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Original Research Paper

Biodegradation of Endosulfan by Bacteria Isolated From Soil

B. S. Anupama*, N. Nandini and S. K. Sarangi***
*****Department of Microbiology and Biotechnology, Bangalore University, Bangalore-560 056, Karnataka, India
******Department of Environmental Science, Bangalore University, Bangalore-560 056, Karnataka, India

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ABSTRACT

In the present study an attempt has been made to enrich and isolate bacterial species capable of degrading endosulfan isomers from different soil habitats. The two potent isolates showed about 51% endosulfan degradation during first eight days of incubation. However, the degradation reached between 63-82% after 16 days of incubation. Further, the degradation of the insecticide increased concomitantly with the growth of the bacteria at pH 8.0 and at 30°C. The results suggest that these novel strains could be used as potent agents for biodegradation of endosulfan pesticides.

INTRODUCTION

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,3,4-benzo-dioxathiepin-3-oxide), an organochlorine insecticide and acaricide, is used for controlling different insect pests of vegetables and fruit crops. It is a mixture of two stereo isomers, alpha and beta endosulfan in the ratio of 70:30. The former is more volatile and thought to be more toxic (Goebel 1982, Siddique et al. 2003a). It is extremely toxic to humans, animals and aquatic organisms and its residues are detected in air, water and soil environments, which gain entry during its usage and manufacture. It is highly hydrophobic and persists in the soil for more than 6 months (Rao & Murthy 1980).

Organic pollutants like endosulfan can serve either as carbon source (Guerin 1999) or sulphur source (Awasthi et al. 1997) for microbial growth. Microorganisms being a potential source of xenobiotic degrading enzyme (Chen & Mulchandani 1998), have been extensively used for the detoxification of endosulfan. Some microbial enzymes are specific to one isomer or catalyse both the isomers at different rates (Kwon et al. 2005). Several studies have been carried out on the isolation of fungi (Shetty et al. 2000, Katayama & Matsumura 1993) and bacteria (Miles & Moy 1979, Sutherland et al. 2000) capable of degrading endosulfan. Endosulfan produces endosulfan sulphate, a toxic product through oxidative pathway (Martens 1976) and endosulfan diol, a non toxic product through hydrolysis pathway. Microbial degradation depends not only on the presence of appropriate degradative enzymes, but also on a wide range of environmental parameters (Hussain et al. 2007, Kumar & Philip 2006). The present study describes the isolation and characterization of bacterial isolates capable of degrading endosulfan.

MATERIALS AND METHODS

Study area: A Chemical farm (CF) of about 6 acres near Bangalore (India), with mango cultivation was selected. Pesticides like endosulfan (35% EC) was used in this land since 10 years and sprayed twice a year to control leaf miners, thrips and caterpillars.

Enrichment and isolation: About 15 g of soil sample was transferred to an Erlenmeyer flask containing nutrient media and kept on a rotary shaker maintained at 150 rpm and at 30°C for 12 hours. The solid particles were allowed to settle down for 1 hour and the supernatant was collected. One mL of the supernatant was inoculated into a flask spiked with endosulfan (technical grade, Sigma Aldrich, USA) and containing freshly prepared nutrient media and kept for incubation at 30°C for 15 days. Further, 1mL of the sample was reinoculated into a fresh flask. This enrichment process (Fig. 1) was carried out thrice and 0.1mL of the enriched broth was transferred to Petri plate containing nutrient medium with agar and endosulfan. The nutrient medium used was the nonsulphur medium consisted of (g/L): KH_2PO_4, 0.225; K_2HPO_4, 0.225; NH_4Cl, 0.225; $MgCl_2.6H_2O$, 0.845; $CaCO_3$, 0.005; $FeCl_2.4H_2O$, 0.005; D-glucose, 1.0; and l mL of trace element solution per litre. The trace element solution prepared for the nonsulphur medium contained (mg/L): $MnCl_2.4H_2O$, 198; $ZnCl_2$, 136; $CuCl_2.2H_2O$. 171; $CoCl_2.6H_2O$, 24; and $NiCl_2.6H_2O$, 24 (Siddique et al. 2003). The pH was adjusted to 8.0.

Fig. 1: Flasks showing enrichment of cultures.

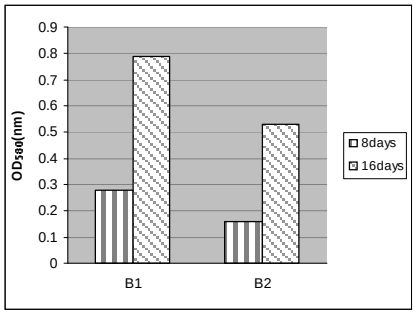

Fig. 2: Bacterial strains isolated from soil for endosulfan degradation.

Screening and identification of bacteria: Screening of the bacterial isolates was carried out to identify the potential endosulfan degraders wherein each of the bacterial isolate was treated with different concentrations of endosulfan spiked into Petri plates containing nutrient agar medium and incubated at 28°C for 24 hrs.

Endosulfan Biodegradation

Culture of bacterial isolates with endosulfan: The bacterial strains isolated by enrichment technique on nonsulphur medium containing endosulfan as a sole sulphur source were used for biodegradation study. Erlenmeyer flask containing nonsulphur media adjusted to pH 8.0, was autoclaved at 121°C for 15 minutes. After cooling to approximately 50°C, endosulfan dissolved in acetone was spiked to the flask to a final concentration of 50mg/L. The flasks were then inoculated with 800µL of the two bacterial isolates (OD_{580} = 0.9) separately and incubated at 30°C on an orbital shaker at 150rpm. Preparation of inoculum was carried out from late log phase cells that were harvested by centrifugation for 10 min at 5000 rpm. Uninoculated controls were also maintained to check abiotic degradation under the same conditions. The experiment was carried out in triplicate.

Extraction and analysis of endosulfan residues: Endosulfan was extracted by adding equal volume of n-hexane with 30 mL broth culture and shaken vigorously to separate the water and hexane layer that contains endosulfan. It was washed thrice and the extract was collected separately. The hexane layer collected was dehydrated by passing through anhydrous Na_2SO_4 and concentrated to dryness using rotary evaporator. Finally, the volume was made up to 5 mL using n-hexane.

The extract containing the endosulfan was analysed with a gas chromatograph (Agilent GC-6890N series) equipped with auto injector (7683b) and a DB-MS capillary column (internal diameter, 0.25mm; film thickness, 0.25 µm). The injector was operated at 290°C and detector was operated at 320°C. The initial oven temperature was 60°C and it was set for a linear increase of 4°C/min to attain the final temperature of 320°C. Carrier gas (nitrogen) was maintained at a flow rate of 1.0 mL/min and the make up gas at a flow rate of 50mL/min.

RESULTS

Enrichment and isolation: Four visibly distinct colonies were isolated and pure cultures were obtained by repeated subculturing. One set was maintained as stock and the other set was used for further study.

Screening and identification of bacteria: Two bacterial isolates (Fig. 2) were selected based on their growth at highest concentration of endosulfan. The bacterial isolates exhibiting maximum biodegradation of endosulfan pesticide were preliminarily identified according to the key of Bergey's Manual of Determinative Bacteriology (Kreig & Holt 1984) through staining and biochemical tests. Both the strains were Gram positive short rods in chains typical of *Bacillus* species.

Endosulfan degradation by bacterial isolates: Substantial degradation of endosulfan was observed by the isolated cultures, which were analysed by GC after 16 days of incubation. During the first seven days of incubation, the degradation of endosulfan was about 51% by both the bacterial isolates, which increased to about 60-82% after 16 days of incubation (Table 1).

Bacterial density: The bacterial density was assessed by

Table 1: Biodegradation of endosulfan by bacterial isolates.

Bacterial Isolates	%Degradation of Endosulfan	
	8 days	16 days
Uninoculated control	5.3 ± 0.3	8.5 ± 0.3
Bacterial isolate 1	49.0 ± 0.6	81.0 ± 0.6
Bacterial isolate 2	50.0 ± 0.6	63.0 ± 0.6
F value	2536***	5700***

Note: ± Standard error; F value followed by one way analysis of variance at ***P < 0.001

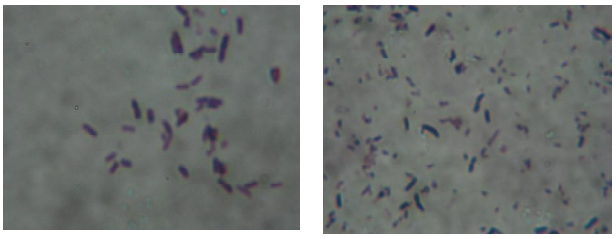

Fig. 3: Changes in the optical density of the culture broth of the bacterial isolates.

recording the changes in the optical density of the culture medium. It was observed that the OD increased proportionately with the increased growth of the bacteria after 16 days incubation. Further, B1 isolate showed highest OD compared to B2 isolate (Fig. 3).

Changes in the pH: The pH of the culture broth was found to be reduced to 3.8 and 5.16 from 8.0 by B1 and B2 isolates, respectively after 16 days of incubation when compared with the 7.47 in uninoculated control (Fig. 4).

DISCUSSION

The present study describes enrichment and isolation of microbial strains capable of degrading endosulfan. Enrichment was carried out by the addition of endosulfan as a sole source of sulphur to the minimal medium containing endosulfan contaminated soil. Kumar & Philip (2006) have successfully enriched and isolated bacterial isolates using endosulfan as a source of energy, which were further used for the degradation of endosulfan with both mixed and pure cultures. In our study endosulfan was used as a sole sulphur source to find out the degradation rate of endosulfan.

A significant difference was observed in the biodegradation of endosulfan among the control and bacterial isolates. The bacterial isolates efficiently degraded endosulfan isomers in the range of 60- 82% after 16 days of incubation, which are higher than the earlier reports, by bacterial isolates utilizing endosulfan either as sulphur source or carbon source (Sutherland et al. 2002a, Awasthi et al. 1997, Shivaramaiah & Kennedy 2006). This might be attributed to the efficient enzymatic system responsible for endosulfan degradation.

In the broth inoculated with B1 and B2 isolates, the pH decreased from 8.0 to 3.82 and 5.16 respectively at the end of the incubation proportionately with the progressive degradation of endosulfan i.e., higher degradation resulted in lower pH and vice versa. The decrease in pH could be due to dehalogenation of endosulfan and subsequent production of organic acids which are in accordance with the earlier reports (Kwon et al. 2002, Awasthi et al. 2003, Hussain et al. 2007). They interpreted that the decrease in pH may be due

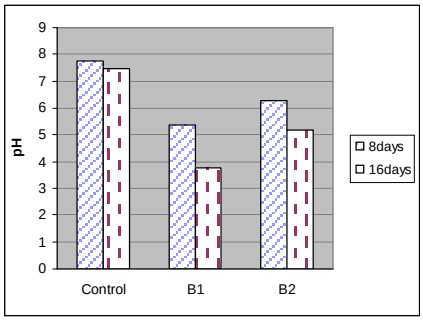

Fig. 4: Changes in the pH of the culture broth of the bacterial isolates.

to the formation of HCl and/or organic acids by the microbes. After the incubation period of 16 days B1 and B2 showed the OD value between 0.78 and 0.532 respectively at pH 8.0 and at 30°C. Sutherland et al. (2000) and Awasthi et al. (1997) also observed a substantial disappearance of endosulfan with a simultaneous increase in bacterial biomass.

In the control, about 15-20% disappearance of the substrate was observed after incubation for 16 days, which indicates that the natural process of degradation of endosulfan is very negligible. Further, endosulfan sulphate, the oxidation product and a persistent metabolite of endosulfan, was not detected in the broth during the study.

These findings demonstrate that the bacterial isolates have the efficiency to degrade endosulfan through a distinct pathway and have valuable applications in the removal of endosulfan from the contaminated sites.

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