WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 9, 660-667. Research Article ISSN 2277– 7105

ENRICHMENT AND ISOLATION OF ENDOSULFAN DEGRADING BACTERIA FROM INDUSTRIAL EFFLUENT

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Article Received on
21 August 2014,

Revised on 14 Sept 2014,
Accepted on 09 Oct 2014

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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. Endosulfan 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 and biochemical test and 16s rRNA sequencing, the isolated strain was identified as *Bacillus oceanisediminis, Tistrella mobilis*, *Parapusillimonas granuli*respectively. From this result that the isolated bacteria could be used for the removal of residues of organochlorine in contaminated area.

KEYWORDS: Industrial effluents, Pesticide Industries, Pollution, Endosulfan, Degradation.

INTRODUCTION

Organochlorine are one the most popular and efficient pesticide used so far. But their extensive use led certain health and environmental issues (Böschen 2002). DDT and Endosulfan are example of such pesticides. Now a day they are banned in certain countries. (Meenakshi et al. 2012; Zitko 2003). The wide applications of organochlorine (OC) pesticide such as these pesticides are employed for plant protection against insect pests (Harish. R et al.2013).

Endosulfan is also an organochlorine insecticide. It is most popular acaricide to control the ticks and moths.(Isaacs, Morrone, and Gajek 2004. Uysal-pala and Bilisli 2006). According to the report of WHO, production of endosulfan was increased gradually during 1980 to

1990. It was noted that upto 1980 approximately 9000M tonnes of endosulfan were produced globally which was reached at 12,800M tonnes at the end of 1990. Knowing the toxic effect of the insecticide it was banned in the year 2000 and production was greatly reduced after the bane. Production, use and sale of the insecticide were banned in India in 2011 after the order of Supreme Court. Endosulfan has various adverse effect of human health. Mostly they affect the endocrines and reproductive system. (Da Cuña et al. 2013; Plunkett 2008). Previous reports also indicated that it may also lead to cancer especially breast cancer.(Bhaskarapillai, Sunilkumar, and Balasubramanian 2012; Mannan 2011)

Degradation of pesticide using microorganism is known as biodegradation of pesticide. If it is applied for removal of pesticide from contaminated area then it is called bioremediation of pesticide using microorganisms (Singh 2008; Megeed & El-nakieb 2008). There several references which show that pesticides can be degraded rapidly using microorganisms. There are certain reasons behind these. (i) There are number of microbes in the nature which degrade either one or another pesticide. (ii) Cultivation of these microbes is comparatively very economic and easy. (iii) Any modifications those are require to enhance their capability could also be applied very easily. (iv) Different microorganisms degrade the pesticide in more than one method yielding various by product which might has economic value. (v) Widely applicable to all kind of environment i.e. air, water, soil etc. It was seen that biodegradation of pesticide mainly involve activity of certain enzymes like hydrolase, dehalogenase, oxidase, reductase etc (Castillo et al. 2011; Singh & Walker 2006). Detail pathways of degradation of popular pesticides are given below.

Most of the methods were able to degrade the endosulfan in more or less quantity. However attempts were also made to study the degradation on soil, water and other contaminated sites. Strains such as *Arthrobacter* sp, *Burkholderia cepacia*, *Pseudomonas* sp and *Rhodococcus* sp are some of the bacteria strains widely used in the majority of biodegradation and bioremediation endosulfan (Diúrak & Kazanici 2001; Singh 2008; Harish et al. 2013; Bhalerao 2012; Karpouzas & Singh 6AD). Many people have also tried using consortia for remove surface contamination (Arunkumar & Chandrasekaran 2013; Fuentes et al. 2010). Results of these studies have shown that consortia can work in better way than the pure culture. Not only this, they have also shown that higher the exposure time will enhance the rate of remediation (Rand et al. 2010).

2. MATERIAL AND METHODS

2.1 **Reagents**

Technical grade Endosulfan (35% pure) was 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 endosulfan 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 endosulfan 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.

2.3 **Enrichment and Selection of Microbial Consortium**

We cultured a microbial consortium capable of Endosulfan degrading pesticides. This microbial consortium had been obtained from pesticide industrial effluents having several applications of Endosulfan. 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 Endosulfan (50 mg/mL), 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 endosulfan. Pesticides. These plates were incubated in incubator (Nova make) at 37℃ for 24-48 hrs. Individual colonies were sub cultured 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 endosulfan pesticides (50 mg/mL) as sole source of carbon and energy. After incubation time, colony characteristics and morphological properties were noted. Endosulfan degrading isolates streaked on to nutrient agar slants and maintained at 4°C 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 Bio Gene-Gujarat Biodiversity Gene Bank, India. Similarity analysis of the 16S rRNA sequences was conducted using the blast function of NCBI Gene Bank.

3. **Results & Discussion**

3.1 **Isolation of Organochlorine Degrading Bacteria**

Numbers of the indigenous pesticide tolerant bacteria was isolated by enrichment technique. From this enrichment cultures, 15 morphologically various strains were isolated on nutrient agar plates containing Endosulfan pesticides. The pesticides degradation ability of these strains was checked on MSM agar plates containing endosulfan pesticides (50 mg/mL). Out of 20 isolated, only four isolated strains (DP-9, DP-3 and DP-4) showed significant degradation of pesticides.

3.2 **Characterization of Bacterial Isolates**

One of the three isolated, Colonies of strain DP-9 on nutrient agar plates appeared with smooth, round and 1–3 mm in diameter, edges are usually irregular with pointed projections. The strain was a Gram-stain-positive, spore-forming, rod-shaped and aerobic bacterium. Second isolate DP-3 is a Gram negative, rod shaped, highly motile with a single polar flagellum, strictly aerobic chemoorganotrophic bacterium. Last isolated DP-4 is a Gram-negative, facultative anaerobic, non-spore-forming, motile and short rod-shaped, beige-coloured bacterium. It grew smooth and circular bacterium colonies after incubation at 35°C for 18 hr. 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 DP-9, DP-3 and DP-4 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-9, DP-3 and DP-4 were identified as *Bacillus oceanisediminis (*Gene Bank accession number- *KM363253), Tistrella mobilis (*Gene Bank accession number- KM363247 *)* and *Parapusillimonas granuli* (Gene Bank accession number- KM363248) respectively, as is showed in Table 3.

Table 1: Biochemical characteristics of organochlorine degrading bacterial isolates

S. No	Biochemical Test	Results		
		Bacillus oceanisediminis	Tistrella mobilis	Parapusillimonas granuli
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 (-)

Table 2: Biochemical characteristics of organochlorine degrading bacterial isolates.

S.No	Biochemical Test	Results		
		Bacillus oceanisediminis	Tistrella mobilis	Parapusillimonas granuli
1	Galactose	-	w	-
2	Fructose	-	+	-
3	Maltose	w	+	-
4	D-Mannitole	+	+	-
5	Sucrose	+	-	-
6	Gluconate	-	+	+
7	Lactose	+	-	-
8	Melibiose	-	-	-
9	Glucose	+	-	+
10	L-Arbinose	-	+	-
11	Rhamnose	+	-	-

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

Table 3: Morphologically diverse bacterial strains were isolated from effluents.

S. No	Name of pesticides	Isolated strains	GeneBank Accession Number
1	Endosulfan	Bacillus oceanisediminis	KM363253
		Tistrella mobilis	KM363247
		Parapusillimonas granuli	KM363248

4. CONCLUSION

Environmental and human health hazardous related with pesticide have forces scientist to find out the ways to get rid of the pesticide pollution. The major challenge for them was to find out most efficient way for a particular pesticide in a particular environment. There are many physical and biological remedies are being applied today to solve the problem. In the initial period around mid-20th century physical and chemical methods were most widely used. Use of microbes for biodegradable became popular after 1970. Now a day it is one of the most popular and efficient method for degradation of pesticides. In this study Endosulfan degrading bacterial strains was isolated from industrial effluents and its contaminated area. According to studied morphological and 16s rRNA sequencing, the isolated strain was identified as *Bacillus oceanisediminis, Tistrella mobilis, Parapusillimonas granuli.* 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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