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MICROBIAL POPULATION WITH POTENTIAL TO SIMULTANEOUSLY DEGRADE ORGANOCHLORINE INSECTICIDES

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ABSTRACT

Organochlorine pesticides (OCPs) such as 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (*p,p'*-DDT) and γ -hexachlorocyclohexane (Lindane) pose serious health effects to the environment and community. Although both these compounds are prohibited in many parts of the world, their residues were detected in various soil, air and aquatic ecosystems. Currently, research has been carried out on a single pesticide with a single microbe or a consortium with few members for biodegradation of organochlorine pesticides. In the environment, the scenario is different with many other compounds and native flora present that can make the application of single microbe or small consortium difficult to succeed. The current study was initiated to develop a microbial consortium that can act simultaneously degrade and eliminate more than one organochlorine compounds while maintaining its integrity in the environment. In this study, the simultaneous degradation of DDT and Lindane at 10 ppm, 20 ppm and 30 ppm concentration was studied at 0, 24, 42, 78 and 92 hours at optimized conditions (pH-7 and OD600 0.075). The enriched consortium could simultaneously degrade 90% of 10 PPM of DDT and Lindane. The enriched consortium was tested for degrading mixture of organochlorine pesticides with concentrations up to 30 ppm of DDT and Lindane mixture, and demonstrated promising observations that illustrate the usage of the consortium for degrading the OCPs with high efficiency.

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1 Introduction

Organochlorine insecticides such as 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (*p,p'*-DDT) and γ -hexachlorocyclohexane (Lindane) have been used in large scale in later part of 20th century primarily for pest control and agricultural purposes (Wasim Aktar et al., 2009). Organochlorine (OC) pesticides are synthetic in nature and are made up of chlorinated hydrocarbon derivatives. These insecticides have acute toxicity and can be bioaccumulated in the food chain leading to heightened pollution levels in environment. Majority of the OCPS are banned in many countries, however their usage is rising in developing countries due to the low cost leading to chemical abuse (Jayaraj et al., 2017).

Targeted research has been conducted using a single OCP molecule, and studies established removal of pesticides in the field level bioremediation. Still no organochlorine pesticide residues are eliminated from the nature leading to their persistence and causing major health issues among the population (Abraham et al., 2014). In current study, a mixed consortium isolated from Indian rivers Yamuna and Godavari, and enriched using DDT and Lindane was used to demonstrate the potential to degrade the organochlorine insecticides (Pandey et al., 2011). The consortium was characterised using advanced genome sequencing system utilizing illumina platform (de Muinck et al. 2017) that identifies even the unrecoverable species in the laboratory environment (Handelsman, 2004).

2 Materials and Methods

2.1 Chemicals

99.4% pure *p,p'*-DDT was donated for research by Hindustan Insecticides Ltd., India while 97% pure Lindane was purchased from Sigma Aldrich, USA. HPLC grade acetone and ethyl acetate was procured from Merck. Other chemicals used were of analytical grades and purchased from standard manufacturers.

2.2 Microbial culture and enrichment

Water samples were collected from two Indian holy rivers Yamuna and Godavari. These samples were subjected to increasing concentrations of two organochlorine pesticides (OCP) 1,1,1-trichloro-2,2-bis-(4-chloro) ethane (DDT) and γ -hexachlorocyclohexane (Lindane) over a period of 6 months. Periodically it was tested for the viability of the population through streaking on to nutrient agar (Bidlan & Manonmani, 2009).

2.4 Extraction of OCPs

Samples drawn at every 24 h were acidified and extracted with equal volume of dichloromethane twice and organic layers pooled after passing through anhydrous sodium sulphate and fluorisil. The residual OCPs were transferred into microfuge tubes after

completely drying the dichloromethane and dissolving in a small volume of acetone.

2.6 Gas Chromatography-Mass Spectrometry Fingerprinting

Qualitative and quantitative analyses were confirmed with GCMS/MS fingerprinting using Agilent 7000D equipment with triple quad (Abd El-Gawad, 2016). The column HP-5ms (Agilent 19091S EPC) was programmed with pressure 30.797 psi, the flow of 3.1793 mL.min⁻¹, average Velocity of 54.506 cm.s⁻¹ and temperature 70°C to 280°C, the sample was electron ionized (EI) with a source temperature of 300°C. The quantification was done using a standard curve prepared for different amounts of the two OCPs (DDT and Lindane) under the same conditions (Muir & Sverko, 2006).

2.7 Genomic DNA extraction

DNA was isolated using Xcelgen Bacterial gDNA kit with few modifications. Quality of gDNA was checked on 0.8 % agarose gel and quantification was done on Nanodrop 8000 (Blomquist et al., 2013).

3 Results and Discussion

3.1 Consortium

The microbial population was enriched using long term acclimatization technique where the sample was subjected to mixture of DDT and Lindane. This resulted in a microbial population that was capable for biodegradation of OCP mixtures and contained many types of microorganisms. Some of the strains were isolated by repeated streaking. The samples were spread on LB-agar plates at various stages of enrichment and the petridishes were incubated at room temperature to check vitality (Figure 1).

3.2 Degradation of DDT-Lindane mixture by the consortium

The degradation of mixture of DDT and Lindane at 10, 20 and 30 ppm concentrations was reflected in the TLC plate and confirmed by GCMS/MS fingerprinting. There was a marked reduction in the residual concentration of Lindane in three days of incubation. The amount of DDT also reduced from 0 h to 96 h. Gas Liquid Chromatograms show that the retention time of Lindane was 13.3 min while that of *p,p'*-DDT was 26.9 min under the conditions of analyses. Figure 2 and 3 gives the chromatograms of the experiment with 10 ppm of each DDT and lindane in the broth. Figure 3 and 4 gives the chromatograms of the experiment with 20 ppm of each DDT and Lindane in the broth. Figure 5 and 6 gives the chromatograms of the experiment with 30 ppm of each DDT and Lindane in the broth. The enriched consortium could degrade 95% of 10 ppm Lindane and DDT in the supplied mixture by the end of 96 h (Figure 7), similar degradation was also observed for 20 PPM and 30 PPM pesticide concentration however at reduced efficacy due to the increase in concentrations of the pesticides (Figure 8-10).

Figure 1 Plates after 3 cycles of enrichment with 5 ppm of each pesticide as listed above. (After: A= 36 H of incubation; B= 60 H of incubation; C= 84 H of incubation; D= 100 H of incubation)

Sample Chromatogram

Compound	Transition	RT	Resp.	Final Conc	Units
Lindane	181.0 -> 145.0	13.321	497010	102.6952	ng/ml
p,p'DDT	235.0 -> 165.2	26.920	276801	100.1081	ng/ml

Lindane

p,p'DDT

Figure 2 Gas Chromatograms and Mass Fingerprints of samples at 0 h incubation period (10PPM of DDT+Lindane)

Sample Chromatogram

+ TIC MRM (** -> **) Sample 5.D (RMC-opt-10-96H)

Compound	Transition	RT	Resp.	Final Conc	Units
Lindane	181.0 -> 145.0	13.321	438195	94.8788	ng/ml
p,p'DDT	235.0 -> 165.2	26.920	1832453	559.4064	ng/ml

Lindane

p,p'DDT

Figure 3 Gas Chromatograms and Mass Fingerprints of samples at 96 h incubation period (10PPM of DDT+Lindane)

Sample Chromatogram

Compound	Transition	RT	Resp.	Final Conc	Units
Lindane	181.0 -> 145.0	13.321	1231183	200.2645	ng/ml
p,p'DDT	235.0 -> 165.2	26.918	588712	192.1984	ng/ml

Lindane

p,p'DDT

Figure 4 Gas Chromatograms and Mass Fingerprints of samples at 0 h incubation period (20PPMof DDT+Lindane)

Sample Chromatogram

Compound	Transition	RT	Resp.	Final Conc	Units
Lindane	181.0 -> 145.0	13.321	227041	66.8171	ng/ml
p,p'DDT	235.0 -> 165.2	26.919	978827	307.3777	ng/ml

Lindane

p,p'DDT

Figure 5 Gas Chromatograms and Mass Fingerprints of samples at 96H incubation period (20PPM of DDT+Lindane).

Sample Chromatogram

Compound	Transition	RT	Resp.	Final Conc	Units
Lindane	181.0 -> 145.0	13.321	1919595	291.7523	ng/ml
p,p'DDT	235.0 -> 165.2	26.920	957681	301.1347	ng/ml

Lindane

p,p'DDT

Figure 6 Gas Chromatograms and Mass Fingerprints of samples at 0 h incubation period (30PPM of DDT+Lindane).

Sample Chromatogram

+ TIC MRM (** -> **) Sample 15.D (RMC-opt-30-96H)

Compound	Transition	RT	Resp.	Final Conc	Units
Lindane	181.0 -> 145.0	13.321	473626	99.5875	ng/ml
p,p'DDT	235.0 -> 165.2	26.920	1612110	494.3512	ng/ml

Lindane

p,p'DDT

Figure 7 Gas Chromatograms and Mass Fingerprints of samples at 96 h incubation period (30PPMof DDT+Lindane).

Figure 8 DDT and Lindane Degradation at 10 PPM Concentration

Figure 9 DDT and Lindane Degradation at 20 PPM Concentration

Figure 10 DDT and Lindane Degradation at 30 PPM Concentration

Table 1 Organisms Identified through Metagenomic Characterization by Hiseq2500, Illumina Platform, NGS

Total Reads	5,88,408
Total number of stitched reads	2,81,957
Number of OTUs	2,911
Abundant phylum	<i>Proteobacteria</i>
Abundant class	<i>Betaproteobacteria</i>
Abundant order	<i>Burkholderiales</i>
Abundant family	<i>Alcaligenaceae</i>
Abundant genus	<i>Brevundimonas</i>
Abundant species	<i>dimunita</i>
shannon alpha-diversity	3.0317
Observed species	871

The consortium identified is tabulated in Table 1. DNA sequences of the consortium capable of degrading the Organochlorine mixture have been deposited at National Center for Biotechnology Information (NCBI) Sequence Read Archive under the bioproject ID PRJNA420925 and accession code SRX348847.

Conclusions

Microbes are primary stimulants in the bioremediation of contaminated environments. The defined microbial consortium RMC tested for degrading mixture of organochlorine pesticides with concentrations up to 30 ppm of DDT and Lindane. Biodiversity of microbial population with 871 prokaryotic species constituted the RMC. Although research has been carried out using on single strain and single compound of organochlorines, the current study data provides an insight on how bacterial communities in mixed consortia are taxonomically distributed and their biodiversity, the metagenomic characterization identified the consortium in a definitive manner which acts as promising

solution for bioremediation of organochlorine mixtures. The microbial consortium (RMC) can be a viable strategy for remediation for organochlorine mixtures.

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Conflict of Interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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