CHANGE OF SOIL BACTERIAL COMMUNITY BY INCUBATING WITH PERSISTANCE INSECTICIDE ALDRIN UNDER ANAEROBIC SEDIMENT CULTURE

Jui-Hung Yen, Tzu-Chuan Chiu and Yei-Shung Wang

Department of Agricultural Chemistry, National Taiwan University, Taipei, Taiwan

Introduction

Aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahyro-1,4-endo-exo-5,8-dimethanonaphthalene), a cyclodiene organochlorine insecticide, was banned by nations and classified as B2 carcinogen by United States Environmental Protection Agency. Because of its chemical stability and lipophilicity, aldrin is regarded as a persistent and recalcitrant compound under aerobic conditions. Biodegradation is a very important mechanism for removal of organic chlorinated hydrocarbons form environment under anaerobic conditions. Microbial reductive dechlorination was thought to be the predominant process of excluding the chlorine atom from chlorinated hydrocarbons

The degradation potential of aldrin by anaerobic microorganisms obtained from the sediment of indigenous river was evaluated[1]. The degradation rate of aldrin by treating with microbial inhibitors, the methane production during the degradation process of aldrin[2] and the effect of environmental factors such as temperatures and nutrients on the aldrin degradation were also investigated in our previous study[3]. This present research is designed to investigate the soil bacterial community structure change under different environment conditions of incubating microorganisms, collected from river sediment, with aldrin . The cluster analysis is also study.

Methods and Materials

Chemicals: Chemical standard aldrin with 97% purity was purchased from Riedel-deHaën Co., Ltd. Germany. *n*-Hexane and acetone, HPLC-grade, were purchased from E. Merck Co, Germany. The stock solutions (1 mg mL^{-1}) were prepared in acetone and then stored at 4℃.

Preparation of anaerobic sediment culture: The river sediment was collected from Er-Jen River located at southern Taiwan. The river had been seriously contaminated with organochlorine chemicals such as PCB and pesticides. A grab sampler was used to collect the sediment in a depth of 0 to 10 cm. After sampling, the sediment was stored in a jar, kept at 4 ℃ and taken to lab for preparation of anaerobic mixed culture. Anaerobic sediment culture was prepared by mixing sediment (100 g) and culture medium (400 mL) in a 1-L serum bottle under a modular atmosphere controller system (Don Whitley Scientific Co, England) filled with N_2, H_2, and CO_2 gases in ratio of 85:10:5 to purge oxygen. The culture medium was slightly modified from Chang et al. (2001), which consisted of (in g/L): NH_4Cl (2.7), $MgCl_2 \cdot 6H_2O$ (0.1), $CaCl_2 \cdot 2H_2O$ (0.1), $FeCl_2 \cdot 4H_2O$ (0.02), K_2HPO_4 (0.27), KH_2PO_4 (0.35), yeast extract (1.0), and resazurin (0.001). The medium was neutralized to approximate pH 7.0, and added titanium citrate to final concentration of 0.9 mM as a reducing reagent.

Batch procedures: Investigation of bacterial community structure was performed by incubating 5 mL anaerobic sediment culture with 45 mL culture medium in a 125 mL serum bottle. After spiking aldrin in 0, 0.5, 2, 5, 10 and 100 μg/mL, separately, to batch culture in serum bottles, the serum bottles were sealed with butyl rubber stopper and capped with aluminum foil, then stored under 30℃ in darkness. At regular intervals (1, 14, 28, 42 and 70 days), 3 mL of samples were removed and centrifuged (6000 rpm). The total DNA was extracted from the pellet, and the genomic DNA of bacteria was extracted using DNA extraction kit in accordance with manufacture's instruction. Extracted DNA was proceeding by electrophoresis in a 1 % agarose gel to remove humic acids. The DNA band was excised from the gel and recovered with a QIAquick gel extraction kit. Purified bacterial 16S rDNA was amplified with primers 968F and 1401R, a GC-rich sequence was attached to the 5' end of primer 968F. The PCR condition was using 35 cycles of 92℃ 1 min, 64℃ 1 min, 72℃ 1 min, followed by a final extension at 72℃ for 10 min.

Denaturing gradient gel electrophoresis (DGGE) analysis: DGGE analysis was performed by using

a D-Code universal mutation detection system (Bio-Rad,, Hercules, Calif.). Samples of 20μL of PCR products were loaded onto 7% (w/v) polyacrylamide gel that containing 40% to 60% denaturing gradient of formamide and urea. The electrophoresis was run at 60℃in 1X TAE for 12 hours at a constant voltage of 75V. After the electrophoresis, polyacrylamide gel was stained with SYBR Green I nucleic acid gel stain and visualized on a UV transilluminator and then photographed with a CCD camera.

Results and Discussion

Incubation of soil bacteria with aldrin for different periods, the result of DGGE pattern of PCR-amplified bacterial 16S rDNA and clustering analysis were shown in Figures 1 and 2. Figure 1 showed the structure of soil bacterial community, which is indicated by DNA bands in DGGE profile, each band represents a dominant bacterial species. Increasing the band numbers and brightly strength as the time elapse was found in the figure. Incubation with aldrin (2 μg/mL), bands 1 to 10 are appeared through the incubation period, but bands 11 and 12 are appeared after incubation for more than 28 days. From the clustering analysis (Figure 2), after incubation in aldrin for 28 to 70 days, similarity of microbial community structure is up to 84%.

Incubation of soil bacteria with different concentrations of aldrin for 42 days, the result of DGGE pattern of PCR-amplified bacterial 16S rDNA and clustering analysis were shown in Figures 3 and 4. Figure 3 showed the lane 2 (no aldrin added) is differet from lanes 3 to 7 (incubated with aldrin) obviourly. Bands 1,2, 3, 8, 10 and 11 in Figure 3 were disappeared as the adrin added which implying the microorganisms was inhibited by addrin, while bands 16, 17, 18, 19 and 20, implying become dominating microbes, are appeared after incubation with aldrin, especial bands 16, 17 and 18. Incubation with 0.5 to 1.0 μg/mL of aldrin, the bands 16, 17 and 18 appeared, but with 100 μg/mL of aldrin, the three bands were disappeared. Higher concentrations (100 μg/mL) of aldrin showed fungicide action. From clustering analysis, in various concentrations of aldrin showed similarity of microbial community structure only 40% between aldrin concentration of 0.5 to 1.0 and 100 μg/mL.

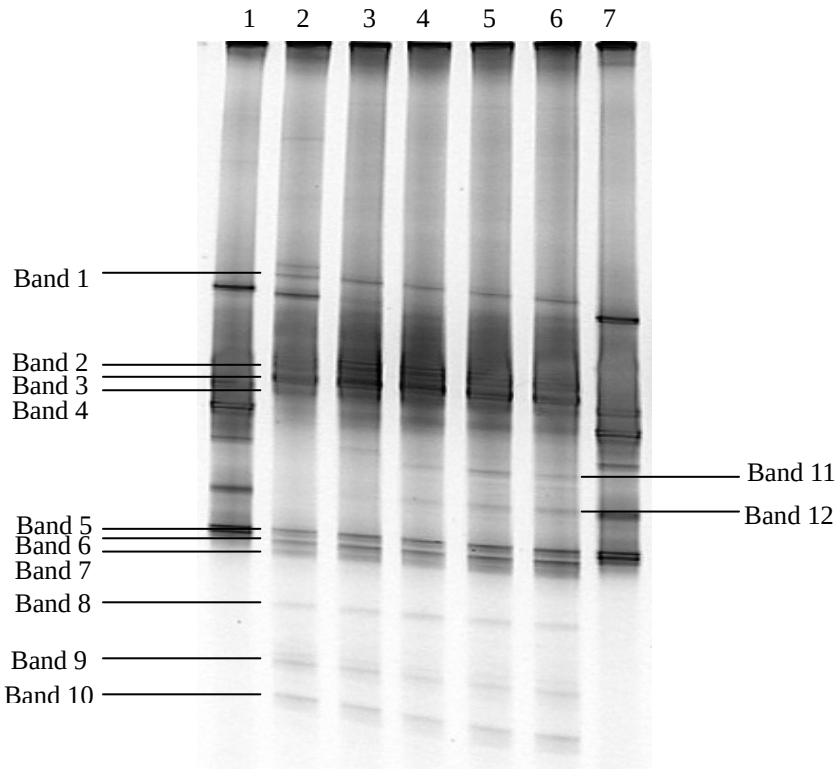

Figure 1. PCR-DGGE analysis of 16S rDNA sequence fragments after incubating with 2 μg/mL of aldrin in the sediment culture for various incubation periods. Lanes 1 and 7 were maker; lanes 2 to 6 were incubated for 1, 14, 28, 42 and 70 days, respectively.

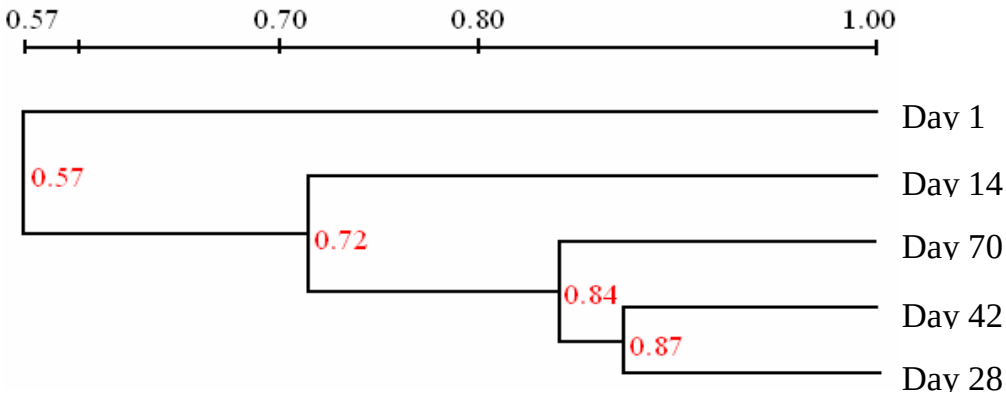

Figure 2. Cluster analysis of microbial community structures by unweighted pairwise grouping method with mathematical averages (UPGMA) after incubating with 2 µg/mL of aldrin in the sediment culture for various incubation periods

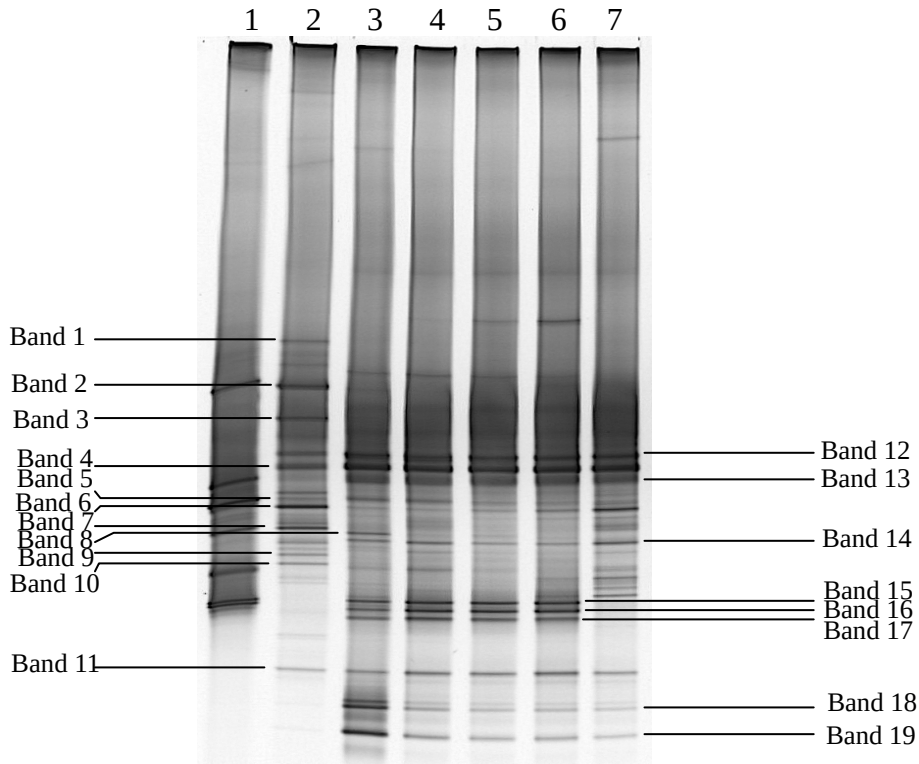

Figure 3. PCR-DGGE analysis of 16S rDNA sequence fragments after incubating with various concentrationos of aldrin in the sediment culture for 42 days. Lane 1 was maker; lanes 2 to 7 were incubated in aldrin concentrations of 0, 0.5, 2, 5, 10 and 100 µg/mL, respectively.

Incubation of microbes in 2 µg/mL of aldrin, and amended with other substrates (such as yeast extracts, sodium acetate or D-glucose) or electric acceptor (such as sodium bicarbonate, sodium sulfate or sodium nitrate), for 42 days and then proceeding PCR-DGGE were also carried out. Anaerobic mixed microbes showed no obvious different on degradation of aldrin among using sodium bicarbonate, sodium sulfate or sodium nitrate as the growth substrate. From the results, amended with different substrate, aldrin presence changed the microbial community structure, but not effect the presence of aldrin-degrading microbes. Amended with different electric acceptor, the dominator microbes and microbial community structure was different.

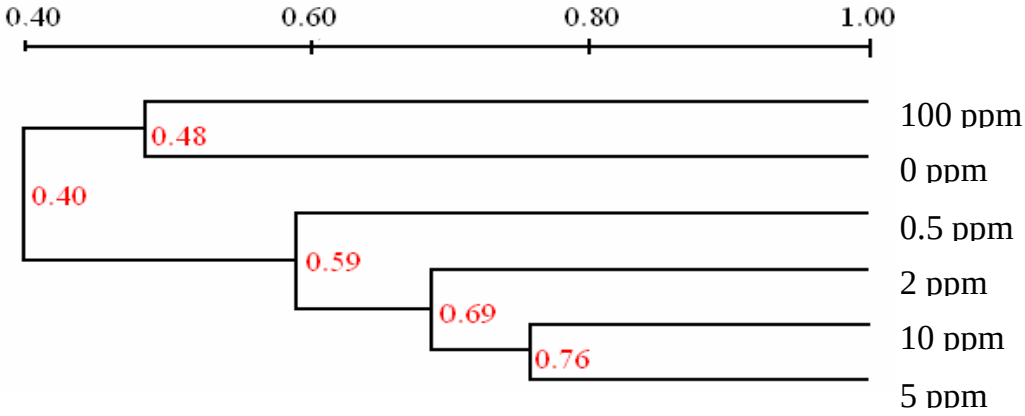

Figure 4. Cluster analysis of microbial community structures by unweighted pairwise grouping method with mathematical averages (UPGMA) after incubating with various concentrationos of aldrin in the sediment culture for 42 days.

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References
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