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Research Article

STUDY OF POTENTIAL ACTIVITY OF SOIL ISOLATED STRAIN OF *BACILLUS SUBTILIS* IN DEGRADING ORGANOPHOSPHATE PESTICIDE ENDOSULFAN

Prafulla Katkar[1], Amol Gargelwar[2], Chetan Wankhede*[3], Shafi Sheikh[4]

[1]*Guru Nanak College of Sconce, Ballarpur, Maharashtra, India*

[2]*Chintamani College of science, Pombhurna, Maharashtra, India*

[3]*Mahatma Gandhi College of Science, Gadchandur, Maharashtra, India*

[4]*Dr. C. V. Raman College of Science, Sironcha, Maharashtra, India*

**Corresponding author: chetan7008wankhede@gmail.com*

ABSTRACT

Endosulfan, which regulates a wide variety of pesticides, is a representative pesticide with Sulphur and chlorine as a functional group. Residues of Endosulfan α and β vary and persist with their oxidation products in air, water and soil. The remediation of novel insecticides is gaining interest, for the characterization of enzymes detoxifying a number organophosphate compound. Enzyme degradation study is essential to provide catalytic mechanism with the assumption that they are able to detoxicate the insecticides. The present work explains the enrichment of an endosulfan degradation by soil bacteria and can be used as bioremediating agent.

The promising degradation of endosulfan have been found when exposed to the soil bacteria. The maximum endosulfan degradation (upto 90%) was observed when incubated with the bacteria. The obtained bacterial isolate found to capable of degrading more of α-endosulfan than β-endosulfan after incubation of 10 days. The maximum growth as observed by the optical density indicated the utilization of endosulfan as a carbon and energy source. Organisms that are isolated from soils polluted by endosulfan by the enrichment process may also be used in soil and water as bio-remediators. The conclusive discovery of a biological cause of Endosulfan degrading activity is an important step in the enzymatic investigation process for endosulfan degradation.

Keywords: Endosulfan, Bioremediation, Gas chromatography, Degradation, Organophosphate, Pesticide.

1. INTRODUCTION

There are varieties of soil microorganism that have ability to degrade the endosulfan. Technical-grade endosulfan is a mixture of two stereoisomers such as β as well as α endosulfan, in 1:7 ratio (Figure 1). Endosulfan degradation of by soil microorganism of family *Bacillus 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%). Like other pesticides, endosulfan persistence and degradation are influenced by the environmental conditions.

Endosulfan is not converted by direct photolysis, and is transformed into alkaline with seawater, by the application of chemical hydrolysis [1]. The use of micro-organisms has shown that endosulfan has been destroyed on the soil [2]. The oxidation is normally poor, however, and metabolism also produces endosulfan, an oxidative metabolite that has been found to be as toxic and permanent as endosulfan, the parent composite. Endosulfan contamination faces considerable environ-mental concerns because of its longevity and toxicity [3, 4]. As a xenobiotic-degrading enzymes source [5], microorganisms have been more and more examined. For more study into enzyme endosulfan bioremediation, we are involved in the isolation of endosulfan degrading bacterium. Fungi, soil bacteria and Actinomycetes have shown the activity of metabolizing endosulfan. Endosulfan sulphate became the main metabolite formed by way of the fungi and endodiol became the fundamental product of the bacteria [6]. Because the removal of carbon moiety dramatically lowers the endosulfan vertebrate toxicity [7], this effects in concurrent cleansing of the insecticide. Outcomes recommend that whilst each isomers might be degraded with the aid of microbial organisms, the degradation substances released counteract the growth of the microorganisms. Most

effective a small amount of C-14 categorized carbon dioxide changed into detected, indicating minimal mineralization. The usage of endosulfan as the only to be had carbon supply, we may enhance soil inoculations of microorganisms that release endosulfan Sulphur, thus creating a carbon-increasing source [8].

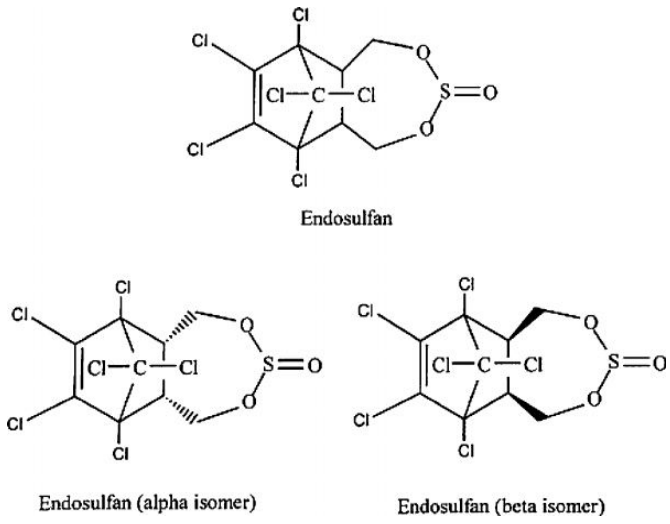


Fig. 1: Structure of endosulfan and its isomers

In order to conduct more research into the enzyme endosulfan bioremediation, we are involved in the isolation of endosulfan degrading bacteria and study here the bacterial culture resulting in endosulfan degrading into a new metabolite caused by chemical hydrolysis. Such results recommend that the obtained bacterial isolates at optimized growth condition are a potential source of an enzymatic bioremediating agent. Microorganisms have been chosen in this research for their ability for releasing endosulfan from the carbon group as well as utilized as a carbon source for growth. In this work we have studied the different process optimization parameters to obtain the maximum degradation.

2. MATERIAL AND METHODS

2.1. Materials and reagents

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

2.2. Sample collection for isolation studies

The soil sample for the enrichment and the isolation of the microorganisms has been gathered from the cotton field near Gadchandur (M.S) India at the end of growing seasons. In general, endosulfan had been applied for at least 2-3 times between September and October 2019. Soil was fertile gray. The top soil gathered from upper layer (approximately 15cm) and stored at 4°C prior to the experimental studies.

2.3. 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 [9]. $CaCl_2$-0.002, $FeSO_4$ $7H_2O$, $MgSO_4$ $7H_2O$-0.5, NaCl-0.5, K_2HPO_4-0.5, KH_2PO_4-0.5, $NaMI_4$-0.001, $CoNO_3$-0.0005, $ZnSO_4$-0.0005, $MnSO_4$-0.0005, Endosulphan-0.001, pH-7.2

2.4. Isolation of endosulfan degrading micro-organisms

Soil perfusion apparatus has been designed for the isolation of endosulfan degrading bacteria. This instrument work on the air pressure created by the vacuum. The small holes were made at the top and sand pebbles were kept over it 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. The soil gets 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 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), and 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 inoculum were plated over the endosulfan enrichment agar. The different population of microorganisms on the endosulfan enrichment agar then achieved.

2.5. Identification of endosulfan degrading microorganisms

For the identification of single strain of isolates ED-B1 following microscopic, morphological and biochemical studies were been carried out.

2.5.1. Microscopic Studies

Microscopic details of the isolate ED-B1 have been done. The given isolates are whether Gram positive or Gram negative also been decided.

2.5.2. Morphological studies

Under the morphological studies, the various colonies characteristics like, shape color and growth pattern have been studied.

2.5.3. Biochemical studies

Following various biochemical tests have been carried out for each isolates; Indole, Methyl Red, Voges Prausker's and Citrate utilization test, Catalase test, Starch utilization test, Urease test, Nitrate reduction test, and Oxidase test.

2.5.4. Sugar Fermentation Test

For the sugar fermentation test 0.5% NaCl. 0.5% peptone and 0.5% of the sugars were been added and incubated with the given isolate ED-B1. The tubes were observed for the production of acid gas after 24 hours.

2.6. Analytical Method

2.6.1. Analysis of bacterial density

Optical densities at λ600 of the endosulfan upgrading media incubated with the given isolates ED-B1 had been measured for evaluating the connection among increase as well as metabolic sports of microorganisms, the bacterial growth of the isolate ED-B1 had been determined in response to endosulfan provided as the carbon supply. The optical density of every isolates turned into measured with the period in-between of two days by way of the visible spectrophotometer and respective readings had been recorded.

2.6.2. Analysis of pH of the Medium

The pH of the endosulfan enrichment media was measured in the order to assess the relationship between growth and metabolic activities of the microorganism. The change in the pH of the endosulfan enrichment media with interim of two days were recorded during the 10 days of incubation. The initial pH of the media was adjusted to 7.2.

2.6.3. Extraction of Endosulfan from the Media

Endosulfan has been extracted from the enrichment media for the degradation studies. Approximately 25 ml culture media sample were taken out from the soil perfusion apparatus and equal volume of acetone (i.e. 25 ml) were added. The acetone - sample mixtures were shaken for 1 hr on the magnetic stirrer. 1ml of the mixture were taken out and transferred to 9 ml of hexane. Next 15 min these mixtures were further shaken [10]. After that sample has been dehydrated by the addition of Na_2SO_4. The sample is then stored in vials at 4°C for the further analysis.

2.6.4. Quantitative Estimation of Endosulfan by Gas Chromatography

Suitable dilutions of the pattern extract were then analyzed via gasoline chromatography-chemito model 1000 GC geared up with electron seize detector by way of the usage of a pitcher column (8 inches length X 0.25 inch diameter). Nitrogen turned into utilized as carrier gasoline at 1.5ml/min glide rate. The injected extent of pattern in GC become 2 µl. Oven temperature turned into programmed for 1minute at 175, accompanied by way of a linear boom of 2.43°C to 260°C for 5 minutes, preserving for 5 minutes at 260°C. 250°C was the temperature of injector whereas 300°C was the temperature of detector.

3. EXPERIMENTAL RESULTS AND DISCUSSION

3.1. Microscopic and Morphological characters

The isolate ED-B1 showed Gram positive rod shaped cells arranged mostly separated (Figure 1B). The colonies on the endosulphan enrichment media were pale yellow/white colored, moist, pleomorphic with rod shape with rounded end (figure 2A). The isolate was found to be motile with swarming movement.

3.2. Biochemical Test

The results of all biochemical test performed with isolate ED-B1 is given in the table 1. The ability of isolate to produce various enzymes is indicated by the positive test. Number of different biochemical test have been performed and from the result obtained the unknown isolate ED-B1 is identified as *Bacillus sp.*

3.3. Sugar Fermentation Test

The result of sugar fermentation test of isolate ED-B1 is given in the table 2. From the result, it has been observed that all the isolate utilize the carbohydrate as a source of carbon and energy through enzymatic breakdown producing acid and gas.

3.4. Identification of isolated strain of bacteria

From of the results of microscopic, morphological and biochemical test, the isolate ED-B1 has been identified as

Bacillus subtilis (Fig. 2B). The obtained result were studied and compared with standard results of respective bacteria [11, 12].

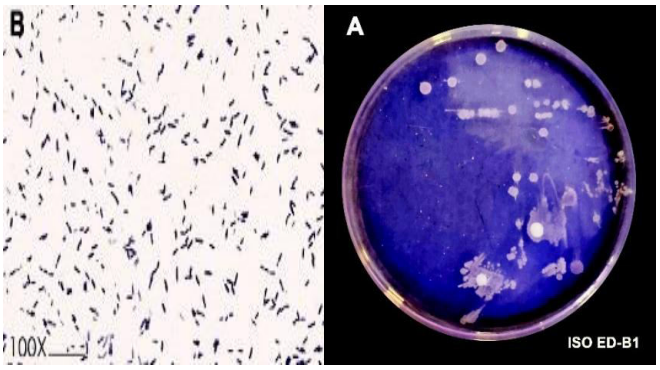


Fig. 2: A) Pure culture of bacterial isolate ED-B1 grown on endosulphan enrichment media isolated from mixed culture B) Microscopic view of Gram positive rod shaped purple coloured bacteria

3.5. Measurement of bacterial density

Optical densities (λ_{600}) of the isolate is represented in the figure.3. Successive progression of bacterial growth observed during the incubation. The highest OD_{600} recorded for ED-B1 was 0.51 as per the result, it has been found that the bacterial strain degrading more endosulfan within the culture media presented high bacterial density. Siddique *et al* [9] was observed the same in that bacterial strain that depleted α and β endosulfan as a sulphur source. There is gradual decrease of endosulfan concentration with the simultaneously increase in the bacterial mass density [13].

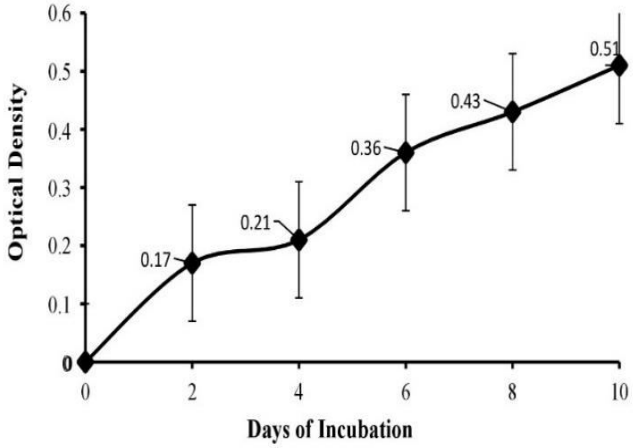


Fig. 3: Variation in the O.D 600 of bacterial culture ED-B1 after 10 days of incubation

Bacterial density obtained with the isolate ED-B1 are quite higher in comparison to Kumar A, *et al* [14] obtained with *Bacillus subtilis* strain and the endosulfan utilization has been attended by the increase optical density (OD_{595}) of the culture media ranging from 0.51 to 0.89 [15].

3.6. Measurement of pH of medium

Figure 4 shows the change in the endosulfan pH enrichment media after 10 days of incubation. These metabolic activities of the growing organism may causes decreased in pH to acidic. The isolate ED-B1 showed the decreased pH of the medium to 3.15 after the 10 days of incubation. It has observed that the decreased in the pH of the medium was found to be associated with enhanced degradation of the endosulfan [10]. With the interim of two day during each pH reading, pH decreased with the bacterial metabolism. The decrease in the pH may be because of endosulfan dehalogenation resulting in the organic acid formation formed by microorganism throughout their metabolic activities.

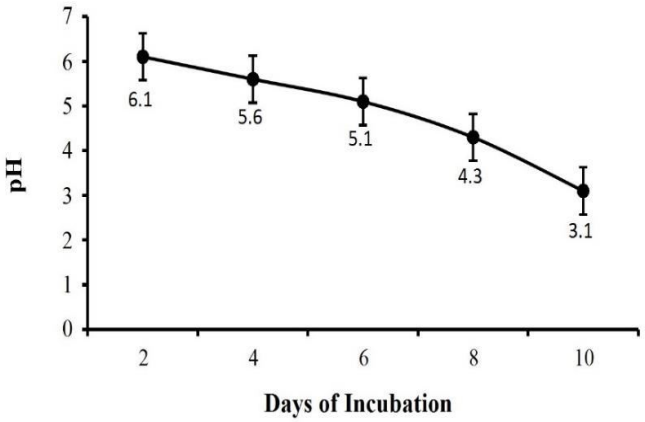


Fig. 4: Variation in the pH of bacterial culture ED-B1 after 10 days of incubation

Certain bacteria that showed the pH value of 8.3 and 8.5 at the end of the experiment [16]. This higher pH value was probably due to the chemical hydrolysis but some of the bacteria were having the low pH values, which indicate that a large portion of degradation was enzymatic. Endosulfan is vulnerable to alkaline hydrolysis occurring with nearly 10-fold increase in hydrolysis with each increased in pH unit. The earlier study is not able to differentiate between biological as well as chemical hydrolysis of endosulfan as microbial development has directed to the increased in alkalinity of the culture media [17].

3.7. Degradation of Endosulfan by the Bacterial Isolate ED-B1

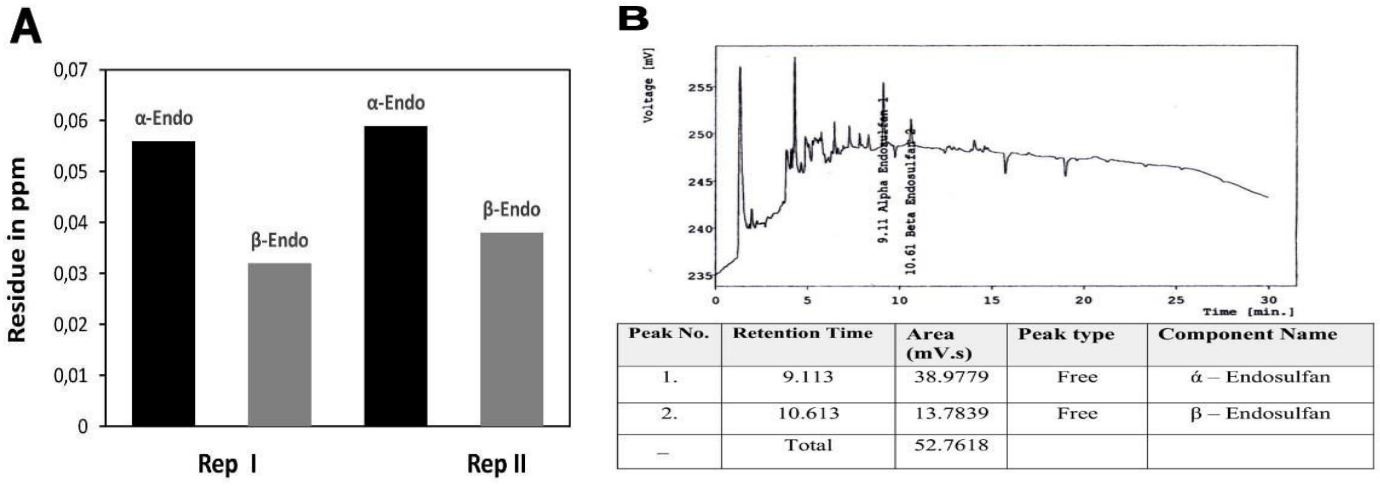
The degradation of endosulfan were confirmed by analyzing the sample by gas chromatography as shown in the figure 4A and table 3. The degradation was determined by monitoring endosulfan disappearance by GLC-ECD detection. The bacterial isolate ED-B1 degraded 90.7% (0.092 ppm) endosulfan after the ten days of incubation. The initial concentration of endosulfan in culture media was 1 ppm. The isolate ED-B1 found to degrade 94.2 % (0.057 ppm) of α-endosulfan and 96.5 % (0.035 ppm) of β-endosulfan. The degradation of β-endosulfan was found to be higher than that of α-endosulfan by ED-B1 isolates and similar results were obtained with *Bordetella sp.* [18] and *Klebsiella sp.* [19]. The result of this study suggest suggests that the ED-B1 isolate is a valuable supply of robust endosulfan degrading enzymes for utilization in enzymatic biodegradation.

The endosulfan was used separately as a carbon source to identify which microorganism prefers endosulfan as a carbon source and to what extent endosulfan is degraded when used as carbon source. This is most likely to the fact that carbon is required by the cells in large amount in the order to develop cell components. The obtained results are much similar to findings of Siddique *et al* [10] who had worked on *Fusarium ventricosum* which degraded α-endosulfan upto 82.2% and 89.0% of β-endosulfan when endosulfan supplied as carbon source. The bacterium Pseudomonas Spinosa and Pseudomonas aeruginosa have been the most effective degraders of both β-endosulfan as well as α-endosulfan as they consumed more than 90% of endosulfan [20] while the other study recorded different results for the *Pseudomonas aeruginosa*. [21]. The less amount of endosulfan detected after degradation by bacteria indicate that the bacteria is hydrolyzing the compound to release the intermediate groups from endosulfan for using them as carbon source for growth and metabolism.

4. CONCLUSION

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. The isolate ED-B1 degrades more endosulfan showed higher growth density which degrade high amount of α-endosulfan and β-endosulfan in the nutrient media utilizing endosulfan as a carbon source. The pH reduction of culture medium is associated with more degradation of endosulfan as it is indicated by the isolate ED-B1. It has been observed that β-endosulfan degradation occurs to a greater extent than α-endosulfan. These results suggest that the obtained bacterial isolate could be a potential source of an enzymatic bioremediating agent.

In overall conclusion, the isolation and enrichment of endosulfan an degrading bacterial isolate that utilize endosulfan as carbon source could be agent for endosulfan bioremediation in contaminated soil. An endosulfan reducing bacterium like this may be used in developing bioremediation processes to minimize the amount of endosulfan in irrigation water.



Peak No.	Retention Time	Area (mV.s)	Peak type	Component Name
1.	9.113	38.9779	Free	ά – Endosulfan
2.	10.613	13.7839	Free	β – Endosulfan
–	Total	52.7618		

Fig. 5: Gas chromatographic analysis of endosulphan degradation by isolate ED-B1. A) Comparative analysis of degradation of alpha and beta isomers of endosulfan. B) Gas chromatogram and retention data of endosulfan isomer

5. ACKNOWLEDGMENT

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Conflict of interest

There is no conflict of interest.

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