Enrichment, Isolation and Identification of Endosulphan Degrading Microorganisms

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Abstract

The chlorinated cyclic sulphite diester endosulfan is a broad spectrum insecticide that has been used extensively for over 30 years on a variety of crops. Endosulfan is often classified as a cyclodiene and has the same primary action and target site as the other cyclodiens[1]. However, it has chemical and physical properties significantly different from other cyclodienes insecticide that affects both its environmental and biological fates. The aim of this research work is the investigation of an enzymatic Method for endosulfan degradation. Enzymatic degradation for pesticide is receiving serious attention as an alternative to existing methods, such as incineration and landfill. In particular, enzymatic insecticide bioremediation is the focus of extensive study after the isolation of enzymes capable of detoxifying a range of organophosphate compounds from several bacterial species. An essential step in the investigation of an enzymatic method for endosulfan degradation is the definitive identification of a biological source of endosulfan degrading activity.

Keywords- Endosulfan, Microorganisms, Gas chromatography, Degradation, oxidation

Introduction:

The chlorinated cyclic sulphite diester endosulfan is a broad spectrum insecticide that has been used extensively for over 30 years on a variety of crops. Endosulfan is often classified as a cyclodiene and has the same primary action and target site as the other cyclodiens[1]. However, it has chemical and physical properties significantly different from other cyclodienes insecticide that affects both its environmental and biological fates. In particular, endosulfan has a relatively reactive cyclic sulfite diester group[2] and, as a consequence, its environmental persistence is lower than that of other cyclodienes. Since the deregistration in many countries of most cyclodiene insecticide, the ongoing availability of endosulfan has become important as an alternative option in resistance management strategies of pest species. Additionally, compared to many other available insecticides, it has low toxicity to many species of beneficial insects, mites, and spiders[3]. However endosulfan is extremely toxic to fish and aquatic invertebrates and it has been implicated increasingly in mammalian gonadal toxicity[4], genotoxicity[5], and neurotoxicity[6]. These environmental and health concerns have led to an interest in post application detoxification of the insecticide.

As with the most pesticides, the persistence of and degradation of endosulfan are affected by the environmental conditions in which it is found. Endosulfan does not undergo direct photolysis but is transformed by the chemical hydrolysis under alkaline condition such as in sea water[7]. In soil, endosulfan has been shown to be degraded by a variety of microorganisms[8]. However degradation rates are usually low and metabolism often results in the formation of endosulfan sulfate, an oxidative metabolite shown to be equally as toxic and persistence as the

parent compound, endosulfan. Because of its persistence and toxicity, endosulfan contamination poses a significant environmental concern.

There are varieties of soil microorganism that have ability to degrade the endosulfan. The degradation of endosulfan by soil microorganism of family *Pseudomonas Sp.* was studied. In microbial degradation of endosulfan under aerobic condition, soil microorganism degrades the endosulfan and yielded the endosulfan sulphate (30-60%), with some endodiol (2.6%) and endolactone (1.2%). The parenthetical numbers refers to the percentage of the applied endosulfan recovered as a metabolite. Sixteen of 28 fungi, fifteen of 49 soil bacteria and three of 10 actinomycetes metabolized greater than 30% of the applied C-14 endosulfan. Endosulfan sulphate was the major metabolite formed by the fungi and endodiol was the predominant product of the bacteria. Only a small amount of C-14 labeled carbon dioxide was detected, indicating minimal mineralization.

Material and Methods:

1. Materials and reagents

Technical grade endosulfan was supplied from Department of microbiology Gurunanak College of science, Ballarpur (M.S.). Technical grade endosulfan (used commercially) is a mixture of two diastereomers, alpha - endosulfan and beta - endosulfan in a ratio of 7:3, hexane (HPLC grade), acetone. Standard chemical were used for the preparation of nutrient media. For the chemical and instrumental analysis, spectrophotometric grade chemical were used.

2. Nutrient media for the enrichment of microorganisms

The endosulfan enrichment media for the isolation of microorganisms was prepared by the addition of following component (gm/lit). This media is actually a basal medium containing the endosulfan as a carbon source. $KH2PO_4$-0.5, $K2HPO_4$-0.5, NaCl-0.5, $MgSO_4$ $7H_2O$-0.5, $FeSO_4$ $7H_2O$, $CaCl_2$-0.002, NaMI4-0.001, CoNO3-0.0005, ZnSO4-0.0005, MnSO4-0.0005, Endosulphan-0.001, pH-7.2

3. Sample collection for isolation studies

The soil sample for the enrichment and the isolation of the microorganisms was collected from the cotton field near Gadchandur (M.S) India at the end of growing seasons. The field had generally received several application of endosulfan in the month of September to October for at least 2-3 times. The soil was fertile gray. The top soil collected from the upper layer (approximately 15 cm) and stored at 4°C prior to the experimental studies.

4. Isolation of endosulfan degrading microorganisms

For the isolation of endosulfan degrading microorganisms, soil perfusion apparatus was designed. This work on the air pressure created by the vacuum. The small holes were made at the top and the sand pebbles bigger in size than the hole were kept over it. The sand pebbles were used for the support and slow perfusion of the soil sample to the medium which is kept at the bottom. The tap water is open to create air pressure; this air pressure is helpful for the aeration to the medium. Due to this air pressure the medium rises above and drifts onto the soil. The soil

moistens with the media and perfused to the medium at the bottom. This process recycles continuously and microorganisms present in the soil enriched into the media. The soil perfusion apparatus and the sand pebbles sterilized at 121°C for 15 minutes. The endosulfan enrichment medium was added to the bottom. the sand pebbles were kept over the holes at the top. The fertile gray soil (approximately 10gm), then the tap water is open such that the medium rises above the soil and soil sample slowly perfused to the medium. The apparatus were kept run for the 10 days. After the 10 days of incubation, the small aliquot of enriched soil inoculums were plated over the endosulfan enrichment agar. The plates were incubated for the 24 hr at 37°C for the growth of microorganisms. After 24hr, the different population of microorganisms cultured on the endosulfan enrichment agar.

5. Isolation of single strain of microorganisms from the mixed culture

Mixed population of bacteria on the endosulfan enrichment agar was plated over the nutrient agar plates for the 24 hrs in the order to obtain the pure culture. The different isolates were subculture on the nutrient agar plates up to 5-6 days until obtain an Isolation of single strain of microorganism. The isolates were stored at 4°C and subculture after 3 weeks.

6. Identification of endosulfan degrading microorganisms

For the identification of single strain of isolates ED-R1, ED-Y1, and ED-P1, following microscopic, morphological and biochemical studies were been carried out.

7. Degradation of Endosulfan by isolate ED-R1, ED-Y1 and ED-P1

The soil perfusion apparatus were sterilized at 121c for 15 min. The endosulfan enrichment media were prepared and approximately 500 ml of media feeded into the apparatus. The Nutrient broth inoculums of the ED-R1, ED-Y1 and ED-P1 (approximately 50 ml) was added to the media. The apparatus were kept run for the 10 days of incubation. The endosulfan was extracted from the media after the incubation period of 10 days.

8. Quantitative Estimation of Endosulfan by Gas Chromatography

The quantitative analysis of endosulfan and its metabolite was done by gas chromatography-chemito model 1000 GC equipped with electron capture detector by using a glass column (8 inches length X 0.25 inch diameter). The instrument operating condition were as follows ; the column temperature was 140°C-2 minutes hold, R1-20 °C-250 °C-0 minutes, the injector temperature was 280 °C, Nitrogen was used as carrier gas at the flow rate of 1.5 ml / min. The injected volume of sample in GC was 2 µl. The extracted endosulfan sample were been analyzed by Insecticide Residue Testing Laboratory, Nagpur.

Result and Discussion:

Identification of Isolated Strain of Bacteria

From of the results of microscopic, morphological and biochemical test, the isolate ED-Y1,ED-R1 and ED-P1were been identified as *Klebsiella sp.*, *Serratia sp.* and

Pseudomonas sp.. The obtained result were studied and compared with standard results of respective bacteria.

2. Degradation of Endosulfan by the Bacterial Isolates

The degradation of endosulfan was been confirmed by analyzing the sample by gas chromatography. The degradation was determined by monitoring endosulfan disappearance by GLC-ECD detection. The bacterial isolate ED-P1 degraded 91.7% (0.083 ppm) which is much higher than that of isolate ED-R1 which degraded 59% (0.417 ppm) and isolate ED-Y1 degraded 79.1% (0.209 ppm) endosulfan after the 10 days of incubation. The initial concentration of endosulfan in the culture media (control) was 1 ppm. The isolate ED-P1 degraded α-endosulfan upto 86% (0.140 ppm) and β-endosulfan upto 97.2% (0.028ppm). The isolate ED-R1 degraded 73.4% (0.266 ppm) of α-endosulfan and 85.5% (0.145 ppm) of β-endosulfan. The 85.1% (0.149 ppm) of α-endosulfan and 93.5% (0.065 ppm) of β-endosulfan was degraded by isolate ED-Y1 (See Figure No.2). The degradation of β-endosulfan was found to be higher than that of α-endosulfan by all the three bacterial isolates. The result of this study suggests that these bacterial isolate are a valuable source of potent endosulfan degrading enzymes for use in enzymatic biodegradation.

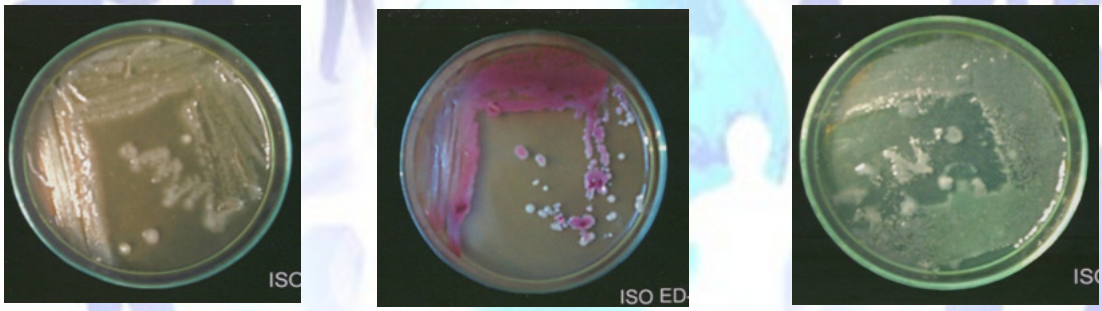
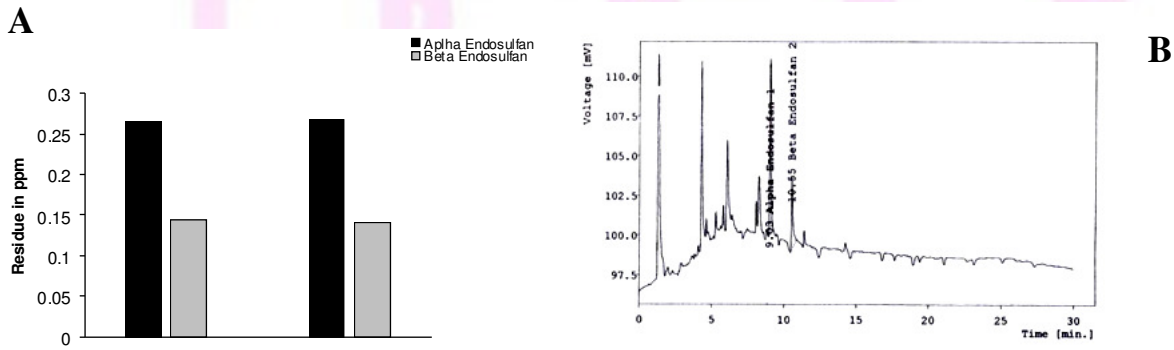


Figure 1 - Pure culture of bacterial isolate ED-Y1, ED-R1and ED-P1 isolated from mixed population of bacteria.



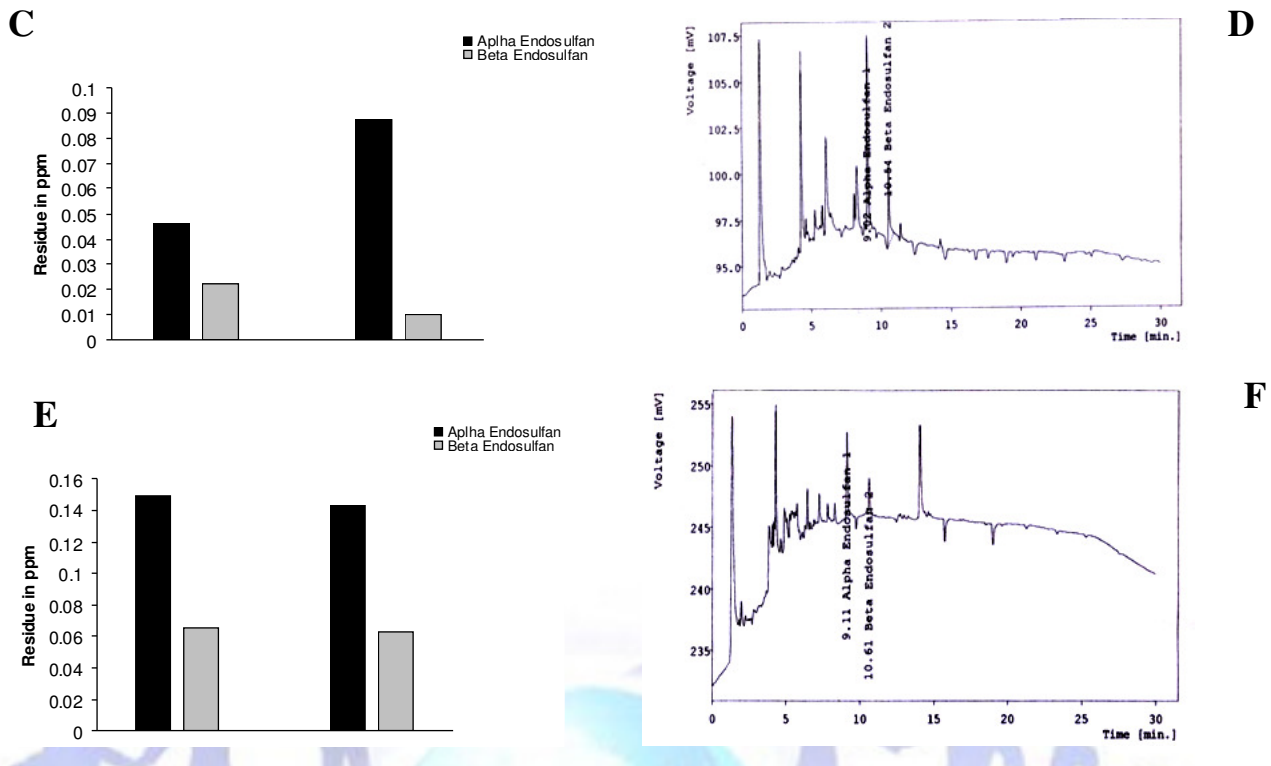


Figure 2 - A) Degradation of Endosulfan by bacterial isolate ED-R1 after 10 days of incubation. **B)** Gas Chromatographic analysis data sheet for degradation of endosulfan by isolate ED-R1 **C)** Degradation of Endosulfan by bacterial isolate ED-P1 after 10 days of incubation. **D)** Gas Chromatographic analysis data sheet for degradation of endosulfan by isolate ED-P1. **E)** Degradation of Endosulfan by bacterial isolate ED-Y1 after 10 days of incubation. **F)** Gas Chromatographic analysis data sheet for degradation of endosulfan by isolate ED-Y1.

Conclusion:

Microorganisms have increasingly been investigated as a source of xenobiotics-degrading enzymes. We are interested in the isolation of endosulfan degrading bacterium for further investigation into enzymatic endosulfan bioremediation. Using endosulfan as the only available carbon source, we can enrich soil inocula for microorganisms capable of releasing the sulfur from the endosulfan, thereby providing a source of carbon for growth. Since the removal of carbon moiety dramatically decrease the vertebrate toxicity of endosulfan, this results in concurrent detoxification of the insecticide. We report here on the resultant bacterial culture that, the culture degrades endosulfan to produce a novel metabolite not reported to occur as a result of chemical hydrolysis. These results suggest that the obtained bacterial isolates are a potential source of an enzymatic bioremediating agent.

We are currently attempting to isolate a pure bacterium from the soil that is capable of detoxifying endosulfan. Such a bacterium would potentially be a valuable source of catalytic enzymes for the development of bioremediating agent to reduce endosulfan residue problems in run-off from irrigation waters.

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