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RESEARCH ARTICLE

ENDOSULFAN TOLERATE / DEGRADING BACTERIA ISOLATED FROM COTTON FIELD SOIL

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Abstract

In this study endosulfan tolerate/degrading bacteria isolated from endosulfan-polluted *Gossypium arboretum* L rhizosphere soil, through repetitive enrichment culture method. The endosulfan was detection by TLC, Spectrophotometric and GC-MS. The TLC and GC-MS chromatography revealed that α - endosulfan, β - endosulfan and endosulfan diol. Therefore, a higher level of endosulfan residues was presented in *Gossypium arboreum* L rhizosphere soil of Tamilnadu, India. Four different bacteria were isolated from enrichment soil sample. This method proved successful in yielding the culture, ISB9 with considerably high potential to tolerate/degrade endosulfan (200 mg L^{-1}). The bacterial isolate of *Chromobacterium violaceum* (ISB9) culture was able to degrade endosulfan to endosulfan diol which is nontoxic as compared to the parent compound.

Introduction

Less than normal rainfall and humid weather have contributed to the increase in mite and pests attacks in crops. Insecticides have become an important tool of crop protection and are often applied several times during one crop season. Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9ahexahydro-6,9-methano-2,3,4-benzodioxathiepin-3-oxide, CAS No. 115-29-7) is an organochlorine pesticide. Technical endosulfan consists of α and β isomers (7:3) and it is a broad spectrum insecticide on cotton crops, field crops such as paddy, sorghum, oil seeds, pulses, as well as vegetables and fruitcrops (Goebel *et al.,* 1982). It has been used in agriculture around the world to control insect pests including whitefly, aphids, leafhoppers, colorado potato beetles and cabbage worms**.** In view of the high usage of endosulfan insecticides on cotton crops throughout the cotton-growing countries including India, Endosulfan contamination has been detected in soils, water, air and food products because of its plentiful usage and latent for ecological transport. Residues of this pesticide have been detected in cotton seed, cotton lint, milk, drinking water and other food stuffs (Masood and Hassan, 1995; Ahad *et al.,* 2000).

Endosulfan causes spermatozoa degeneration (Nath and Kumar, 2007) as well as declined testosterone level and ovarian nuclear degeneration (Sahay *et al.,* 2007). Biochemical changes in endosulfan treated testes of rats were observed by Sinha *et al*. (1995). Endosulfan is extremely toxic to fish and aquatic invertebrates. It has been implicated increasingly in mammalian gonadal toxicity, genotoxicity, neurotoxicity (Siddique *et al.,* 2003) and persists for a relatively long period with half-life of 60–800 days (Rao and Murty, 1980).

Soil is a dynamic living system. It is consists of micro, macro flora and fauna including microorganisms. Microorganisms represent one of the largest reservoirs for essential nutrients. They are considered a living pool of organic matter, which is vital for maintenance of soil health. These organisms have a primary catabolic role in the degradation of plant and animal residues in the environment, which contributes to the cycling of nutrients. Microbes, being in intimate contact with the soil microenvironment, make ideal monitors of soil pollution (Brooks, 1995). A wide variety of soil microorganisms have been reported cable of degrading endosulfan such as *Klebsiella pneumoniae* KE-1 (Kwon *et al.,* 2002); *Rhodococcus* strain (Verma *et al.,* 2006); *Staphylococcus* sp., *Bacillus circulans* (Mathava Kumar and Ligy Philip, 2006), *Stenotrophomonas maltophilia*, *Rhodococcus erythropolis* (Koel Kumar *et al.,* 2007); *Ochrobacterum* sp., *Arthrobacter* sp., *Burkholderia* sp., *Pseudomonas alcaligenes, Pseudomonas* sp

(Kumar *et al.,* 2008), *Achromobacter xylosoxidans* (Wen Li *et al.,* 2009); *Aspergillus niger* (Tejomyee *et al.,* 2007; 2012), *Aspergillus sydoni* (Supriya Goswami *et al.,* 2009). In this study the isolation of bacteria capable of tolerate/degrading endosulfan from endosulfan-polluted *Gossypium arboreum* L rhizosphere soil, through repetitive enrichment culture method.

MATERIALS AND METHODS

Soil sample collection

Soil was obtained from *Gossypium arboreum* L rhizosphere soil at Alangudi (Latitude: 11°6'26.13"N; Longitude: 79°32'46.36"E) near Thanjavur, Tamilnadu, India.

Physico-chemical parameters and microbial status of soil

Soil was air-dried, ground and passed through a 2mm pore sieve and was stored in sealed containers at room temperature. Soil was characterized for physico-chemical (APHA, 1995) and microbial status was analyzed (Cappuccino and Sherman, 1999).

Extraction and detection of endosulfan from collected soil

One gram of dried soil was transferred to a test tube and extracted with 3 ml of ethyl acetate by vortexing. The ethyl acetate layer was decanted after 5 min. this extraction was repeated two more times. The ethyl acetate fractions were pooled, passed through anhydrous sodium sulfate and evaporated at room temperature. The efficiency of extraction was 85±2% (Awasthi *et al.,* 2000). The TLC was developed in hexane:chloroform:acetone (9:3:1), on silica gel 60 F plates, and separated spots were analyzed by $AgNO_3$ chromogenic reagent (Kovacs, 1965). Spectrophotometric determination of endosulfan using thionin and methylene blue as chromogrnic reagents (Chand Pasha and Badiadka Narayana, 2008). The endosulfan was monitored by GC-MS (Shimadzu, 2200). The compounds were identified on the basis of mass spectra and retention time using the NIST library.

Enrichment and identification of microorganisms

One gram of soil was added to 100 mL of basal medium (Koel Kumar *et al.,* 2007) with 10 mg endosulfan and kept on an orbital shaker for 3 days at 150 rpm and ambient conditions (Sutherland *et al.,* 2000). 10 mL of the soil suspension in basal medium was transferred into a fresh flask containing enrichment medium with 20 mg L^{-1} endosulfan. The cultures developed in the flask were successively transferred to fresh enrichment medium containing increasing concentrations of endosulfan upto 100 mg L^{-1}. The isolated bacteria from the enrichment medium were then further purified on nutrient agar (peptic digest of animal tissue - 5 g; NaCl - 5 g; beef extract - 1.50 g; yeast extract - 1.50 g; agar - 15 g; endosulfan - 0.20 g/L), and incubated at 28±1ºC for 48 h. Isolated colonies were picked and streaked on nutrient agar to obtain the pure culture. The effective bacterial isolates were subjected to cultural, morphological and biochemical (Hi25^{TM} identification kit, Himedia Laboratories Pvt. Ltd., Mumbai, India).

RESULTS AND DISCUSSION

The physico chemical property of collected soil sample is presented in table – 1. The data indicates presence of organic carbon, nitrogen, phosphorus, sulphate, calcium, chloride, sodium, potassium and magnesium in soil. The soil is clay loam in nature and bulk density of soil sample 1.20 g/cc. The chloride of the soil is 198.52±11.57 (mg/g), significant increase of free chloride from the soil amended with endosulfan clearly indicates the degradation of the endosulfan. Verma *et al.* (2006) also demonstrated the same results. The microbial characterization of soil is presented in Table - 2. The data indicates the presence of bacteria, fungi and actinomycetes in soil. The qualitative detection of technical endosulfan was measured by TLC and GC-MS. The TLC chromatography revealed that α, β endosulfan and endosulfan diol (Fig 1). The R_f for α-endosulfan, β-endosulfan, and endosulfan diol were 0.67, 0.4, and 0.2, respectively. Similarly Tejomyee *et al.,* (2007) TLC analysis the formation of various intermediates of endosulfan metabolism including endosulfan diol, endosulfan sulfate, and a unidentified metabolite. The present GC-MS chromatography revealed that retention times for α - endosulfan, β-endosulfan, were 27.4 and 28.7 min, respectively (Fig 2). The addition of isolated bacterial cells to contaminated soils causes an enhanced degradation of endosulfan isomers. Various factors, including the additional presence of carbon sources, pH, moisture content, concentration of endosulfan, and size of inoculum, influenced the degradation of endosulfan isomers (Niranjan *et al.,* 2000). The presence of high levels of α-endosulfan and β-

endosulfan residues in the locations of soil samples used in the present study could be explained by the ability of the two isomers of endosulfan persisting for a long time and then accumulating in the environment especially in the soil. These residual quantities were in agreement with those reported by Jayakumar (2000) and Carey and Douglas (1971) who have reported higher levels of endosulfan residues in the soils tested indicating that endosulfan isomers do not easily dissolve in water, but highly dissolve in fat and they have a capacity to be adsorbed to soil particles. Adsorption of endosulfan to soil particles, in addition to its low mobility in the soil and low solubility in water would explain the presence of higher levels of endosulfan residues in the upper layers of soils analyzed than in the lower layers. Moreover, the soil type plays an important role in the adsorption of organochlorine insecticides to the soil particles (Smith *et al.,* 1991). Therefore, a higher level of endosulfan residues was presented in *Gossypium arboreum* L rhizosphere soil of Tamilnadu, India.

Table – 1 Physico-Chemical characteristics of *Gossypium arboreum* L rhizosphere soil

Parameter	Soil (M±SD) n=3
pH	7.51±0.20
Moisture (%)	8.77±0.09
Temperature (ºC)	27
Cation Exchange Capacity	0.27±0.008
% Organic Carbon	1.03±0.01
Phosphorus (mg/g)	10.00±0.29
Kjeldahl Nitrogen (mg/g)	74.22±5.73
Sulphate (mg/g)	2.73±0.20
Calcium (mg/g)	115.96±3.29
Chloride (mg/g)	198.52±11.57
Potassium (mg/g)	100.5±2.06
Sodium (mg/g)	22.21±0.38
Magnesium (mg/g)	43.36±0.84

Values are expressed (Mean± Standard Deviation)

Table – 2 Microbial characteristics of *Gossypium arboreum* L rhizosphere soil

Parameters	Number of Colonies/g
Total Bacterial count	$64\pm16.87 \times 10^6$
Total Fungal count	$7.33\pm1.24\times10^3$
Total Actinomycetes count	$124\pm1.68\times10^4$

Values are expressed (Mean± Standard Deviation)

Table - 3 Morphological and biochemical characteristics of *Chromobacterium violaceum* strain

S. No.	Characteristics	Isolated bacterial strains
		ISB9
1	Grams staining	-
2	Cell shape	Rod
3	Cell size (µm)	0.2-1.0

4	Cell diameter (µm)	0.4-6.0
5	Motility	-
6	Indole test	-
8	Methyl red test	-
9	VogesProskaur test	-
10	Citrate utilization test	+
11	TSI	A/G
12	Urease test	+
13	Starch	-
14	Lipase	-
15	Hydrogen sulphide production test	-
16	Catalase test	+
17	Oxidase test	-
18	Nitrate reduction test	+
19	Casein	-

+ - Positive; - - Negative; A/G – Acid/Gas; ± - Variable

Fig. 1 Screening of endosulfan from *Gossypium arboreum* L rhizosphere soil by TLC. aES – α Endosulfan; bES – β Endosulfan; ED – Endosulfan diol; T1 – Standard; T2 – Sample.

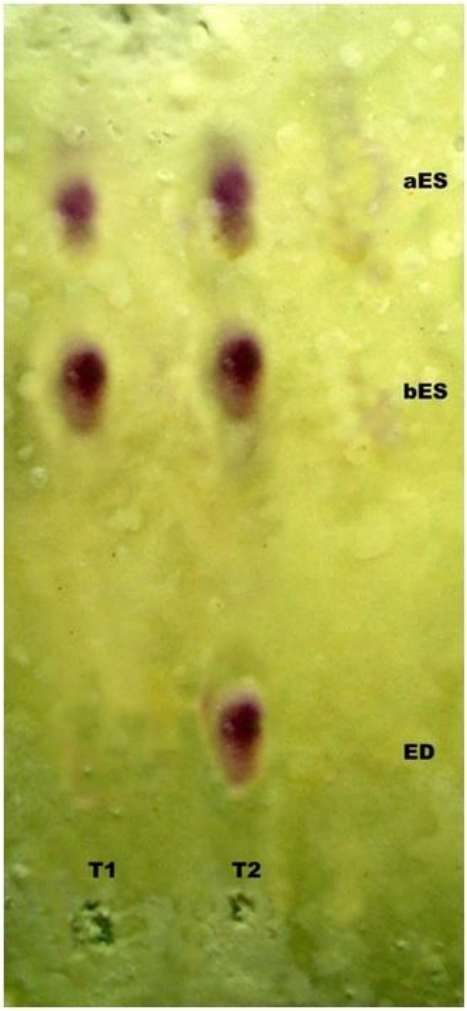
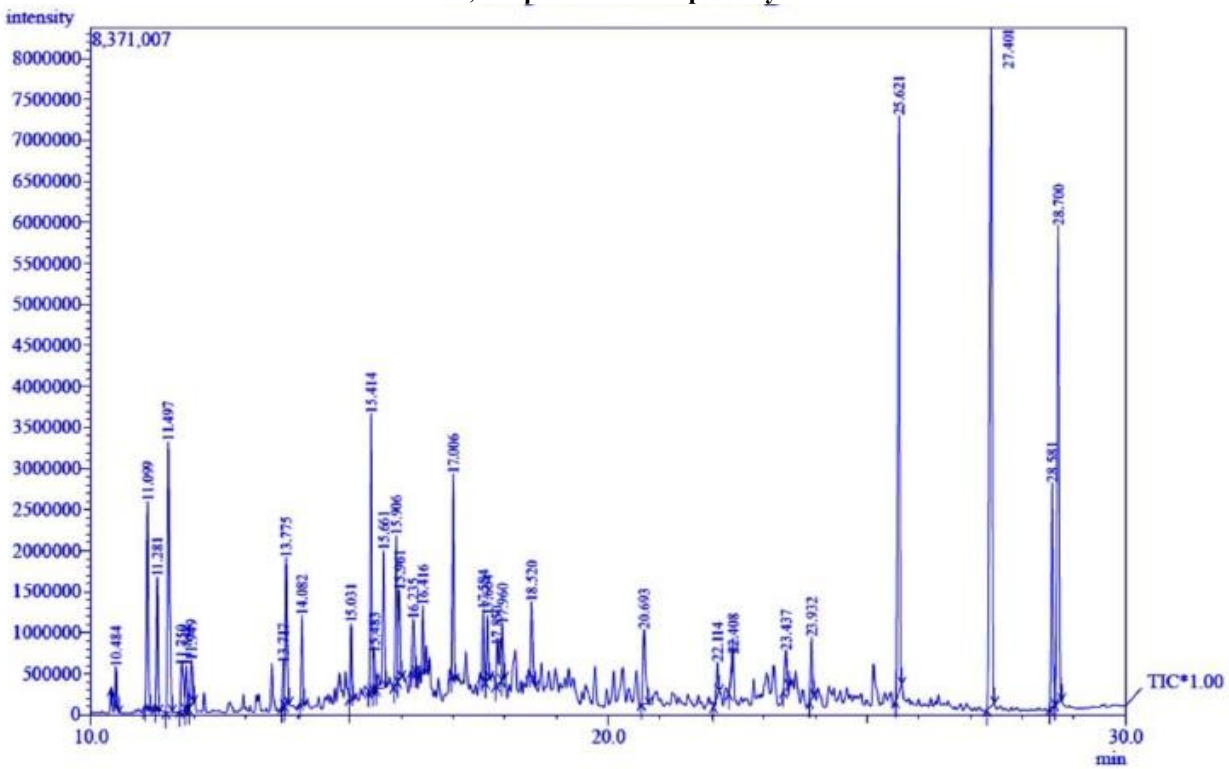


Fig. 2 Detection of endosulfan from cotton field (*Gossypium arboreum* L) soil by GC-MS. Retention time of 27.4 and 28.7 for α, and β endosulfan respectively.



Four different bacteria were isolated from enrichment soil sample. The isolates were transfer to fresh enrichment medium containing increasing concentration of endosulfan up to 200 mg L^{-1}. The maximum level of endosulfan tolerating/degrading culture is ISB9 compare than other isolates. This method proved successful in yielding the culture, ISB9 with considerably high potential to tolerate and degrade endosulfan (200 mg L^{-1}). Strain ISB9 was gram negative, citrate utilization, catalase positive and oxidase negative observed in identification kit (Table 3) (Hi25^{TM}, Himedia). It produces the characteristic purple pigment violacein (Antonisamy *et al.*, 2009). The bacterial isolate of *Chromobacterium violaceum* culture was able to degrade endosulfan to endosulfan diol which is nontoxic as compared to the parent compound. Further studies are required for the evaluation of exact mechanism of endosulfan biodegradation by *Chromobacterium violaceum.*

CONCLUSION

A bacterium strain ISB9 was isolated from enrichment sample of *Gossypium arboretum* L rhizosphere soil. The strain survives at high concentration (200 µg mL^{-1}) of endosulfan compare than other enrichment isolates. Hence, it can potentially be utilized for bioremediation of endosulfan.

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