Biotransformation of Aldrin and Chlorpyrifos-methyl by *Anabaena* sp. PCC 7120

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A cyanobacteria species, *Anabaena* sp. PCC 7120, was tested to assess its biotransformation ability on two widely used insecticides, aldrin and chlorpyrifos-methyl, in the culture medium. The blue-green alga metabolized aldrin mainly to dieldrin by an epoxidation reaction with the participation of cytochrome P450-dependent monooxygenase in the cyanobacteria. The blue-green alga also produced chlorpyrifos-methyl oxon as a primary metabolite from chlorpyrifos-methyl via a desulfuration reaction, presumably conducted by cytochrome P450-dependent monooxygenase. Therefore, two insecticides might be possibly dissipated by cytochrome P450-dependent monooxygenases in the blue-green algae in the contaminated environments.

Key Words: *Anabaena* sp. PCC 7120, aldrin, chlroyrifos-methyl, cytochrome P450-dependent monooxygenase

INTRODUCTION

The use of chemical protectants to control pests in the soil environment is widespread. This control may be largely dependent on the concentration of the pesticide to which the pests are exposed. However, some chlorinated pesticides may receive much attention because of their long persistency in the soil environment. Aldrin is strongly toxic to the soil-dwelling insect pests and is used for the protection of woods from the attack by the termites. By the termites, the estimated damage has been reported to be about US$1.7 billion in the United States (Gold et al., 1993). Although the use of aldrin has been severely restricted or banned in many countries, it is still used in termite control in some countries (Meister, 1989). Aldrin fate in soil has been well documented as its removal from the soil can occur by the peroxidation reaction to dieldrin or evaporation

to air (Khan, 1980). To form the peroxides of aldrin, various soil microorganisms can involve this oxidation reaction in the soil environment via a cytochrome P450-dependent monooxygenase system (Khan, 1980). The oxidation product of aldrin, dieldrin, can be subjected further metabolic reaction to aldrin diol or aldrin ketone via biotic or abiotic catalysts (Menzie, 1969) (Fig. 1).

Epoxide hydroxylase may be involved in the production of aldrin diol, but an additional oxidation reaction can produce keto-aldrin. In addition, Baxter (1990) has found the dechlorinaed aldrins under anaerobic conditions.

Chlorpyrifos-methyl, CM(S), is an organophosphate insecticide containing three chlorine atoms of the pyridine ring. Its huge use as a soil fumigant for the control of soil-dwelling insect pests can increase the persistency in the soil. Many studies have conducted to show its dissipation patterns from the soil after application (Racke et al., 1994; Racke et al., 1996; Baskaran et al., 1999). Racke et al. (1994) has showed that the dissipation half-life of CM(S) in soil-incorporated application at the recommended rate

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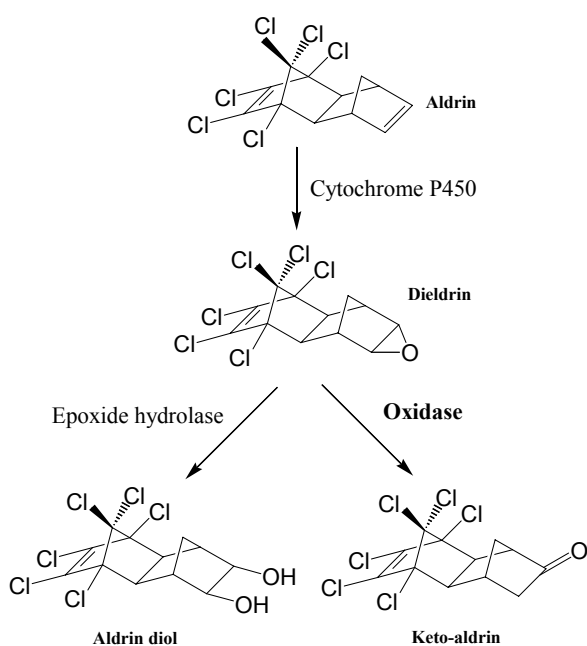E-mail: selpest@hanmail.net

Fig. 1. A presumable biotransformation pathways of aldrin in the soil environment.

ranges 4 to 8 weeks. A principal dissipation mechanism for CM(S) has been introduced as hydrolytic reaction under alkaline conditions. In aquatic environments, CM(S) can be easily degraded to 3,5,6-trichloro-2-pyridinol (TCP) (Baskaran et al., 1999).

Cyanobacteria are free living, photoautotrophic microorganisms and have shown their capabilities to degrade both naturally occurring compounds and synthetic chemicals, especially pesticides (Megharaj et al., 1987; Megharaj et al., 1994; Yan et al. 1998). Therefore, cyanobacteria have been considered as the potent alternative organisms for the chemical and physical treatments to transform the environmentally persistent, toxic materials. Herein, we report aldrin and CM(S) biotransformation by *Anabaena* sp. PCC 7120 in a culture medium and identify their biotransforming products. We also postulate the role of *Anabaena* species in soil or waterways to dissipate aldrin and CM(S).

MATERIALS AND METHODS

Microorganisms

Anabaena sp. PCC 7120 was obtained from America Type Culture Collection (ATCC). *Anabaena* species was grown in Allen's liquid medium without nitrate on shaker at room temperature in the light intensity of

about 1700 Lux by fluorescent lamps with a 12 h: 12 h (light: dark) cycle. Their growth was monitored by measurement of chlorophyll a content (Mackinney, 1941). All operations were carried out under sterile conditions in order to avoid bacterial contamination.

Chemicals

Aldrin, dieldrin, $NaNO_3$, K_2HPO_4, $MgSO_4 \cdot 7H_2O$, $CaCl_2$, ferric citrate, and ethylene diamine tetraacetate (EDTA) were purchased from Sigma Chemical Co. (St. Louis, MO). CM(S) and chlorpyrifos-methyl oxon, CM(O), were purchased from Chem. Service Inc. (West Chester, PA). All chemicals used were of highest grade commercially available.

Determination of aldrin and CM(S) degradation and metabolite production

Experiments were carried out in batch cultures. One hundred ml of Allen's medium in 250 ml flasks covered with cotton plugs were inoculated with *Anabaena* species. Four days later, aldrin or CM(S) was supplemented to the inoculated medium to give a final concentration of 10 mg/L. Uninoculated culture medium was served as control. All the flasks were sealed and incubated on shaker at room temperature in the light intensity of about 1700 Lux by fluorescent lamps with a 12 h: 12 h (light: dark) cycle. Algal growth was monitored by measurement of chlorophyll a content (Mackinney, 1941). Degradation was assessed by measurement of aldrin and its metabolite dieldrin or CM(S) in triplicate flasks. Sampling was done at various periods of incubation and analyzed by gas chromatography with electron capture detector (GC-ECD).

The remaining aldrin or CM(S) was extracted from 5 mL of the crushed bacterial suspension by glass homogenizer with equal volume of nanograde hexane by vortexing for 30 s twice. The organic layer was collected in vial and dried with nitrogen gas. A 2 uL volume of each hexane extract was subjected to GC-ECD analysis using a Hewlett-Packard Model 5890 gas chromatograph, equipped with a 30 m × 0.32 mm i.d. (d_f = 0.25 m) DB-1 bonded-phase fused-silica capillary column (J&W Scientific, Folsom, CA) and a ECD was used for determination of concentrations. The injector and detector temperatures were 170℃ and 250℃, respectively. The oven temperature was

programmed from 35℃ (4 min isothermal) to 230℃ (held for 25 min at final temperature) at 2℃/min. The linear velocity of the helium carrier gas was 36 cm/s (30℃) at a split ratio of 1:20. A standard curve was prepared in the range of 0-5 pmol for aldrin and dieldrin

Determination of chlorpyrifos-methyl oxon from the growth media

Chlorpyrifos-methyl oxon [CM(O)] produced by desulfuration reaction of CM(S) in the cyanobacteria was determined based on its capacity to inhibit acetyl-cholinesterase (AChE) by product of the oxidation of CM(S) insecticide by the method of using a Varian model CARY 2200 spectrophotometer (Hüber, 1986; Lee and Lees, 2001). The reaction was initiated by the addition of 200 mL of the growth media to give a total volume of 0.5 mL. Control reaction mixtures were set up without the growth media and contained 0.17 to 0.36 mL of phosphate buffer.

RESULTS AND DISCUSSION

The algal growth in the medium was checked up to 15 days after inoculation, and then the growth stopped (Fig. 2). The pH increase in the medium occurred until 10 days after inoculation in the flask. The maximum growth was until chlorophyll a content reached to about 50 mg/L and the maximum pH in the medium was around 12.5. Aldrin or CM(S) was spiked after 7 days of inoculation of *Anabaena* sp. PCC 7120. There were no changes in both chlorophyll
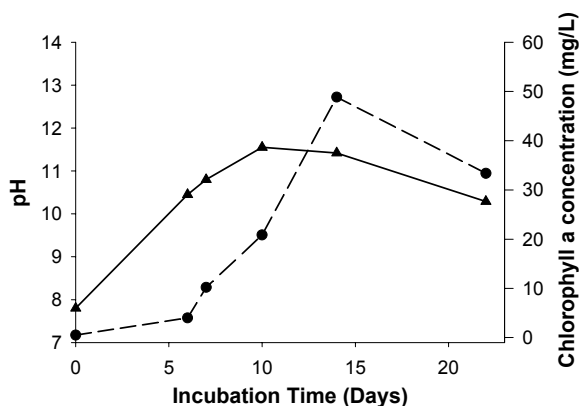
a content and pH in the control medium (data not shown). Aldrin decreased immediately after spiking, then after 70 h incubation aldrin was remained about 30% from the initial incubation time (Fig. 3).

According to the GC analysis after spiking in the medium containing the algae, aldrin spiked in control was not metabolized. The major metabolite for the aldrin metabolism by the cyanobacteria was dieldrin (Fig. 3), and several unidentified metabolites were also formed with trace amounts. On the other hand, CM(S) spiked into *Anabaena* sp. PCC 7120–containing medium disappeared and found less 10% of the insecticide after 70 h incubation (Fig. 4). By specto-photometric analysis of the metabolites from CM(S), chlorpyrifos-methyl oxon, CM(O) was determined (Fig. 4).

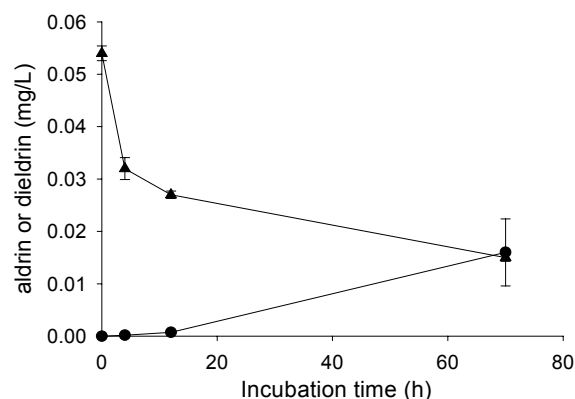Cyanobacteria can degrade both naturally occurring

Fig. 3. Aldrin biotransformation after spiking in the Allen's medium inoculated with *Anabaena* sp. PCC 7120: aldrin (▲); dieldrin (●).

Fig. 2. Growth of *Anabaena* sp. PCC 7120 under an aerobic condition (●) and pH changes in the Allen's medium (▲).
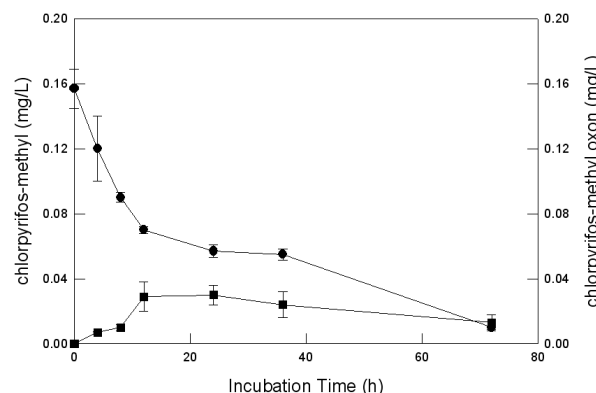
Fig. 4. Chlorpyrifos-methyl (●) degradation and chlorpy-rifos-methyl oxon (■) production after spiking in the Allen's medium inoculated with *Anabaena* sp. PCC 7120.

aromatic hydrocarbons and man-made xenobiotics. For example, *Oscillatoria* sp. strain JCM degrades naphthalene and transformed it to 1-naphthol (Cerniglia et al., 1980). Three blue-green algal species such as *Synechococcus elongates*, *Nostoc linckia* and *Phormidium tenue* have potent abilities to degrade monocrotophos and quinalphos in the soil environment (Megharaj et al., 1987). However, several studies have revealed potential inhibitory effects of pesticides on the growth of cyanobacteria. Dimethoate and endosulfan inhibited algal growth and decreased survivability of *A. doliolum* (Mohapatra and Mohanty, 1992). Atrazine and hexazinone also inhibited the growth of *A. flos-aquae* and *Selenastrum capricornutum* (Abou-Waly et al., 1991). Therefore, some impact of aldrin and chlorpyrifos on the growth of *Anabaena* species may reduce the role of *Anabaena* sp. PCC 7120 for the removal or dissipation of two chlorine-containing insecticides in soil or waterways.

Our results have indicated two interesting metabolic pathways of aldrin and CM(S). *Anabaena* sp. PCC 7120 produced dieldrin from aldrin as a primary product. Dieldrin has much longer persistency in soil than aldrin. Thus, the biotransformation of aldrin by the blue-green algae may increase the environmental impact. In addition, cytochrome P450-dependent monooxygenase activity of the algae plays a pivotal role in dieldrin production from aldrin. This mechanism has been introduced. For the transformation of CM(S), pH increase in the medium may be an important factor because of the occurrence of chemical hydrolysis. However, the authors did not find the hydrolytic metabolite, TCP. Even though chemical hydrolysis may influence on the reduction of CM(S), cytochrome P450-dependent monooxygenase activity also can degrade the insecticide. But, a possible metabolite of CM(S) via desulfuration reaction cannot be extracted because it can completely bind to esterase enzymes in the algae. To prevent chemical hydrolysis of insecticide, the use of strongly buffered culture medium (pH 6.6) was suggested and the medium containing a detergent Tween 80 for increasing the amount of endosulfan in contact with mixed bacteria was used (Sutherlund et al., 2000). No growth of bacteria was detected in control cultures in the absence of endosulfan as carbon source. In the presence of endosulfan, growth of mixed bacteria occurred concomitant with endo-sulfan decrease.

There are several more considerable factors for degrading pesticides by soil microorganisms. As many studies suggested, cyanobacteria including *Anabaena* species are present in soil. In agricultural soils, high water potential is determined as high concentration of salt ions present. This strong water potential may influence pesticide fate in the soil environment. Han and New (1994) suggested the maximal removal of 2,4-dichlorophenoxyacetic acid (2,4-D) by soil organisms occurred at the highest water potential (ψ) of −0.1 MPa and the degradation rate decreased progressively when the water potential down to ψ = −5.5 MPa with no breakdown at ψ = −22 MPa. Awasthi et al. (2000) also suggested moisture content was one of the influenced factors in endosulfan degradation.

Anabaena sp. PCC 7120 possesses dechlorination activity of lindane (Kuritz and Wolk, 1995; Kuritz et al., 1997), leading to the formation of 2,3,4,5,6-pentachloro-1-cyclohexene, 1,2,3- and 1,2,4-trichlorobenzene. *Anabaena* sp. can transform 2,4,6-trinitrotoluene (TNT) to azoxy-tetranitrotoluene and hydroxyaminodinitrotoluene (Pavlostathis and Jackson, 1999). In this study, we report that *Anabaena* sp. PCC 7120 degrade aldrin to dieldrin and chlorpyrifos. Further studies will be conducted using the strongly buffered medium to compare the difference between the pH-controlled or non-controlled medium in the degradation of CM(S). Additionally, all of the metabolites produced from aldrin and CM(S) by the cyanobacteria were not determined in this study, although dieldrin and CM(O) production were determined by spectrometric analysis. Therefore, additional studies will be conducted to assess minor metabolites of aldrin and CM(S) when they are degraded by the microbes.

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