Isolation and characterization of organophosphorus degrading bacteria from pesticide industrial effluent

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Abstract— **Development of new industries or expansion of existing industrial establishments resulted in the disposal of industrial effluents which discharge untreated causing air, water, soil and solid waste pollution and adversely affect biological growth. Organophosphorus (Monochrotophos, Dichlorovos, Dimethoate) degrading bacterium was isolated from pesticide industrial effluent samples which collected from Ahmedabad (India) and its surrounding pesticide contaminated area. On the basis of morphological, cultural characteristics, biochemical test and 16s rRNA sequencing, the isolated strain was identified as *Pseudomonas stutzeri,* Tistrella mobilis and Sphingosinicella microcystinivorans respectively. From this result that the isolated bacteria could be used for the removal of residues of organophosphorus in contaminated area.**

***Index Terms*—Industrial Effluents, Pesticide industries, Pollution, Organophosphorus, Degradation**

I. INTRODUCTION

The pesticide industry is an important part of the economy. The Indian Pesticide industry with 85,000 tonnes (T) of production during financial year (FY) 07 is ranked second in Asia (behind China) and twelfth globally (McKenna,1987).It is found that one-third of the total water pollution in India comes in the form of industrial effluent discharge, solid wastes and other hazardous wastes (Pravin et al. 2011). The main causes of surface water and groundwater contamination are industrial discharges and agricultural activities (Celalettin et al. 2008).Most major industries have treatment facilities for industrial effluents. But this is not the case with small scale industries, which cannot afford enormous investments in pollution control equipment as their profit margin is very slender (Lokhande et al. 2011). Pesticides usually have direct adverse effect on the living organisms. These compounds are toxic and carcinogenic in nature even at low concentration (Serkan et al.2008). Industries that use large amounts of water for processing have the potential to pollute waterways through the discharge of their waste into streams and rivers, or by run-off and seepage of stored wastes into nearby water sources. Industrial Effluents can also contain inorganic wastes such as brine salts and metals (Inventory Report to Congress).

Organophosphorous pesticide is one of the major chemicals responsible for the contamination and deterioration of soil and groundwater, particularly in the close vicinities of agricultural fields (Harish.R and Jyoti chauhan, 2013). It mainly affects the nervous system by disrupting the enzyme which is responsible for acetylcholine activity (Leibson and Lifshitz 2008). Most organophosphates are used as insecticides to get rid of insects. They were first introduced during the early 19th century (Gupta 2006; Meenakshi et al. 2012). They also have some side effects and the major of them is their similar effects on humans as well (Meenakshi et al. 2012; Zitko 2003). Some are those much poisonous that they were used as a weapon in World War II (Johanningsmeier 2000; Zacharia 1996).

The process of pesticide removal from industrial wastewater is of great importance because of pesticide resistance to microbial degradation and its ability of accumulation in the environment as well as possible carcinogenic and mutagenic properties. Microorganisms play an important role in the conversion of cyclodiene insecticides in soil to nontoxic products. In the natural environment microorganisms may provide some protection against toxicity of pesticide (Abdelbagi et al. 2010). The prevalence of such materials into the environment has an increased interest in studying microbes involved in their biodegradation (Zhuang et al. 2003). Microbial degradation of pesticides is one of the important ways to remove the environmentally harmful compounds and this biodegradation is carried out by different organisms like Bacteria, Fungus, and Algae (Varsha1 et al. 2011). Microorganisms use components of the effluent as their "food" and in doing so break them down to fewer complexes and less hazardous compounds. The aim of this research was to isolate and characterize microorganisms that could biodegrade organochlorine pesticides from the industrial effluents. The standard method for isolating microorganisms with the ability to degrade environmental pollutants is to enrich them from contaminated soils.

II. Material and Methods

2.1 Reagents

Technical grade Dimethoate (30% pure), Monochrotophous (36% pure), Dichlorovos (76% pure) were purchased from pesticide manufacturer. All chemicals and solvents used in the study were of analytical grade and purchased from standard manufactures.

2.2 Culture medium

Luria-Bertoni (L.B.) medium used for screening of pesticide degrading bacteria consisted of (Yeast extract,10 g;Peptone,5 g; Agar 20 g; Distilled water 1000 mL;pH-7.2) .Nutrient agar used for primary screening of selected different pesticides degrading bacteria consisted of (peptone 10 g ;Beef extract 3.0 g; Nacl (sodium Chloride) 5 g; Agar 15 g; Distilled water 1000 mL,pH-7.6).All these media were sterilized by autoclaving at 121 ℃ for 15-20 mins.

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2.3 Enrichment and selection of microbial consortium

We cultured a microbial consortium capable of selected pesticides. This microbial consortium had been obtained from pesticide industrial effluents having several applications of different pesticides including Monochrotophos, Dichlorovos, Dimethoate. These effluent samples were collected from pesticide manufacture industry in Ahmedabad (INDIA) and its surrounding area. To An aqueous suspension of the effluent samples were inoculated into 100 mL of Luria-Bertoni broth in 250 mL Erlenmeyer flasks containing 10 mg/mL selected pesticides. The flasks were kept on an orbital shaker for 7 days at 150 rpm and ambient conditions. After incubation, 10 mL of the culture suspensions from L.B. medium were inoculated into fresh 100 mL of Luria-Bertoni broth in 250 mL Erlenmeyer flasks containing 20 mg/mL selected pesticides. The flasks were incubated for another 7 days under the same conditions. The cultures were gradually acclimated to increasing concentration of selected different pesticides ranging from 20-50 mg/mL at weekly intervals. At about a final concentration of 50 mg/mL of different pesticides, the pesticides tolerant cultures were picked up for further degradation study. (Fang and dong *et al*, 2010).

2.4 Isolation and characterization of microbial consortium

The bacterial cultures were isolated from mixed cultures by streaking method on nutrient agar plates containing 50 mg/mL of different pesticides .These plates were incubated in incubator (Nova make) at 37℃ for 24-48 hrs. Individual colonies were subcultured into nutrient agar plates containing same concentration of pesticides until pure culture was isolated. Once all the isolates obtained were purified, the pesticides utilization ability of these strains was checked on MSM agar plates containing organophosphorus pesticides (50 mg/mL) as sole source of carbon and energy. After incubation time, colony characteristics and morphological properties were noted. All pesticide degrading isolates streaked on to nutrient agar slants and maintained at 4℃ and also subculture after every three months. The Metabolic characteristics were studied by using various biochemical media, according to Bergey's manual of systemic Bacteriology (Bergey's manual of systemic Bacteriology, 1989).

2.5 Identification of microbial consortium

The purified bacterial strains were identified based on its morphological, biochemical and 16S rRNA sequence. The 16S rRNA sequencing from isolated strains was accomplished by BioGene-Gujarat Biodiversity Gene Bank, India. Similarity analysis of the 16S rRNA sequences was conducted using the blast function of NCBI Gene Bank.

III. Results & discussion

3.1 Isolation of organophosphorus degrading bacteria

Numbers of the indigenous pesticide tolerant bacteria was isolated by enrichment technique. From this enrichment cultures, 60 morphologically various strains were isolated on nutrient agar plates containing organophosphorus such as Monochrotophos, Dichlorovos, Dimethoate pesticides. The pesticides degradation ability of these strains was checked on MSM agar plates containing organophosphorus pesticides (50 mg/mL). Out of 50 isolated, only three isolated strains (DP-7, DP-1 and DP-8) showed significant degradation of pesticides.

3.2 Characterization of bacterial isolates

One of the three isolated, Colonies of strain DP-7 on nutrient agar plates appeared with non-fluorescent yellowish, wrinkled, dry, coherent, and ridged with concentric spreading areas and a mucoid colony together with various intermediate types colonies. The strain was a Gram negative, aerobic, rod shaped, motile and single polar flagellated. Second isolate DP-1 is a Gram negative, rod shaped, highly motile with a single polar flagellum, strictly aerobic chemoorganotrophic bacterium. Last isolate DP-8 is a Gram negative, aerobic and rod shaped and non- spore- forming bacterium. Their colony characteristic on nutrient agar plates is observed to be yellow, round, smooth, wet and slightly raised. Biochemical test for all these isolates according to Bergey's manual are describing in Table 1 & 2.

3.3 Identification of bacterial isolates

For Identification of bacterial strain to carry out by 16S rRNA sequence analysis from the isolates 2 (N), 1(O), and 6(Q) have been submitted in the NCBI Gene Bank database under the accession number. On the basis of morphological, biochemical characteristics and 16S rRNA sequences, isolated strains DP-7, DP-1 and DP-8 was identified as *Pseudomonas stutzeri (*Gene Bank accession number- *KM363251), Tistrella mobilis (*Gene Bank accession number- KM363245 *)* and *Sphingosinicella microcystinivorans (*Gene Bank accession number- KM363252 *)* respectively, as is showed in Table 3.

Table 1: Biochemical characteristics of organophosphorus degrading bacterial isolates

No.	Biochemical Test	Results		
		Pseudomonas stutzeri	*Tistrella mobilis*	*Sphingosinicella microcystinivorans*
1	Catalase	+	+	+
2	Oxidase	+	+	+
3	Indole production test	-	+	-
4	Methyl red test	-	-	-
5	Voges proskauer's test	+	-	-
6	Citrate utilization test	+	-	+
7	Nitrate reduction test	+	+	-
8	Urease test	-	-	-
9	H_2S production	-	-	-
10	Gelatin liquefaction	-	+	-
11	Starch hydrolysis	-	-	-
12	Casein hydrolysis	-	-	-
13	Motility test	+	+	+

Key: - positive (+), negative (-) assimilate

Table 2 Biochemical characteristics of organophosphorus degrading bacterial isolates

No.	Biochemical Test	Results		
		Pseudomonas stutzeri	*Tistrella mobilis*	*Sphingosinicella microcystinivorans*
1	Galactose	-	w	-
2	Fructose	+	+	-
3	Maltose	-	+	+
4	Saccharose	+	-	-
5	D-Mannitole	-	+	-
6	Sucrose	-	-	+
7	Gluconate	-	+	+
8	Lactose	-	-	-
9	Melibiose	-	-	+
10	Glucosamine	+	-	-
11	Glucose	+	-	+
12	L-Arbinose	-	+	+
13	Rhamnose	-	-	+

Key: - positive (+), negative (-), w (weak reaction)

Table 3 Three morphologically diverse bacterial strains were isolated from effluents**.**

NO.	Name of pesticides	Isolated strains	GeneBank accession number
1	Monochrotophos	*Pseudomonas stutzeri*	*KM363251*
2	Dichlorovos	*Tistrella mobilis*	KM363245
3	Dimethoate	Sphingosinicella microcystinivorans	KM363252

Conclusion

Organophosphorus compounds have become increasingly popular for agricultural, industrial and home use and represent a significant potential health risk. Organophosphorus pesticide degrading bacterial strains was isolated from industrial effluents and its contaminated area. According to studied morphological, physiological characteristics, biochemical test and 16s rRNA sequencing, the isolated strain was identified as *Pseudomonas stutzeri,* ***Tistrella mobilis***, Sphingosinicella microcystinivorans. This identified bacterial mixtures will be apply in pesticide contaminated field. So, we can improve and restore biodiversity in soil and water and also use enhancing N-fixation and mineralization in soil and making organic farming possible on the decontaminated areas.

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