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Microbial degradation of pesticides: the degrading enzymes and genes

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This study arouses me interest in two respects; the application of the microbial abilities to degrade pesticides to the bioremediation and the evolutional appearance of new enzymes attacking xenobiotics. In this meeting, I will describe the microbial degradation of four pesticides; a rodenticide monofluoroacetate, a herbicide Dalapon, an insecticide Diazinon, and a microbicide hinokitiol. Enzymes degrading these pesticides and their genes will be elucidated.

1. Monofluoroacetate : Fluoroacetate, which has been found in nature as a toxic component in a plant Gifblaar in South Africa, is known for its toxic action by the lethal synthesis of fluorocitrate, a potent inhibitor of the Krebs cycle enzyme aconitase. The compound is extremely toxic to animals, so that it has been used for control of noxious animals such as rats, rabbits, and opossums in Australia and New Zealand. The fluorine-carbon bond in fluoroacetate is chemically much more stable than other halogen-carbon bonds and withstands boiling in concentrated sulfuric acid. We isolated some soil bacteria that utilized fluoroacetate as a sole carbon source. These bacteria have enzymes, known as haloacetate dehalogenase, which catalyze the hydrolytic cleavage of halogen-carbon bonds as follows:

$XCH_2COOH + H_2O \rightarrow HOCH_2COOH + HX$ (X = halogen)

Moraxella sp. B has two haloacetate dehalogenases, H-1 and H-2, which differ in halogen specificity; H-1 acts preferentially on fluoroacetate and H-2 acts on chloro-, bromo-, and iodoacetates but not on fluoroacetate. Both enzymes are encoded on a conjugative plasmid pUO1 (65-kb). The plasmid suffered frequently the spontaneous deletion of an about 5-kb DNA containing the H-2 gene. The nucleotide sequence analysis of the deletion region has revealed that the H-2 gene is held between two 3.2-kb repeated sequences. This sequence, which contains an open reading frame that seems to be a transposase gene and is flanked by 110-bp inverted repeats, is nearly identical to IS1071, which was found in chlorobenzoate catabolic transposon Tn5271. With respect to the structure, the H-2 gene flanked by two IS1071 is considered to be a class I composite transposon.

Fluoroacetate poisoning causes substantial loss of livestock in Australia, Africa and Central America. In Australia, around 40 species of trees and shrubs produce fluoroacetate, which is lethal to sheep and cattle. To protect sheep from fluoroacetate poisoning, a rumen bacterium has been genetically modified using the H-1 gene, to allow ruminal defluorination of fluoroacetate.

2. Dalapon : Over the last several decades, a large amount and a great variety of chlorinated

organic compounds used as agricultural and industrial chemicals have been released into the environment. Since most of them are resistant to microbial attack, they remain as environmental pollutants. The herbicide Dalapon, 2,2-dichloro-propionate, is one of them and the use of it is prohibited now in many countries.

Pseudomonas sp. B7aM that was isolated from soil as a 2-chloropropionate utilizing bacterium had a 2-haloacid dehalogenase, which could dechlorinate both D- and L-2-chloropropionate producing L- and D-lactate, respectively, and 2,2-dichloro-propionate (Dalapon) was also dechlorinated to pyruvate. The fact that the dehalogenase is specified by a 60-kb conjugative plasmid harbouring in this strain has been proved by the curing and conjugal transfer of the plasmid. The enzyme gene has been cloned and sequenced, and it has been found that this gene also is accompanied with IS1071.

3. Diazinon : Diazinon, one of organophosphorus pesticides, is currently used in large quantities in golf links because of the relatively low persistence. However, this compound is toxic to mammalian as well as insect by inhibiting acetylcholin-esterase, so that the leakage of it from golf links into surrounding rivers might cause environmental problems.

Sphingomonas sp. No.6 isolated from golf links soil can degrade diazinon to produce 2-isopropyl-6-methyl-4-pyrimidinol as an intermediate. Thiophosphate esterase solubilized from the cell membrane hydrolyzes diazinon and other organophosphorus triesters such as parathion, tolclofos-methyl and fenitrothion. A large (50-kb) plasmid harbouring in the organism was not concerned with the diazinon degradation. The gene for diazinon hydrolase (*dzd*) was cloned and expressed in *E. coli*. The nucleotide sequence (1095-bp) of the gene showed 88% similarity to the parathion hydrolase gene of *Flavobacterium* sp. A mutant losing the hydrolase activity frequently appeared on the curing experiment. Analysis of the sequences flanking *dzd* revealed the presence of two IS6100 at the both sides of the gene. This suggests that the diazinon hadrolase gene is carried on a transposon.

4. Hinokitiol : Hinokitiol (4-isopropyltropolone) shows antimicrobial and insecticidal activities. A hinokitiol-degrading *Pseudomonas* sp.was isolated, in which we found a novel enzyme that converts hinokitiol that is a seven-membered ring compound to 3-isopropylphenol, a six-membered ring compound.