Osong Public Health Res Perspect 2015 6(1), 39–46
http://dx.doi.org/10.1016/j.phrp.2014.12.003
pISSN 2210-9099 eISSN 2233-6052

ORIGINAL ARTICLE

Isolation and Identification of Endosulfan-Degrading Bacteria and Evaluation of Their Bioremediation in Kor River, Iran

Farshid Kafilzadeh*, Moslem Ebrahimnezhad, Yaghoob Tahery

Department of Biology, Jahrom Branch, Islamic Azad University, Jahrom, Iran.

Received: October 13, 2014
Revised: November 15, 2014
Accepted: December 4, 2014

KEYWORDS:
acinetobacter,
endosulfan,
gas chromatography/mass spectrometry,
Klebsiella,
Kor River

Abstract
Objectives: Endosulfan is a lipophilic insecticide, which causes severe health issues due to its environmental stability, toxicity, and biological reservation in organisms. It is found in the atmosphere, soil, sediments, surface waters, rain, and food in almost equal proportions. The aim of this study was to isolate and identify endosulfan-degrading bacteria from the Kor River and evaluate the possibility of applying bioremediation in reducing environmental pollution in the desired region.
Methods: Samples of surface sediments and water were collected from three different stations in two seasons (summer and autumn), as these are areas with high agricultural activity. Isolated bacteria were identified by various biochemical tests and morphological characteristics. The amounts of degradation of endosulfan isomers and metabolites produced as a result of biodegradation were then analyzed using gas chromatography/mass spectrometry.
Results: In this study, the following five bacterial genera were able to degrade endosulfan: *Klebsiella, Acinetobacter, Alcaligenes, Flavobacterium,* and *Bacillus*. During biodegradation, metabolites of endosulfan diol, endosulfan lactone, and endosulfan ether were also produced, but these had lesser toxicity compared with the original compound (i.e., endosulfan).
Conclusion: The five genera isolated can be used as a biocatalyst for bioremediation of endosulfan.

1. Introduction

During the past 50 years, pesticides have been the essential part of the agricultural world. Although the demand for production and distribution of pesticides to increase the quality and efficiency of the agricultural industry is evident, use of improper and unreasonable pesticides is likely [1]. Despite their benefits, pesticides are compounds that may have toxic side effects, causing potential environmental risk [2]. Endosulfan is an

*Corresponding author.
E-mail: Kafilzadeh@jia.ac.ir

organochlorine pesticide, which belongs to the family of polycyclic chlorinated hydrocarbons. For more than 30 years, it has been used extensively in agriculture, horticulture, and forestry [3]. Endosulfan contains two stereoisomers [alfa- and beta-endosulfan (ratio, 3:7)] and has been registered under the trade names Thiodan, Cyclodan, Thimol, Thiofar, and Malix [4]. Endosulfan contamination and its persistence in soil and water environments cause it to accumulate in cells of phytoplankton products, zooplankton, fishes, and vegetables [5]. Endosulfan persists in soil and water for 3−6 months or longer [6]. Endosulfan attaches to gamma-aminobutyric acid receptors located on the membrane of neurons and reduces flow of chloride. Endosulfan poisoning causes seizures. All of these aforementioned problems encouraged the scientific community to develop biological methods to remove endosulfan instead of incineration and landfill methods [7].

Biodegradation is an efficient bioremediation technique in microorganisms that grow in different ecosystems and through symbiosis with xenobiotics, these microorganisms are able to survive even in incompatible conditions. Various studies have used endosulfan as a source of sulfur for microbial growth and as a carbon resource in bioremediation. Endosulfan is decomposed into endosulfan sulfate by oxidation pathway and into endosulfan diol by hydrolysis. Endosulfan sulfate is also toxic and stable as the major component (endosulfan). Endosulfan diol can be converted to endosulfan ether, endosulfan hydroxyl ether, endosulfan dialdehyde, and endosulfan lactone. However, these metabolites are less toxic [8]. There are many reports on the degradation of endosulfan by bacteria [9]. *Klebsiella pneumoniae* has the capability to biologically degrade (biodegradation) endosulfan. *Pandoraea* sp. degrades around 95−100% of alfa- and beta-endosulfan without producing endosulfan sulfate when incubated for 18 days. *Klebsiella oxytoca* was reported to degrade 145−260 mg of endosulfan in 6 days [6]. Some Gram-positive bacteria such as *Bacillus* are able to convert endosulfan to endosulfan sulfate [10]. Natural adsorbents such as sediments can control water quality indirectly by absorbing or releasing pesticides. Surface waters are good environments for degrading pesticides, particularly when microorganisms are capable of binding to the surface contour of the water, sediment, rocks, and plants. Many types of compounds decompose slowly in aerobic regions [11].

Kor River is one of the largest surface water resources in the Fars Province, with thousands of farmers depending on its water for agricultural use. In addition, the river provides a high percentage of drinking water to the regions of Shiraz, Marvdasht, and other villages along its way. The river also provides water to industries and factories located nearby. Urban, industrial, and agricultural activities are the main causes of pollution of this river. This water is mainly polluted by the Fars meat industrial complex, petrochemical industries, sugar mills, refineries, glazed tile factories, industrial towns, and wastewater of Marvdasht [12]. Urban and industrial wastewaters have adverse effects on fish breeding, environmental health, and particularly on drinking water of downstream residents. Thus, protecting the river is particularly important. The aim of this study was to isolate and identify endosulfan-degrading bacteria from the Kor River and also to evaluate the use of bioremediation techniques to improve the environmental status of this river.

2. Materials and methods

2.1. Sampling range

The study site is located in Fars Province in the southwestern region of Iran. Because of intense agricultural activities in the surrounding Kor River, three sampling stations were chosen.

2.2. Sampling method

Water and sediment samples were collected from areas with high agricultural activities at three different stations (3 times in each station) in two seasons [summer and autumn (fall)]. The samples were packaged in sterile plastic containers and flasks filled with ice and were transported to the laboratory within 4 hours [13].

2.3. Counting the bacterial colonies

Laboratory chemical manufactured by Merck Company (Darmstadt, Germany) were used in this study. After transporting the soil samples to the laboratory, bacteria were counted using the total viable plate count method. During the procedure, sediment and water samples were diluted with normal saline (from 10^{-1} to 10^{-9}). Then 0.1 mL of each dilution was taken and surface cultured on two medium of nutrient agar: one containing the toxin and the other without toxin (control). The cultures were incubated for 48 hours at 37°C. Once the colonies appear, plates with identifiable and countable colonies were selected and the number of colonies was counted [14].

A mixture of different culture media, such as the mineral broth (1 g KH_2PO_4, 1 g K_2HPO_4, 1 g of NH_4NO_3, 0.2 g $MgSO_4$, 0.02 g $CaCl_2$, and 0.01 g $FeSO_4$), deionized water, nutrient agar, and biochemical medium were used in the experiments [6].

2.4. Enrichment and isolation of endosulfan-degrading bacteria

To enrich endosulfan-degrading bacteria, 5 g of sediment of each station was added to an Erlenmeyer flask containing 50 mL of mineral medium and 50 μg/mL of endosulfan pesticide. The mixture was then incubated in a shaking incubator at 30°C at 150 rpm. After 10 days, 5 mL of the mixture from each flask was added to a fresh medium containing 50 mL of mineral medium and 50 μg/mL of endosulfan pesticide, and then incubated in a shaker incubator as described earlier.