INFLUENCE OF ORGANOCHLORINE PESTICIDE DDT ON CHANGE BACTERIAL COMMUNITY IN RIVER SEDIMENT UNDER ANAEROBIC CONDITIONS

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

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

Introduction

DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane) is one of the persistent organochlorine pesticides which was with widespread use over several decades. Due to its highly lipophilicity and bioaccumulative, DDT was absorbed readily on soil particles and sediment. Many reports showed that DDT was detected in river sediment. Barra et al. reported that a relatively new occurrence of unknown source *p,p'*-DDT was found in a watershed in Chile[1]. Doong et al. reported that *p,p'*-DDT was still residue in river sediment of Taiwan in trace amounts[2]. Osuna-Flores and Riva reported that some organochlorine pesticide residues were present in shrimps and sediment from Ohuira Bay in concentrations above legally permitted levels[3]. Besides, DDT can be distributed into the atmosphere and contaminate areas which never applied DDT through air currents. Therefore, to investigate the impact of DDT residues in the environment is of serious concern by countries. In this study, we attempt to evaluate the impact of DDT on community of anaerobic microorganisms in river sediment. DDT might change the bacterial structure and lead to the adverse effect on aquatic ecosystem under the lower oxygen environment. The impact of DDT on diversity of microorganisms is useful for environmental risk assessment. However, traditional culture-based methods are difficult to explain the interaction between DDT and community of anaerobic microorganisms. A culture-independent method named DGGE (denaturing gradient gel electrophoresis), is which based on analyzing the 16S rDNA variable region of bacteria, and has been used to investigate bacterial communities[4]. Herein, PCR-DGGE was used to realize the change of bacterial community structure following spiked of DDT.

Materials and Methods

Chemicals

DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] with 98% purity was purchased from Riedel-deHaën Co, Germany. HPLC-graded solvents used in this experiment including *n*-hexane and acetone were purchased from E. Merck Co, Germany. The stock solutions (1 mg/mL) were prepared in acetone and then stored in cold.

Culturure

Sediment was collected from Er-Jen River, a serious contaminated river located at southern Taiwan. Sample was gathered from sediment surface to 10 cm depth by using a grab sampler. The collected sample was soaked with river water and sealed in a jar to avoid in contacting with oxygen, and then stored at 4 for preparing of anaerobic mixed culture. Anaerobic mixed culture was prepared in a 1-L serum bottle by adding sediment (100g) to culture medium (400mL) to a slurry-like culture in a modular atmosphere controller system (dwscientific Co, England) filling

with N_2, H_2, and CO_2 gases (85:10:5). The culture medium was slightly modified from Chang et al[5], which is consisted of (in g/L): NH_4Cl (2.7), $MgCl_2$ $6H_2O$ (0.1), $CaCl_2$ $2H_2O$ (0.1), $FeCl_2$ $4H_2O$ (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 about 7.0, and added titanium citrate to final concentration of 0.9 mM as a reducing reagent.

Batch procedures

Experiments for Bacterial community structure were performed by incubating 5 mL anaerobic mixed culture with 45 mL culture medium in a 125 mL serum bottle. After spiking DDT in range of 0 ~ 100 ìg / mL, separately, to batch culture, serum bottle was sealed with butyl rubber stoppers and capped with aluminum foil, then stored in bottle at 30^o in darkness. At regular intervals, 3 mL of sample was removed from serum bottle and centrifuged in 6000 rpm, after removing the supernant, the total DNA was extracted from the pellet. The total genomic DNA of bacteria was extracted using DNA extraction kit in accordance with manufacture's instruction. After the total DNA was extracted, humic acids was removed by electrophoresis of the DNA extracts in a 1 % agarose gel. 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 ^{o} 1 min, 64 ^{o} 1 min, 72 ^{o} 1 min, followed by a final extension at 72 ^{o} for 10 min.

Denaturing gradient gel electrophoresis analysis

DGGE analysis was performed by using a D-Code universal mutation detection system (Bio-Rad,, Hercules, Calif.). Samples of 20ìL 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 ^{o} 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

Extraction of genomic DNA and PCR amplification

After total genomic DNA was extracted, the extracted DNA was purified by excised the band on agarose gel for removal of the potential PCR inhibitor in sediment. The optimum PCR condition was sought for avoiding the potential interference band in DGGE. Figure 1 shows the result of PCR under different programmatic conditions. From this result, side band was appeared when annealing temperature lower than 60 ^{o}. The optimum annealing temperature for completely removal of side band was observed at 64 ^{o}.

DGGE analysis

The result of DGGE pattern of PCR-amplified bacterial 16S rDNA and clustering analysis were shown in Figure 2. Figure 2A indicated DNA bands in DGGE profile, each band represents a dominant bacterial species. After spiking DDT (2 ìg / mL), for one day, there is only nine distinct bands was observed. But after day 5, thirteen bands were appeared. Among which, band 4 disappeared at day 5, and never re-appeared during 70 days of incubation periods. Band 5 appeared at day 5 and remained thereafter. Band 10, 11, 12 appeared at day 5 and disappeared at day 70. The prominence of thirteen distinct bands may be represent some DDT-degrading microorganisms were proliferated. To avoid personal error in viewing bands on DGGE

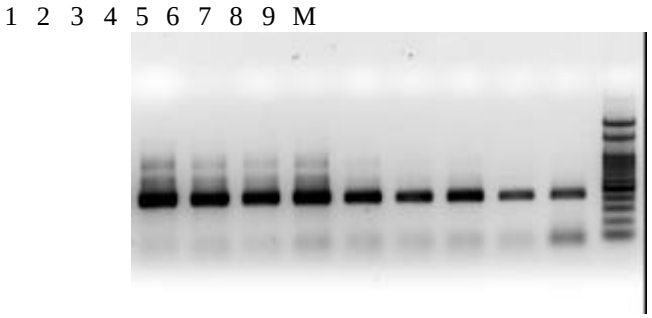
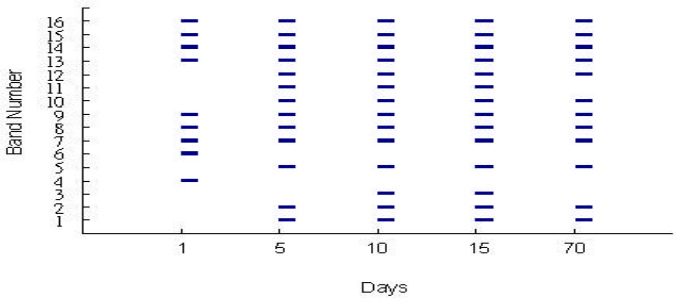


Figure 1. PCR amplification products obtain from sediment by changing annealing temperature. Lane 1 ~ 9 indicated different annealing temperature of 57, 58, 59, 60, 61, 62, 63, 64 and 65 °, respectively.

-(A)



(B)

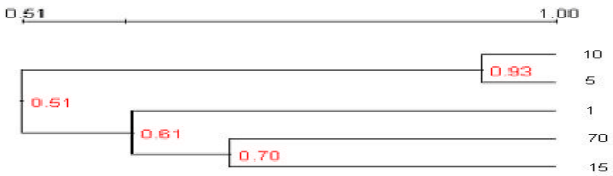


Figure 2. DGGE pattern of 16S rDNA fragments in anaerobic mixed culture incubated with *p,p'*-DDT for different days (A) and the genetic similarity of microbial-community in DGGE profile

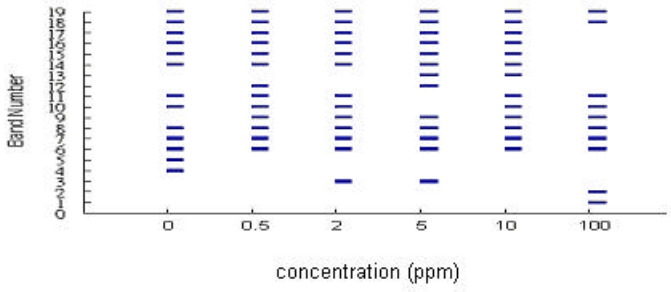


Figure 3. DGGE pattern of microbial community incubated with different concentrations of *p,p'*-DDT.

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profile, cluster analysis was used to analyze the genetic similarity of bacterial structures. Figure 2B showed 93 % of similarity in the bacterial communities between day 5 and the day 10. At day 70, the structure of bacterial communities was more similar to the day 1 than day 5 or day 10. The results can be thought of the bacterial communities return to original stage following decrease of DDT. For investigating the influence of different concentrations of DDT on bacterial community, DGGE analysis was done after 15 days of incubation periods. The DGGE pattern was showed in Figure 3. This result reveals that bacterial community in range of 0 ~ 10 ìg / mL DDT concentrations were similar. In contrast, there is a significant difference in bacterial community structure was observed in concentration of 100 ìg / mL. In our previous study, anaerobic microorganisms attacked on DDT for dechlorination in range of 0.5 ~ 10 ìg / mL concentrations, but dechlorination of 100 ìg / mL DDT was significantly inhibited. The results suggested that higher concentration of DDT may cause toxicity on microorganisms. In the research, we attempt to access the feasibility of using PCR-DGGE to describe the effect of DDT on bacterial dynamics under anaerobic condition in sediment. Moreover, the change of microbial diversity is also can be used on environmental risk management.

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