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Preliminary Study of an Enzyme Extracted from *Alcaligenes* sp. Strain YF11 Capable of Degrading Pesticides

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Synthetic pyrethroids are an important group of insecticides used for the control of agricultural pests as well as arthropods of medical and veterinary importance. Despite some environmental deficiencies and toxic effects on a number of non-target organisms, such as fish and bees, there is no evidence to suggest that the importance and use of pyrethroid insecticides is likely to decline in the foreseeable future. Organophosphorothioate insecticides such as parathion, parathion-methyl, and fenitrothion are also widely used in the control of agricultural pests. Due to environmental concerns associated with the accumulation of these insecticides in food products and water supplies, there is a great need to develop safe, convenient, and economically feasible methods for their detoxification. As a promising strategy, the use of microbial enzymes in pesticide detoxification has been gained much attention (Nannipieri and Bollag 1991; Richins et al. 1997; Karam and Nicell 1997; Chen and Mulchandani 1998; Chen et al. 1999). Numerous investigations have been made on microbial and enzymatic degradation of organophosphorothioate insecticides. A number of bacterial species capable of degrading organophosphorothioate insecticides have been isolated, and parathion hydrolases have been extracted and purified (Munnecke 1976; Lal 1982; Ou and Sharma 1989; Mulbry and Karns 1989; Nannipieri and Bollag 1991; Chen et al. 1998; Chen and Brűhlmann 1999). Although many studies on the metabolism of synthetic pyrethroids and their transformation products have been made (Mulbry and Kearney 1991; Maloney et al. 1988; 1992), few report exists on microbial mineralization of synthetic pyrethroids. As a consequence, with the exception of cell-free preparation from *Bacillus* sp. strain DC-1 and *Bacillus cereus* Sm3, which catalyzed the hydrolytic cleavage of a permethrin analog (Maloney et al. 1988; 1993; Yu et al. 1996), the enzymatic activities of microorganisms in the hydrolysis of synthetic pyrethroids have not been reported.

In previous study, we isolated and identified a bacterial strain YF11 of *Alcaligenes* sp. and demonstrated that this strain can effectively degrade a number of synthetic

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pyrethroids and organophosphorothioate insecticides such as fenvalerate, fenpropathrin, cypermethrin, deltamethrin, permethrin, cyhalothrin, parathion, parathion-methyl, fenitrothion (Yu et al. 1997). Here, we report preparation of the crude enzyme from *Alcaligenes* sp. strain YF11 and the enzymatic degradation of synthetic pyrethroids and organophosphorothioates.

MATERIALS AND METHODS

An enzyme extract, which contained degradative activities for synthetic pyrethroids and organophosphorothioates, was obtained from a culture of *Alcaligenes* sp. strain YF11. The bacteria were grown in a rich medium (peptone, 10 g; beef extract, 5 g; NaCl, 5 g; fenvalerate, 0.1 g; H_2O, 1000 mL; pH 7.2) in 250-mL Erlenmeyer flasks at 30°C with shaking at 150 rpm. After 5 days, the bacteria were harvested by centrifugation. The precipitate was washed twice with potassium phosphate buffer (0.1 M, pH7.0), and then suspended in the same buffer. The bacterial suspension was sonicated at 4°C for 6 min, and then centrifugated. The supernatant, which contained degradative activities, was stored at 4°C until use. The protein concentration in the preparations was determined with the method described by Bradford (1976).

For the measurement of enzymatic degradation, 0.3 mL of the crude enzyme (0.299 mg soluble protein/mL) was added to 2.7 mL of preincubated 0.05 M potassium phosphate buffer containing synthetic pyrethroid or organophosphorothioate at known concentration. The pesticide residues were extracted 3 min after the addition. Synthetic pyrethroids were extracted thrice with 3 mL of redistilled petro-ether (60-90°C). The organophosphorothioates were extracted thrice with 3 mL of CH_2Cl_2, followed by transfer to acetone. The organic phases were dehydrated with anhydrous Na_2SO_4 and the pesticides were measured by gas chromatography.

ECD gas chromatography was performed with Hewlett Packard Gas Chromatograph (HP 6890) for the determination of synthetic pyrethroids. A HP-5 glass capillary column (25 m × 0.25 mm I. D.) was employed. Operating temperatures were as follows: injection port, 230 °C; column, 200 °C; detector, 230 °C. Nitrogen of 1.2 kg/cm^2 was used as carrier gas at a spilt flow ratio of 1:20. For the measurement of organophosphorothioates, PE gas chromatograph (Sigma 2000) equipped with NPD was employed. A glass column (1 m × 2 mm I. D.) with 5% OV-17 on 80/100 mesh chromosorb W DMCS was used. Operating conditions were as follows: injection port, 230 °C; column, 220 °C; detector, 250 °C; carrier gas (nitrogen), 227.4 kPa; air, 104.7 kPa; hydrogen, 137.8 kPa.

RESULTS AND DISCUSSION

The effect of pH on activities of the crude enzyme for degradation of the insecticides was measured with the insecticides of 20 ppm at 30 °C. The optimal pH for degradation of the insecticides ranges from 7.5 for cyhalothrin to 9.0 for

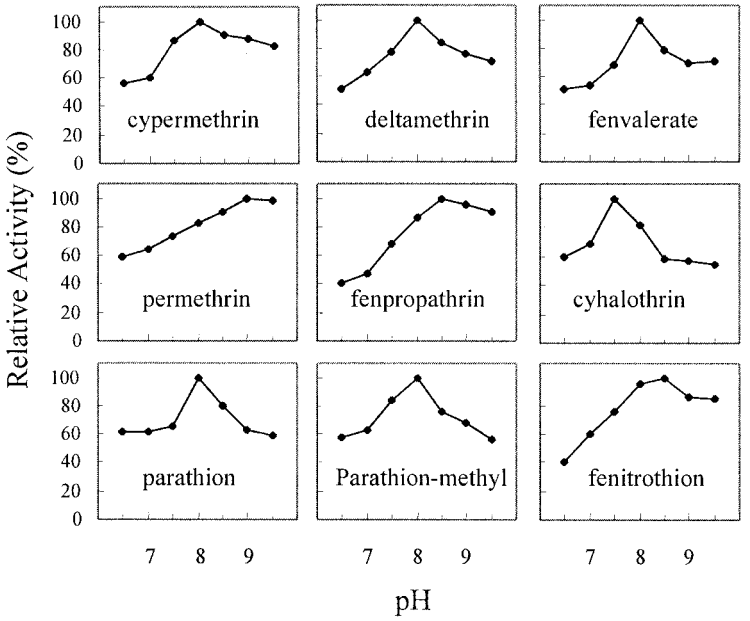

Figure 1. Effect of pH on the enzymatic activities for degradation of the insecticides (20ppm)

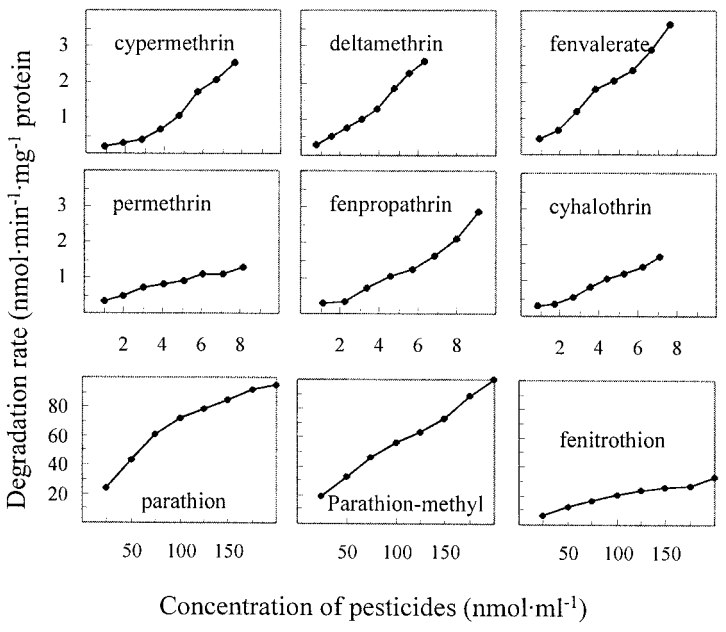

Figure 2. The effect of pesticide concentration on enzymatic degradation

permethrin (Fig.1). The stability of the enzyme under different pH was determined with fenvalerate as substrate at 30 °C. It was observed that the crude enzyme was inactivated at pH 5.0 and 10.5 because of protein denaturation.

The optimal temperature of the crude enzyme for degradation of fenvalerate at concentration of 20 ppm was measured to be 32.5 °C, which is similar to that for the growth of *Alcaligenes* sp. strain YF11 (Yu et al. 1997). The crude enzyme was found to be stable at 25°C, 99% of initial activity was detected after preincubation for 30 hours. However, the stability was decreased with increasement of temperature, only was 16.4% of initial activities retained after preincubation for 30 hours at 50 °C.

The enzymatic degradation rates of the insecticides at different concentrations were measured. Fig. 2 shows the effect of pesticide concentration on enzymatic degradation. Based on Lineweaver-Burk equation, V_{max} was calculated to be 194.6, 173.7, 64.1, 19.0, 29.3, 13.4, 76.5, 1.87, 2.63 nmol/min, and K_m 187.2, 205.5, 212.4, 41.4, 136.8, 64.4, 222.8, 5.2, 8.67 for parathion, parathion-methyl, fenitrothion, fenvalerate, fenpropathrin, cypermethrin, deltamethrin, permethrin, cyhalothrin, respectively. The data indicate that organophosphorothioates are degraded enzymatically more rapid than synthetic pyrethroids.

The degradation pathway of fenvalerate was demonstrated as a mineralization process (Yu et al. 1998). The mineralization of parathion was also observed in our experiments. It could be concluded that the enzymatic degradation of the pesticides might be a detoxification. All of the pesticides used in this study were degraded rapidly by the crude enzyme. The enzyme involved in detoxification of the insecticides has a wide range of substrates.

Our results suggest that the enzyme from *Alcaligenes* sp. strain YF11 may be used under a wide range of environmental conditions for *in situ* detoxification of the pesticides where they cause environmental pollution; for example, in the treatment of production wastewater and agricultural application, and also in the degradation of widely used synthetic pyrethroid moth-proofing agents in the effluent from carpet and wool processing industries. Nevertheless, environmental stability of the enzyme, e. g., immobilization of the enzyme, effects of bio-factors and chem-physical factors on stability and enzymatic kinetics, needs to be investigated further.

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REFERENCES

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248-254

Chen W, Brűhlmann F, Richins RD, Mulchandani A (1999) Engineering of improved microbes and enzymes for bioremediation. Curr Opin Biotech 10:137-141

Chen W, Mulchandani A (1998) The use of live biocatalysts for pesticide detoxification. Trends Biotechnol 16:71-76

Karam J, Nicell JA (1997) Potential applications of enzymes in waste treatment. J Chem Tech Biotechnol 69:141-153

Lal R (1982) Accumulation, metabolism and effect of organophosphorus insecticides on microorganisms. Adv Appl Microbiol 28:149-200

Maloney SE, Maule A, Smith ARW (1988) Microbial transformation of the pyrethroid insecticides: permethrin, deltamethrin, fastac, fenvalerate, and fluvalinate. Appl Environ Microbiol 54:2874-2880

Maloney SE, Maule A, Smith ARW (1992) Transformation of synthetic pyrethroid insecticides by a thermophilic *Bacillus* sp. Arch Microbiol 158:282-286

Maloney SE, Maule A, Smith ARW (1993) Purification and preliminary characterization of permethrinase from a pyrethroid-transforming strain of *Bacillus cereus*. Appl Environ Microbiol 59:2007-2013

Mulbry WW, Karns JS (1989) Purification and characterization of three parathion hydrolases from gram-negative bacterial strains. Appl Environ Microbiol 55:289-293

Mulbry WW, Kearney PC (1991) Degradation of pesticides by microorganisms and the potential for genetic manipulation. Crop Protect 10:334-348

Munnecke DM (1976) Enzymatic hydrolysis of organophosphoate insecticides, a possible pesticide disposal method. Appl Environ Microbiol 32:7-13

Nanniperi P, Bollag JM (1991) Use of enzymes to detoxify pesticide-contaminated soils and waters. J Environ Qual 20:510-517

Ou LT, Sharma A (1989) Degradation of methyl parathion by mixed bacterial culture and a *Bacillus* sp. isolated from different soils. J Agric Food Chem 37:1514-1519

Richins RD, Kaneva I, Mulchandani A, Chen W (1997) Biodegradation of organophosphorus pesticides by surface-expressed organophosphorus hydrolase. Nature Biotechnol 15:984-987

Yu YL, Chen H (1998) Biodegradation of fenvalerate by *Alcaligenes* sp.YF11. Environ Pollut Cont 6:5-8

Yu YL, Fan D, Chen H (1996) Current status and developing of pesticide degradation by microorganisms. Adv Environ Sci 4:28-36

Yu YL, Sheng GY, Fu JM, Chen H, Fan D (1997) Degradation of fenvalerate by microorganism and its enzyme. Chinese J Environ Sci 2:5-8