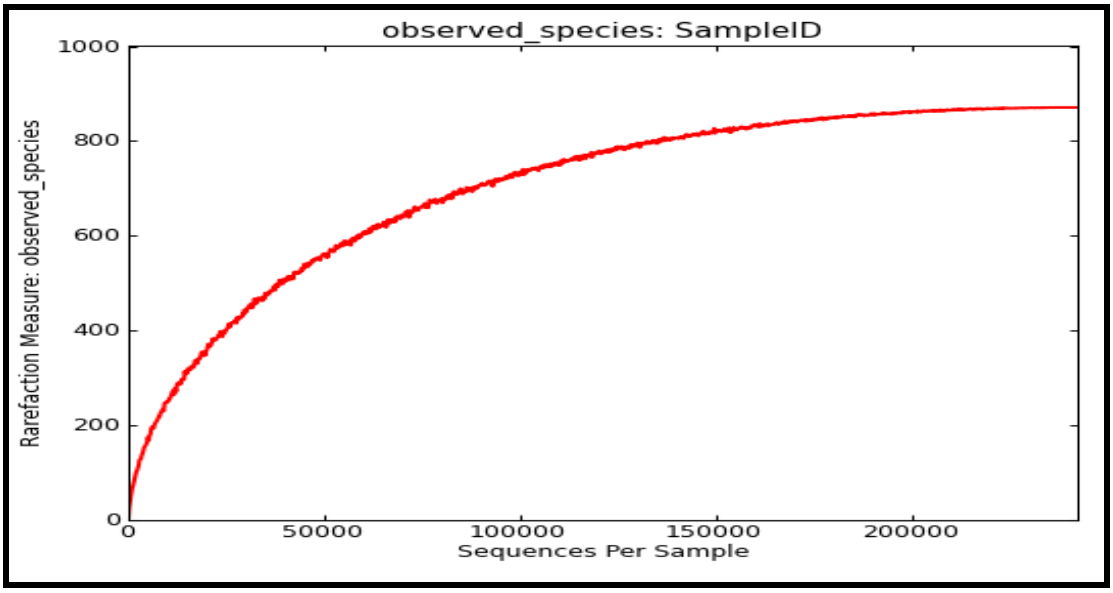

Figure 1: Rarefaction plot for the Observed Species in the Rivers Yamuna and the Godavari

Biodiversity of the bacterial community was studied using Quantitative Insight into Microbial Ecology (QIIME) Analysis[10]. The most abundant classes were found to be *Betaproteobacteria* followed by *Alphaproteobacteria* (Figure 2)

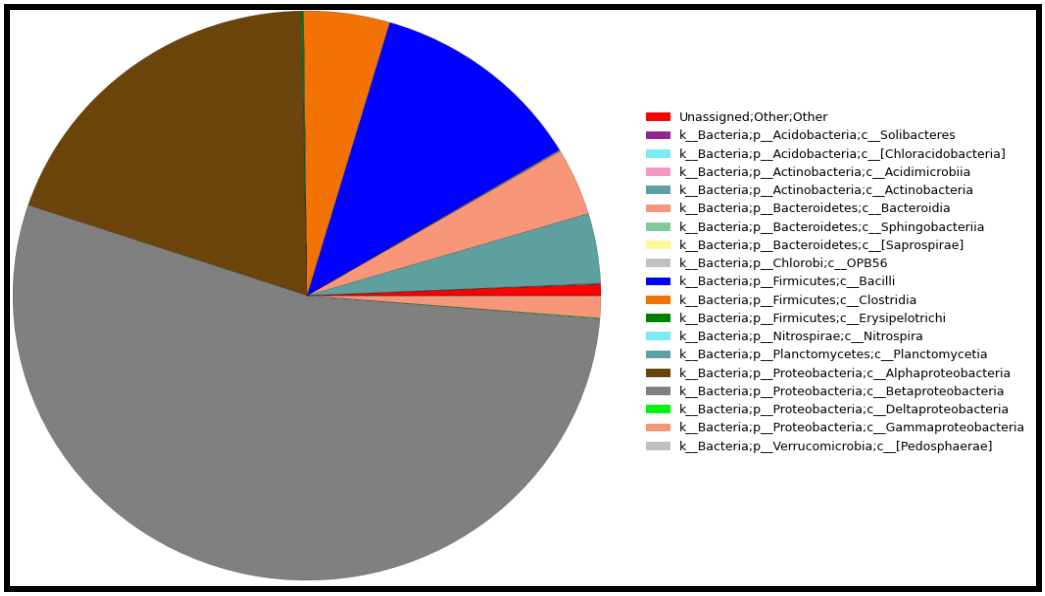

Figure 2: Pie chart showing the relative abundance of each class within themicrobial community

The most abundant family from the bacterial community profiled is found to be *Alcaligenaceae* followed by *Caulobacteraceae* (Figure 3).

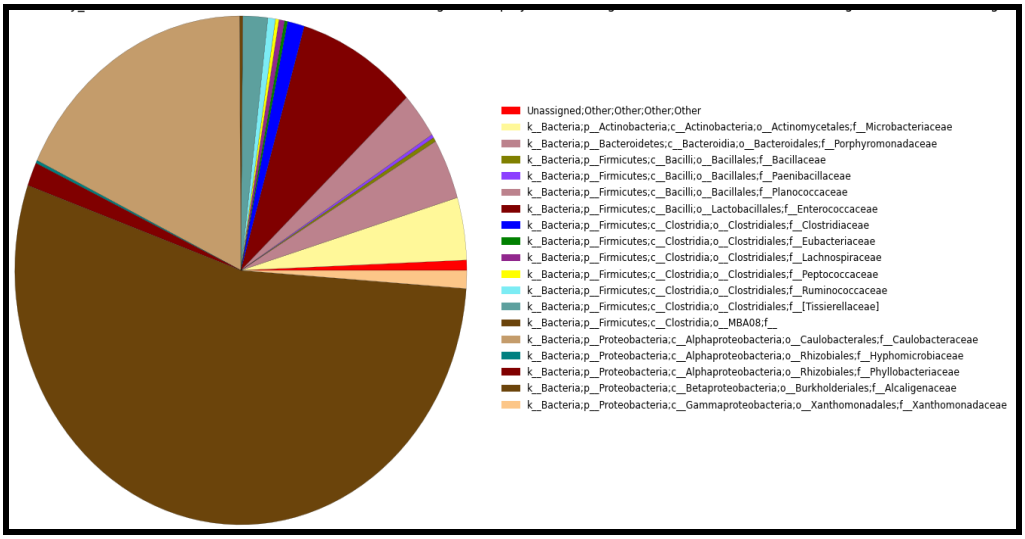

Figure 3: Pie chart showing the relative abundance of each family within themicrobial community. From the figure, it can be inferred that Alcaligenaceae are most abundant followed by Caulobacteraceae

A total of 871 species were profiled in the River Yamuna and Godavari from the OCP enriched consortium. The bacterial populations with highest abundance ratio are found to be *Brevundimonas diminuta, Alcaligenes faecalis, Stenotrophomonas acidaminiphila, Bacillus cereus* and *Desulfosporosinus meridiei* (Figure4).

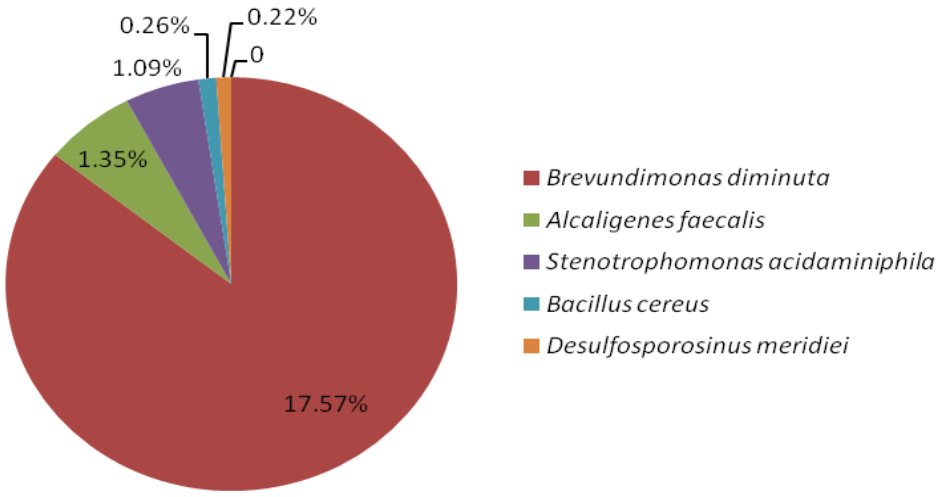

Figure 4: Species identified with higher abundance ratio

A total of 871 species were profiled in the River Yamuna and Godavari from the OCP enriched consortium. The next generation sequencing facilitated identification of any unculturable organisms in the rivers which otherwise would have been impossible to identify using growth based techniques[11,12]. The profiling of microbial population from the water samples collected from Rivers Yamuna and the Godavari identified diverse specifies and the organisms identified with higher abundance are provided (Table 2).

Table 2: Taxonomic List of Species identified in River Yamuna and Godavari from the Microbial Consortium Enriched with OCPs

Taxonomy	Abundance Ratio (More Than 0.1%)
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Leucobacter;s__	3.9%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__;s__	3.8%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus;s__cereus	0.3%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae;g__Paenibacillus;s__	0.2%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae;g__Lysinibacillus;s__boronitolerans	2.9%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Enterococcaceae;g__Enterococcus;s__	8.5%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__;g__;s__	0.1%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Alkaliphilus;s__	1.2%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Eubacteriaceae;g__Garciella;s__	0.2%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Coprococcus;s__	0.4%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptococcaceae;g__Desulfosporosinus;s__meridiei	0.2%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__;s__	0.5%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Oscillospira;s__	0.1%

k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Tissierellaceae];g__;s__	0.9%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Tissierellaceae];g__Sedimentibacter;s__	0.1%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Tissierellaceae];g__Tissierella_Soehngenia;s__	0.7%
k__Bacteria;p__Firmicutes;c__Clostridia;o__MBA08;f__;g__;s__	0.2%
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__;s__	0.1%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Brevundimonas;s__diminuta	17.6%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Brucellaceae;g__Ochrobactrum;s__	0.1%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Devosia;s__	0.2%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;g__;s__	1.5%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__;s__	51.2%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Achromobacter;s__	1.0%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Alcaligenes;s__faecalis	1.4%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Denitrobacter;s__	0.1%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Stenotrophomonas;s__acidaminiphila	1.1%

The present study profiled the bacterial community of the Rivers Yamuna and Godavari using NGS Illumina metagenomic platform[13]. The study provided a holistic understanding of the biodiversity of the bacterial communities in these rivers, and identified various species using novel metagenomic amplicon analysis. The consortium was found capable of degrading mixture of organochlorine pesticides[14-16], hence each of identified species can be further explored and can be promising candidates for potential biodegrades of various organochlorines and other compounds.

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