J. Pesticide Sci. **16**, 97–100 (1991)

Note

Metabolism of Fenitrothion, Parathion and Cyanophos by Isolated Salithion-Degrading Bacteria from Soil

Kazuhito ITOH

Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Takatsukasa, Takarazuka 665, Japan

(Received September 28, 1990)

INTRODUCTION

Three bacterial strains of *Agrobacterium* sp. (B-7, B-15 and B-17) and one of *Acinetobacter* sp. (B-60) isolated from Ushiku loam upland soil metabolized organophosphorus insecticide salithion (2-methoxy-4*H*-1,3,2-benzodioxaphosphorin-2-sulfide; SLT) *via* cleavage of the *P-O*-aryl and *P-O*-aralkyl linkages and/or demethylation,[1] and the metabolic reactions by them were stereoselective,[2] B-7 and B-17 cleaved the *P-O*-aryl and *P-O*-aralkyl linkages of the $(S)_P$-enantiomer of SLT faster than those of the $(R)_P$-enantiomer, whereas B-60 cleaved the $(R)_P$-enantiomer faster. Furthermore, the $(R)_P$-SLT and $(S)_P$-SLT were stereoselectively demethylated by B-17 and B-60, respectively.

This report deals with metabolism of other organophosphorus insecticides by the above four SLT-degrading bacterial strains and the substrate specificity in cleaving the *P-O*-aryl or *P-O*-alkyl linkage of phosphorus esters. Chemicals used were SLT, fenitrothion [*O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate; SMT], parathion (*O,O*-diethyl *O*-4-nitrophenyl phosphorothioate; PRT) and cyanophos (*O,O*-dimethyl *O*-4-cyanophenyl phosphorothioate; CYN), which were uniformly labeled with ^{14}C at the phenyl ring.

MATERIALS AND METHODS

Each of the isolated SLT-degrading bacteria was inoculated into 30 ml of 1/10 diluted nutrient broth (Difco) in a 100-ml Erlenmeyer flask and incubated at 30°C with shaking. The overnight culture was taken into four test tubes with equal volumes (3 ml), and the ^{14}C-preparation of SLT (42.5 mCi/mmol, >99.9%), SMT (60.5 mCi/mmol, >99.9%), PRT (19.0 mCi/mmol, >99.9%), or CYN (30.2 mCi/mmol, >99.9%) in dimethyl sulfoxide (10 μl) was applied into each culture at a concentration of 20 ppm. The applied cultures were continuously incubated under the same conditions. Liquid media without inoculation were also applied and incubated as controls in the same manner. Samples (10 μl) of the culture solutions were directly analyzed by liquid scintillation counting and thin-layer co-chromatography (TLC) after 0, 4, 8, 24 and 48 hr of incubation. Radioassay and TLC were done according to the methods reported previously.[3-5]

^{14}C-PRT was purchased from NEN Research Products, and other ^{14}C-preparations were synthesized in our laboratory. The following unlabeled compounds were used as authentic standards: SLT, 2-hydroxy-4*H*-1,3,2-benzodioxaphosphorin-2-sulfide (SLT-DM), 2-hydroxybenzyl alcohol (Sal-alc), 2-hydroxybenzoic acid (Sal-acid), SMT, *O*-methyl *O*-hydrogen *O*-(3-methyl-4-nitrophenyl) phosphorothioate (SMT-DM), 3-methyl-4-nitrophenol (MNP), *O,O*-dimethyl *O*-(3-methyl-4-aminophenyl) phosphorothioate (SMT-NH_2), *O,O*-dimethyl *O*-(3-methyl-4-acetylaminophenyl) phosphorothioate (SMT-NHAc), PRT, *O*-ethyl *O*-hydrogen *O*-4-nitrophenyl phosphorothioate (PRT-DE), 4-nitrophenol (NP), *O,O*-diethyl *O*-4-aminophenyl phosphorothioate (PRT-NH_2), *O,O*-diethyl *O*-4-acetylaminophenyl phosphorothioate (PRT-NHAc), CYN, *O*-methyl *O*-hydrogen *O*-4-cyanophenyl phosphorothioate (CYN-DM), 4-cyanophenol (CP), *O,O*-dimethyl *O*-4-carbamoylphenyl phosphorothioate (CYN-$CONH_2$) and *O,O*-dimethyl *O*-4-carboxyphenyl phosphorothioate (CYN-COOH). SLT, Sal-alc, Sal-acid, SMT, MNP, PRT, NP, CYN and CP were purchased from Wako Pure Chemical Industries, Ltd. (Osaka), and other reference samples were synthesized according to the previously reported procedures[6-8] with slight modifications.

RESULTS AND DISCUSSION

No other organophosphorus insecticide examined was metabolized as fast as SLT by all of the strains. Metabolite distribution in the cultures after 48 hr of incubation is shown in Tables 1–4.

As previously reported,[1] each of the strains metabolized SLT *via* cleavage of the *P-O*-aryl and *P-O*-aralkyl linkages and/or demethylation.

Table 1 Metabolite distribution in cultures[a] with salithion after 48 hr of incubation.

Compound	% of the applied ^{14}C				
	B-7	B-15	B-17	B-60	Control
SLT	70.6	73.7	57.7	1.3	88.1
SLT-DM	3.0	2.6	4.7	27.3	2.7
Sal-alc	10.2	9.1	12.8	33.6	3.5
Sal-acid	1.3	1.0	4.0	0.5	nd[b]
I	2.3	1.9	3.6	2.3	2.0
II	4.5	3.7	8.6	22.2	0.5
III	2.6	2.1	1.8	nd	nd
IV	0.5	0.4	0.6	1.0	nd
Origin	6.1	4.2	6.9	11.5	0.7
Others	1.4	0.8	1.3	2.1	0.7
Total	102.5	99.5	102.0	101.8	98.3

[a] B-7, B-15 and B-17 were *Agrobacterium* sp., and B-60 *Acinetobacter* sp.
[b] Not detected.

Table 2 Metabolite distribution in cultures[a] with fenitrothion after 48 hr of incubation.

Compound	% of the applied ^{14}C				
	B-7	B-15	B-17	B-60	Control
SMT	95.1	95.6	94.1	91.8	100.5
SMT-DM	3.2	3.2	3.4	3.7	2.7
MNP	0.7	1.0	1.0	2.6	nd[b]
SMT-NH_2	nd	nd	nd	0.4	nd
SMT-NHAc	nd	nd	nd	0.2	nd
Origin	0.1	0.1	0.1	0.2	nd
Others	0.6	0.4	0.5	0.8	0.7
Total	99.5	100.3	99.1	99.7	103.9

[a] B-7, B-15 and B-17 were *Agrobacterium* sp., and B-60 *Acinetobacter* sp.
[b] Not detected.

Table 3 Metabolite distribution in cultures[a] with parathion after 48 hr of incubation.

Compound	% of the applied ^{14}C				
	B-7	B-15	B-17	B-60	Control
PRT	91.0	89.9	92.9	93.3	98.2
PRT-DE	0.9	0.9	0.9	1.1	0.8
NP	nd[b]	nd	nd	2.8	nd
PRT-NH_2	nd	nd	nd	1.5	nd
PRT-NHAc	nd	nd	nd	0.8	nd
Origin	0.3	0.4	0.2	1.2	nd
Others	2.4	2.9	2.4	2.2	1.0
Total	94.6	94.1	96.4	102.9	100.0

[a] B-7, B-15 and B-17 were *Agrobacterium* sp., and B-60 *Acinetobacter* sp.
[b] Not detected.

Table 4 Metabolite distribution in cultures[a] with cyanophos after 48 hr of incubation.

Compound	% of the applied ^{14}C				
	B-7	B-15	B-17	B-60	Control
CYN	95.2	85.6	93.9	92.7	98.5
CYN-DM	3.1	2.8	3.1	3.9	2.5
CP	0.4	0.2	0.5	2.1	nd[b]
CYN-$CONH_2$	2.4	9.7	2.0	0.2	nd
CYN-COOH	0.2	0.3	0.1	nd	nd
Origin	0.1	0.1	<0.1	<0.1	nd
Others	0.3	0.5	0.2	0.6	0.9
Total	101.7	99.2	99.9	99.6	101.9

[a] B-7, B-15 and B-17 were *Agrobacterium* sp., and B-60 *Acinetobacter* sp.
[b] Not detected.

Metabolites **I** and **II** were *O*-methyl *O*-(2-hydroxymethylphenyl) hydrogen phosphorothioate and *O*-methyl *O*-(2-hydroxybenzyl) hydrogen phosphorothioate, respectively. **III** and **IV** were unidentified metabolites, which were more hydrophilic than **I** and **II**.

SMT was slightly metabolized by the four SLT-degrading isolates, with 91.8–95.6% of the applied ^{14}C being recovered as SMT within 48 hr. MNP, resulting from the cleavage of the *P-O*-aryl bond of SMT, amounted to 0.7–2.6%, showing that the bacteria were weakly active in hydrolyzing SMT. The amount of SMT-DM (3.2–3.7%) in the inoculated cultures was little larger than that (2.7%) in the control cultures, suggesting that SMT was mainly demethylated *via* non-biological reactions in the liquid media. The activity of B-60 was also very weak in reducing the nitro group of SMT to SMT-NH_2 followed by acetylation to form SMT-NHAc. The total amount of these metabolites was less than 0.6% after 48 hr of incubation. The other three bacteria were not active at all in reductive metabolism of SMT. All of the identified metabolites have been also found in the metabolism studies of SMT in soil[4,9] and by SMT-degrading microorganisms.[4]

PRT was metabolized by B-60 *via* cleavage of the *P-O*-aryl linkage or reduction of the nitro group followed by acetylation. The degrading activity was weak, and the amounts of NP, PRT-

NH_2 and PRT-NHAc were 2.8, 1.5 and 0.8%, respectively. In the cultures with the other three strains, no radioactive spots other than those of PRT and PRT-DE were detected on the autoradiograms, but radioactivity in the origin and others fractions was slightly higher than those in the control culture. The amount of PRT-DE was almost equal in all of the inoculated cultures, as well as in control cultures, suggesting that deethylation of PRT in the cultures was due to non-biological reactions. NP and PRT-NH_2 have been reported[10,11] as degradation products by PRT-degrading microorganisms, and acetylation of the amino group leading to PRT-NHAc was supposed to be a metabolism similar to that of SMT-NH_2, although acetylation of the amino group of PRT-NH_2 by microorganisms may not have been reported.

As in the metabolism of SMT, the *P-O*-aryl or *P-O*-methyl linkage of CYN was cleaved by all of the SLT-degrading bacteria, but to a small extent. On the other hand, hydrolysis of the cyano group of CYN leading to CYN-COOH *via* CYN-$CONH_2$ was observed as a main metabolic reaction, especially by *Agrobacterium* sp. (B-7, B-15 and B-17). After 48 hr of incubation, CYN-$CONH_2$ amounted to 9.7% in the culture with B-15, which had the highest activity among the strains examined. In a previous degradation study of CYN in soil,[12] cleavage of the ester linkages was a main degradation pathway and the cyano group was not transformed. No nitrile transformation of CYN has been reported in metabolism by mammals,[13] plants,[12] and photodecomposition,[5] although hydrolysis of aromatic nitrile by microorganisms has been well examined in metabolism of nitrilic herbicides such as bromoxynil[14-16] and ioxynil.[15,17] It is known that nitrile hydrolysis by microorganisms proceeds *via* two types of transformations: a two-step reaction from the cyano group to the corresponding carboxylic acid *via* amide intermediate[18,19] and a single reaction of the cyano group to the corresponding carboxyl group.[14,15,20,21] Transformation of the cyano group of CYN by the SLT-degrading bacteria seemed to proceed *via* the former metabolic reaction.

A recent investigation with an organophosphate-degrading enzyme, parathion hydrolase,[22] shows that the enzyme has a broad substrate range for dialkylthiophosphates. Insecticides hydrolyzed by this enzyme include methyl parathion, diazinon, fenitrothion, cyanophos, chlorpyrifos and coumaphos, in addition to parathion. Another two *Pseudomonas* strains isolated from soil and sewage were able to utilize eight organophosphorus insecticides as sole phosphorus sources, showing a broad substrate range.[23] Munnecke[24] has also demonstrated that the crude phosphoesterase obtained from a mixed bacterial culture hydrolyzed at least nine organophosphates.

Three SLT-degrading *Agrobacterium* sp. strains (B-7, B-15 and B-17) hydrolyzed SMT and CYN to a small extent, but not PRT. One *Acinetobacter* sp. strain (B-60) with the highest SLT-degrading activity was slightly more active than the *Agrobacterium* sp. strains in hydrolyzing SMT and CYN. It also hydrolyzed PRT. These results suggest that enzymes catalyzing the cleavage of the *P-O*-aryl linkage of SLT in the bacterial strains used were fairly specific to the cyclic organophosphorus insecticide SLT and that they are different from the phopshoester-hydrolyzing enzymes described above in terms of substrate specificity. The SLT-demethylating enzyme in B-60 also seemed to be considerably specific for substrate since the *P-O*-alkyl linkage of SMT, PRT and CYN was hardly cleaved by the strain. This high substrate specificity might be due to the unique cyclic structure of SLT.

REFERENCES

1) K. Itoh: *J. Pesticide Sci.* **16**, 77 (1991)
2) K. Itoh: *J. Pesticide Sci.* **16**, 85 (1991)
3) K. Itoh: *J. Pesticide Sci.* **15**, 561 (1990)
4) Y. Takimoto, M. Hirota, H. Inui & J. Miyamoto: *J. Pesticide Sci.* **1**, 131 (1976)
5) N. Mikami, H. Ohkawa & J. Miyamoto: *J. Pesticide Sci.* **1**, 273 (1976)
6) M. Sasaki, T. Ooishi, T. Kato, C. Takayama & K. Mukai: *J. Pesticide Sci.* **9**, 737 (1984)
7) N. K. Van Alfen & T. Kosuge: *J. Agric. Food Chem.* **22**, 221 (1974)
8) J. Miyamoto, K. Kitagawa & Y. Sato: *Jpn. J. Exp. Med.* **36**, 211 (1966)
9) N. Mikami, S. Sakata, H. Yamada & J. Miyamoto: *J. Pesticide Sci.* **10**, 491 (1985)
10) N. Sethunathan, R. Siddaramappa, K. P. Rajaram, S. Barik & P. A. Wahid: *Residue Rev.* **68**, 91 (1977)
11) I. C. MacRae: *Rev. Environ. Contam. Toxicol.* **109**, 1 (1989)
12) M. Chiba, S. Kato & I. Yamamoto: *J. Pesticide Sci.* **1**, 179 (1976)
13) A. Wakimura & J. Miyamoto: *Agric. Biol. Chem.* **35**, 410 (1971)
14) K. E. Mcbride, J. W. Kenny & D. M. Stalker: *Appl. Environ. Microbiol.* **52**, 325 (1986)

15) D. B. Harper: *Biochem. J.* **167**, 685 (1977)
16) A. E. Smith & D. R. Cullimore: *Can. J. Microbiol.* **20**, 773 (1974)
17) J. C. Hsu & N. D. Camper: *Can. J. Microbiol.* **22**, 537 (1976)
18) P. A. Collins & C. J. Knowles: *J. Gen. Microbiol.* **129**, 711 (1983)
19) J. M. Miller & C. J. Knowles: *FEMS Microbiol. Lett.* **21**, 147 (1984)
20) D. B. Harper: *Biochem. J.* **165**, 309 (1977)
21) A. K. Bandyopadhyay, T. Nagasawa, Y. Asano, K. Fujishiro, Y. Tani & H. Yamada: *Appl. Environ. Microbiol.* **51**, 302 (1986)
22) P. C. Kearney, J. S. Karns & W. W. Mulbry: "Pesticide Science and Biotechnology," ed. by R. Greenhalgh & T. R. Roberts, Blackwell Scientific Publications, London, p. 591, 1987
23) A. Rosenberg & M. Alexander: *Appl. Environ. Microbiol.* **37**, 886 (1979)
24) D. M. Munnecke: *Appl. Environ. Microbiol.* **32**, 7 (1976)

要　約

土壌から単離したサリチオン分解菌によるフェニトロチオン，パラチオンおよびシアノホスの代謝

井藤和人

サリチオンを *P-O*-アリールおよび *P-O*-アラルキル結合の開裂，および脱メチル化により分解する3株の *Agrobacterium* sp. および1株の *Acinetobacter* sp. は，フェニトロチオン，パラチオンおよびシアノホスの *P-O*-アリールおよび *P-O*-アルキル結合の開裂においてほとんど活性を示さないか弱い活性しか示さず，単離されたサリチオン分解菌が基質特異性の高い分解関連酵素を有していることが認められた．一方，*Acinetobacter* sp. は，フェニトロチオンおよびパラチオンのニトロ基のアミノ基への還元，およびそれに引き続くアミノ基のアセチル化に弱い活性を示した．また，3株の *Agrobacterium* sp. はシアノホスのシアノ基を2段階に加水分解し，シアノホスのアミド体およびカルボキシル体が培養液中に認められた．