DEGRADATION OF PERSISTENT ENDOSULFAN BY ISOLATED ACTINOMYCETES AND *Rhodococcuss jostii*, RHA1

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SUMMARY: The property of organochlorine endosulfan which is persistent and toxic can cause environmental pollution and pose human health and has raised concern worldwide. Hence, biodegradation serves as promising tool in overcoming the problem of pesticides pollution. The effective approach of removal of endosulfan from the environment is by using Gram-positive microorganisms such as actinomycetes, predominant microorganisms found in soil habitat are less exploited and studied in biodegradation of endosulfan. The diverse metabolic diversity and unique enzymatic capabilities of actinomycetes has gained interest in biodegradation to remove the endosulfan from the environment. This study aims to isolate indigenous actinomycetes from soil habitat and screening the ability of isolated actinomycetes to degrade the endosulfan. Cameron Highland where intensive agricultural activities were carried out was chosen as the sampling site for isolation of actinomycetes that could have potential in degradation of endosulfan. Screening of the ability of endosulfan degradation by isolated actinomycetes in sulphur free broth culture and followed by determination by residual endosulfan by using GC-ECD analysis which is sensitive and specific assay in detecting the endosulfan.
Keywords-Actinomycetes, Degradation, Endosulfan, Persistent

INTRODUCTION

Endosulfan(6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a-hexahydro- 6,9-methano-2,4,3-benzodioxathiepine-3-oxide) is a broad spectrum chlorinated cyclodiene insecticide that has been widely used on agricultural crops. The nature of endosulfan is persistence in nature and it is categorized as US EPA priority pollutant [1]. The endosulfan residues have been detected in the atmosphere, soils, sediments, surface, rain waters and foods due to the extensive use in agriculture [2, 3]. The hydrophobic nature of endosulfan makes it easier to be get absorbed into the soil and sediments easily [4]. Although many countries imposed a ban on endosulfan usage and consumption, however it is still one of the priority chemicals used extensively in pest control in developing countries [5]. In Malaysia, researchers found the high concentrations of endosulfan in Cameron Highland where extensive agricultural activities have been carried out. The existing technologies used in detoxification of pesticides include incineration and landfills. The drawback of technologies that makes it not widely implemented are costly and associated with environmental pollution problems. Detoxification, degradation and mineralization by biological means receiving serious attention as an alternative method to existing technologies because it is cost effective and environmental friendly [6]. However, little information are available on biodegradation, transformation of endosulfan using Gram-positive bacteria especially actinomycetes. Although there are research carried out to studied the tolerance of *Streptomyces* on organochlorine pesticides including endosulfan, however the metabolic pathway and molecular basics in biodegradation and biotransformation of endosulfan are less studied [7]. Based on previous research, the toxicity of endosulfan and the residues of endosulfan presence in the Cameron Highland, bioremediation serve as the tool to minimize the concentration of endosulfan residues in the environment. Hence, intensive research to study on isolate the potential actinomycetes on the ability to degrade the endosulfan is needed to address the endosulfan problem.

2. MATERIALS AND METHODS

2.1 Microbial Strain

Rhodococcus jostii, RHA1 was previously isolated from hexachlorocyclohexane-contaminated soil.
Rhodococcus *jostii*, RHA1 have remarkably known to degrade a very broad range of polychlorinated biphenyls (PCBs) [8].

2.2 Collection and Pretreatmnt of Soil Samples

Soil samples was collected from the agricultural field that has the history of endosulfan contamination by using soil sampling tools. Physical method of pre-treatment of soil samples is by air-drying the soil samples at room temperature overnight. Then, the air-dried soil samples are preheated to 55°C for one hour in the hot air oven and then stored at room temperature in plastic bags.

2.3 Direct Isolation of Actinomycetes by using McBeth Scales Starch Mineral Agar

5g of pre-treated soil samples were suspended in 50mL of sterile distilled water and homogenized for 20mins in a shaker. Serial dilution of the homogenized soil samples suspension was prepared by 10 fold dilution until dilution factor 10^{-3}. 0.1ml of inocula from dilution $10^{-3,}$ 10^{-2} and 10^{-1} was plated on Mc-Beth Starch Mineral Agar supplemented with cycloheximide (20µg/mL) and nalidixic acid (30µg/mL) spread by using a Hockey stick spreader. The inoculated plates were incubated at room temperatures for 5 to 7 days.

2.4 Qualitative Screening of Endosulfan Degrading Actinomycetes Isolates

Preliminary qualitative screening was carried out in non-sulphur agar medium supplemented with 100µg/ml of

endosulfan. 100µg/mL of endosulfan dissolved in acetone was added into the autoclaved non-sulphur agar medium by membrane sterilization technique. Pure actinomycetes isolates was streaked onto the non-sulphur agar supplemented with endosulfan dissolved in acetone (100µg/mL). The growth of the actinomycetes was observed after incubated for 7 days at 28 °C.

2.5 Quantitative Screening of Ability of Isolated Actinomycetes and *Rhodococcus jostii,* RHA1 to degrade endosulfan in broth culture

The endosulfan used in non-sulphur broth was used as the sole sulphur source. Endosulfan dissolved in acetone (100µg/mL) was aseptically added into the sterile *Erlenmeyer* flask and the left inside the laminar flow to evaporate off acetone. Uninoculated flask served as negative control in the experiment.

2.6 Extraction of residual endosulfan and endosulfan intermediate metabolites

After 10 days of incubation, the whole broth culture was harvested and centrifuged at 10000 rpm to separate the pellets and supernatants. The cell free culture broth was acidified with 1N hydrochloric acid and extracted with n-hexane: acetone (80:20) by liquid-liquid partition. Finally, the extracted samples was dehydrated by passing through anhydrous sodium sulphate and stored at -4°C for further analysis.

2.7 Analytical Technique

The extracted samples were analysed using Gas Chromatography using Electron Capture Detector. Agilent gas chromatography 7820A equipped with Ni^{63} ECD electron capture detector was used to analyse the residual endosulfan.

3. RESULTS AND DISCUSSIONS

3.1 Isolation of Actinomycetes

A total of 25 actinomycetes were isolated from the agricultural soil samples in Cameron Highland.

3.2 Qualitative Screening of Endosulfan Degrading Actinomycetes Isolates Result

In this screening test, endosulfan was utilized as the sole source of sulphur. The screening test result showed that all actinomycetes isolates were able to grow and tolerate with the endosulfan (100µg/mL) supplemented on the sulphur free screening media. The screening test result is not valid since the all actinomycetes isolates also showed growth on non-sulphur medium without addition of endosulfan.

3.3 Quantitative Screening Result of Ability of Isolated Actinomycetes and *Rhodococcus jostii,* RHA1 to degrade endosulfan in Non-Sulphur Broth Culture

The GC-ECD chromatogram of technical grade endosulfan which depicted two prominent peaks which represent by retention time 9.785 and 11.717 which represents alpha and beta isomer of technical grade of endosulfan. The screening test result showed that all actinomycetes and RHA1 able to tolerate the endosulfan presence and showed growth in non-sulphur broth. However, from the analysis of GC-ECD showed that the alpha and beta endosulfan presence in relative abundance of 34.14% and 39.30% respectively. Therefore, preliminary deduction that can be made is all the isolated actinomycetes does not showed good ability in degradation of endosulfan however they can grow and tolerate to the endosulfan presence in non-sulphur broth. Some actinomycetes isolates such as A14, A15, A23 and A24 which depicted the new peak based on GC-ECD chromatogram which showed retention time 6.285, 9.283 and 13.235, might showed the isolate resistance ability towards the endosulfan presence in non-sulphur broth through detoxification mechanism. *Rhodococcus jostii*, RHA1, well known PCB degrader showed ability in degradation in endosulfan as depicted in GC-ECD screening result since it showed reduction in the relative abundance of one isomer of endosulfan. Moreover, the large genome size of *Rhodococcuss jostii*, RHA1 can harbor the endosulfan degrading gene and the ability of production of surfactant can overcome the hydrophobicity of endosulfan.

Hence, ongoing confirmation work of degradation of endosulfan by using GC-MS analysis and authentic standard of individual alpha and beta endosulfan and degradation metabolites will be published in the next paper.

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