January 2009 *Volume 4 Issue 2*

Trade Science Inc.

Environmental Science

An Indian Journal

Current Research Paper

ESAIJ, 4(2), 2009 [69-71]

Biodegradation-end of the road for endosulfan

Rakhee Nair, Priya R.Iyer

Women's Christian College, College Road, Chennai-600006, (INDIA)

E-mail : brajuraj@yahoo.com

Received: 9th May, 2008 ; Accepted: 14th May, 2008

ABSTRACT

An Endosulfan-degrading mixed culture (Bacteria & Fungi) was enriched from soil with a history of endosulfan exposure, using NSM (non sulfur media) as media; where endosulfan is the sole source of sulfur. Degradation of insecticide occurred with growth of bacteria and fungi. Bacteria and Fungi were identified and the samples were analyzed for degradation using Thin Layer Chromatography (TLC) and Gas Chromatography (GC). The optimum conditions (pH, temperature, substrate concentration and lighting condition) for degradation were also found. The plasmid was isolated from bacteria by Alkaline Lysis Method and was transformed to *Escherichia Coli* DH 5α and colonies were inoculated in NSM to check degradation ability.

KEYWORDS

Endosulphan;
Nonsulphur media;
TLC;
GC.

INTRODUCTION

Endosulphan is the commonly used pesticide for the fruiting plants like cashew. The pesticide has been found in the fruits and the soil in the region where it is used abundantly. The pesticide has also affected the pregnant mothers and children in the region. Degradation of the pesticide is very slow and the biomagnification is rapid. Degradation of the pesticide was taken as a challenge to reduce the number of people being affected by the pesticide[1].

MATERIALS AND METHODS

Soil sample

The soil sample was collected from a cashew plantation where endosulfan has been sprayed about three years ago from Kasaragod District, Kerala. The soil sample was collected from different areas. It was stored in dark for one month prior to enrichment[11].

Isolation of endosulfan degrading organism from soil sample

Two types of Media were used - NSM (Non-Sulphur Media) and FTW.

Chemicals

NSM (g\L)	FTW (g\L)
Di potassium hydrogen phosphate - 0.225g	Di potassium hydrogen phosphate - 0.225g
Potassium dihydrogen Phosphate - 0.225g	Potassium dihydrogen Phosphate - 0.225g
Ammonium Sulphate 0.225g	Ammonium Chloride 0.22
$MgSo_4$ 7H20 - 0.05g	Magnesium Chloride - 0.845g
Ferrous Chloride - 0.005g	Ferrous Chloride- 0.005g

Current Research Paper

NSM (g\L)	FTW (g\L)
Calcium Carbonate - 0.005g	Calcium Carbonate - 0.005g
-	Glucose - 1.0g
Trace element solution (mg/L)	Trace element solution (mg/L)
$MnSo_4$ - 169 mg	$MnCl_2$ - 198 mg
$ZnSo_4$ - 288 mg	Zn Cl_2 - 236 mg
Copper Sulphate - 250 mg	$CuCl_2$ - 24 mg
Cobalt Sulphate - 28 mg	$Nicl_2$ - 24 mg
Na_2 Mo O_4 $2H_20$ - 24 mg	-

pH = 7.2

Both media (NSM and FTW) were prepared and pH was adjusted to 7.2. The media were autoclaved and poured into sterile boiling tubes. 1.5ml of technical grade endosulfan was added to all tubes. One tube was kept as control. Two gram of soil was added to tubes and incubated in dark for 40 days, at room temperature[4].

Following tests were done to study the morphological characteristics of isolated Organism[10].

1. **Simple Staining** - This method is useful to observe the microorganism's morphology and cell arrangement.
2. **Grams Staining** - This method is used to differentiate gram positive and gram negative bacteria.
3. Isolated Organisms were sub cultured to get a pure culture. (Fungi and Bacteria).
4. **Catalase test** - To identify the bacteria that produces enzyme catalase, which splits hydrogen peroxide into oxygen and water. .
5. **Oxidase test** - To identify the organism producing oxidase,that oxidizes the reagent to a coloured product.
6. **Motility test** - It is used to demonstrate bacterial motility.
7. **Citrate Utilization test** - To identify the bacteria which utilize citrate as sole source of carbon.
8. **Indole test** - To determine the ability of an organism to produce indole.
9. **Nitrate Reduction (NR) test** - To determine the ability of an organism to reduce nitrates to nitrites.
10. **Methyl Red**-Voger Proskauer (MR-VP) test - To determine the change pH of medium and production of acetone.
11. **Gelatin liquefaction test** - To identify the organism which utilizes gelatin, resulting in hydrolysis and liquefaction of gelatins.
12. **Starch Hydrolysis test** - To determine whether the organism produced extracellular amylase.
13. **Lipid Hydrolysis test** - To determine whether the organism produces the extra cellular enzyme, lipases.
14. To identify unknown fungi; Lactophenol Cotton blue test was done.

Standardization tests were carried out to find out the optimum conditions of degradation- pH, temperature and substrate concentration[9].

Analytical methods[7]

Both TLC and GC analysis were done for quantitative analysis of amount of endosulfan monaldehyde. For TLC, plates coated with Alumina were used. The solvent used was Petroleum ether: acetone (4:1) or chloroform: ethyl acetate (3:1). Plates were observed by spraying silver nitrate. For GC, the column was made up of silica. Compared to TLC, GC was more sensitive.

Transformation

Plasmid DNA was isolated using Alkaline Lysis Method. Competent Cells were also prepared and Transformation was done. Test to determine the degradation of Endosulfan by transformed bacteria, non-transformed bacteria and isolated bacteria were also done.

RESULTS

A mixture of Endosulfan degrading microorganisms were isolated (Bacteria and fungi). Following the biochemical tests; the bacteria were identified as *Renibacterium salmoninarum* and K*urthia* species. Fungi were identified as *Aspergillus niger* and *Fusarium* species[2,3]. After standardization, it was found that the optimum conditions for degradation of endosulfan were: Optimum pH-7.2; Optimum temperature-25ºC; Optimum substrate concentration-1.5 ml.

Using both TLC and GC method, it was found out that endosulfan (Total Endosulfan, α endosulfan ?and β endosulfan) were degraded to Endosulfan Monaldehyde. Plasmid DNA was isolated and was confirmed by Agarose gel electrophoresis (AGE).Blue coloured colonies were observed after transformation.The Transformed *E.coli* was found to degrade Endosulfan. Therefore, degradation is a plas-

Test	Culture 1	Culture 2
Catalase test	+	+
Oxidase Test	+	+
Motility Test	Non-motile	Motile
Citrate Utilization test	+	+
Indole Test	-	-
NR Test	+	-
Detection of H2S production using TSI medium NoH2S and gas production NoH2S and gas production	Acid butt, Alkaline Slant NoH2S and gas production	Acid butt, Alkaline Slant NoH2S and gas production
MR - VP Test	-	-
Gelatin liquefaction test	-	-
Lipid Hydrolysis Test	-	-
Starch Hydrolysis Test	+	-

mid borne character and transformed *E.coli* showed acquisition of this new character.

DISCUSSION

The organisms which were isolated from soil with endosulfan, were able to degrade endosulphan[6]. The oxidation reaction favoured the alpha-isomer. Hydrolysis involved a novel intermediate, tentatively identified as endosulfan monaldehyde, on basis of gas-chromatography. The accumulation and decline of metabolites suggest that the parent compound was hydrolyzed to the putative monaldehyde, thereby releasing the sulfite moiety required for growth[5]. It was also found that the degradation is a plasmid borne character and is transformed to *E.coli* DH5α.

REFERENCES

[1] N.Aswathy, N.Manickam, A.Kumar; Appl. Microbiol.Biotechnol., **62(2-3)**, 279-83 **(2003)**.

[2] Cappuccino, Sherman; Microbiology-A Laboratory manual, **221-226**, 133-195, **(2001)**.

[3] T.Sundararaj; Microbiology-Laboratory manual, 48-64 **(2000)**.

[4] T.C.Dubey; 'A Textbook of Biotechnology', 364-366 **(2002)**.

[5] G.A.Elzorgani, M.E.H.Omer; Bull.Environ. Contam.Toxicol., **12(2)**, 182-5 **(1974)**.

[6] J.Heritage, E.G.V.Evans, R.A.Killington; Introductory Microbiology, 200-240 **(1996)**.

[7] H.Maier-Bode; Properties, Effect, Residue and Analysis of Insecticides, Endosulfan Residue Rev., **22**, 1-44 **(1968)**.

[8] Sambruke, Russel; Molecular Cloning, **1**, 1-150 **(1989)**.

[9] Tara.D.Sutherland, Irene Horne, Michael J.Laury, Rebecca L.Harcourt, Robyn.J.Russell, John.G.Luke Sholl; Applied Environmental Microbiology, **72(5)**, 3524-30 **(2006)**.

[10] T.D.Sutherland, I.Horne, R.L.Harcourt, R.J.Russell, J.G.Oakeshott; J.Appl.Microbiol., **93(3)**, 380-9 **(2002)**.

[11] Tariq Siddique, C.Benedict, Muhammad Arshad, William.J.Frankenberger; Journal of Environmental Quality J.Environ.Qual., **32(1)**, 47-54 **(2003)**.