APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1987, p. 1685–1689
0099-2240/87/071685-05$02.00/0

Vol. 53, No. 7

Bacterial Detoxification of Diisopropyl Fluorophosphate

HUBERT ATTAWAY,[1]† JUDD O. NELSON,[2] ANA M. BAYA,[1] MARY J. VOLL,[1] WILLIAM E. WHITE,[3] D. JAY GRIMES,[1] AND RITA R. COLWELL[1]*

Departments of Microbiology[1] and Entomology,[2] University of Maryland, College Park, Maryland 20742, and Biotechnology Division, Chemical Research, Development and Engineering Center, U.S. Army, Aberdeen Proving Ground (EA), Maryland 21010-5423[3]

Received 11 November 1986/Accepted 29 April 1987

The ability of 18 gram-negative bacterial isolates to detoxify diisopropyl fluorophosphate, a structural analog of the agents soman and sarin, was investigated. Detoxification by both frozen cell sonicates and acetone powders was assayed by two methods, i.e., the hydrolytic release of fluoride, measured by a fluoride-specific ion electrode, and the disappearance of acetylcholinesterase inhibition in vitro. Frozen cell sonicates for all strains exhibited some activity (F^- ion release). In general, acetone powder preparations produced higher activity than frozen cell sonicates did, and the highest activities were exhibited by strains with known parathion hydrolase activity. Two ranges in activity were observed, low level, ranging from 0.1 to 7.0 μmol/min per g of protein, and high level, detected only in parathion hydrolase-producing strains, from 47 to >300 μmol/min per g of protein. Results indicate that parathion hydrolase was nonspecific in phosphoesterase activity. Also, it was an effective detoxicant at low concentrations and near-neutral pH.

Diisopropyl fluorophosphate (DFP) has provided a useful model for biodegradation of toxic organophosphates by both eucaryotic (6–10) and procaryotic systems (1, 8, 13, 21). At the present time, biodegradation of DFP is of interest, since the compound is very similar in structure to the agents soman (1,2,2-trimethylpropyl methylphosphonofluoridate) and sarin (isopropyl methylphosphonofluoridate). Detoxification of these toxic compounds is important for field decontamination and for destruction of existing stockpiles of such agents. Traditional methods involving oxidation or alkaline treatment of toxic organophosphates are effective but extremely corrosive, especially for plastics and metals. (D. Mason, D. Coleman, R. Stafford, and T. Lawler, U.S. Army technical report CRDEC-CR-87025, Feb., 1987). Thus, a biocatalytic system operating at neutral pH and having little or no toxicity is needed.

Diisopropyl fluorophosphatases (DFPases) have been studied extensively, and at least two major classes are now recognized. One is stimulated by Mn^{2+} (Mazur), while the other is unaffected or slightly inhibited by Mn^{2+} (Squid) (8). Enzyme systems hydrolyzing DFP, whether of microbial or animal origin, hydrolyze soman at a significant rate, i.e., 30,000 times the background hydrolysis rates (8) and are thus good candidates for detoxification.

A variety of bacteria have been shown to metabolize organophosphorus compounds by attacking the phosphorus moiety (1–3, 12, 13, 15, 16, 18), including nonfluorinated analogs of sarin and soman (4). Enzyme extracts prepared from bacteria have been characterized with respect to rate and specificity of organophosphate substrate (2, 8, 15, 17, 18, 21).

Parathion hydrolysis has been a focus of research, which has led to successful cloning of plasmid-encoded genes for hydrolysis from two separate isolates (14, 17). Results of the cloning experiments show identical restriction maps and genome size for the parathion-hydrolyzing genes but not for the rest of the plasmids in the two isolates *Flavobacterium* sp. ATCC 27551 (PAR^+ strain) and *Pseudomonas diminuta* MG (14).

In this study, we describe the DFPase activities of several bacterial isolates, including those of the two parathion-hydrolyzing strains (PAR^+ and MG) and a plasmid-cured *Flavobacterium* sp. (PAR^-), and confirm the parathion hydrolysis ability of strains PAR^+ and MG, showing association of that ability with DFPase activity.

MATERIALS AND METHODS

Nutrient broth (Difco Laboratories, Detroit, Mich.) was used for growth of all organisms examined in this study. For

TABLE 1. Sources of strains

Strain	Source or reference(s)
Flavobacterium sp. PAR^+[a]	W. W. Mulbry (14)
Flavobacterium sp. PAR^-	W. W. Mulbry (14)
Pseudomonas diminuta MG	D. T. Gibson (17, 18)
Escherichia coli HB101	Maniatis et al. (11)
K-12	UMMMCC[b]
H3	A. M. Baya
C	UMMMCC
ATCC 25922	American Type Culture Collection
K-12 N6399	J. L. Rasner
K-12 CGSC 5346	CGSC[c]
K-12 LE392	Maniatis et al. (11)
K-12 ML1410	Tanaka et al. (19)
Vibrio alginolyticus F-46	Chesapeake Bay sediment
Vibrio alginolyticus F-47	Chesapeake Bay sediment
Vibrio parahaemolyticus	UMMMCC
Pseudomonas aeruginosa PAO	B. Hollaway
Pseudomonas putida PRS 2015	Wheelis and Ornston (20)
Pseudomonas putida PgGl900 (RP4)	R. Bever

[a] Available from the American Type Culture Collection, Rockville, Md., as ATCC 27551.
[b] University of Maryland Marine Microbiology Culture Collection.
[c] *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.

* Corresponding author.
† Present address: Manville Research and Development Center, P.O. Box 5108, Denver, CO 80217-5108.

TABLE 2. DFPase activity of frozen sonicates and acetone powders as measured with a fluoride electrode with or without manganous ion

Strain or mean	DFPase activity (μmol/min per g of protein) ± SD of[a]:			
	Frozen sonicate		Acetone powder	
	+ Mn^{2+}	− Mn^{2+}	+ Mn^{2+}	− Mn^{2+}
Flavobacterium sp. PAR^+	33.90 ± 2.80 (6)	47.18 ± 6.38 (2)	57.45 ± 10.45 (2) 243.00[b]	72.00 312.00[b]
Flavobacterium sp. PAR^-	6.01 ± 0.92 (5)	3.31 ± 0.57 (3)	3.04	
Pseudomonas diminuta MG	46.70	59.17	64.90[b]	174.07[b]
Pseudomonas aeruginosa PAO	2.05 ± 1.22 (2)	1.11	9.87 ± 3.55 (2)	NR
Pseudomonas putida PRS 2015	0	0.19	0.13	NR
Pseudomonas putida PGG 1900 (RP4)	0.67	0.17	0	0
$\bar{x}$ *Pseudomonas* group I[c]	1.19 ± 0.72 (4)	0.49 ± 0.31 (3)	4.97 ± 3.18 (4)	<0
Escherichia coli HB101	2.89 ± 0.63 (15)	0.67 ± 0.16 (2)	NR	NR
K-12	2.80 ± 0.53 (13)	0.31 ± 0.07 (2)	–	–
H3	6.38	1.25	–	–
C	2.66 ± 1.48 (3)	0.80	NR	16.49
ATCC 25922	4.09 ± 0.44 (8)	2.01 ± 0.82 (4)	NR	18.34
K-12 N6399	2.35	–	–	–
K-12 GTSC 5346	3.82	–	–	–
K-12 LE 392	6.33	–	–	–
K-12 ML1410	0.38 ± 0.38 (2)	–	–	–
$\bar{x}$ *E. coli*	3.12 ± 0.32 (45)	1.21 ± 0.38 (10)	–	17.42 ± 0.93 (2)
Vibrio alginolyticus F-46	0.91 ± 0.75 (3)	–	–	–
Vibrio alginolyticus F-47	0.20	0.17	1.82	3.25
Vibrio parahaemolyticus	0.60	–	–	–
$\bar{x}$ *Vibrio*	0.70 ± 0.43 (5)	–	–	–

[a] Parenthetical values are the number of replicates. –, No measurement made; NR, negative rate, implying that F^- is bound faster than it is generated.
[b] Acetone powders which had been redissolved in buffer and centrifuged to give a clear supernatant, which gave less total protein and thus, higher rates.
[c] Group I includes *P. aeruginosa* and *P. putida* strains; *P. diminuta* belongs to rRNA homology group IV.

Vibrio spp., 1% NaCl (wt/vol) was added to the broth. The strains used in this study and their sources are listed in Table 1.

Cell culture and preparation of extracts. Pure cultures of each strain were grown in 100 ml of nutrient broth in 250-ml flasks incubated at 30°C on a rotary shaker (75 rpm) for 24 h, with the exception of the *Flavobacterium* sp. PAR^+ and *P. diminuta* MG, both of which required incubation for 48 h. The cultures were centrifuged and washed three times with a phosphate-buffered salts solution (PBS) consisting of 17 g of NaCl, 18.2 g of Na_2HPO_4, and 3.0 g of KH_2PO_4 dissolved in deionized water and adjusted to pH 7.0. The cell pellet was suspended and diluted 1:5 in the buffer of Hoskin and Prusch (9) (referred to below as Hoskin buffer) without $MnCl_2 \cdot 4H_2O$. The cell suspension was sonicated in an ice bath in three 1-min bursts, alternating with 1-min rest periods. The sonicate was stored at −70°C.

Acetone powder preparation of cells. Cultures of each strain, in 100-ml volumes grown as above, were centrifuged, and the cells were washed once with acetone-PBS (50:50; 15-ml volume of each) and twice with 30-ml volumes of pure acetone and allowed to air dry. The preparations were stored at room temperature. To reconstitute the cell powder, 10 mg of the dry powder was mixed with 1 ml of the Mn^{2+}-free Hoskin buffer (9), vortexed for 1 min, and then centrifuged (8,800 × *g*) for 15 to 30 s in a microcentrifuge. The supernatant was used for all subsequent analyses. Protein measurements (Bio-Rad Protein Assay Kit; Bio-Rad Laboratories, Richmond, Calif.) were made of the frozen cell sonicates and acetone powder reconstitutions.

DFPase assay. DFP hydrolysis was measured as described by Hoskin et al. (9, 10). To a 10-ml vial fitted with a magnetic stirring bar were added 1.0 ml of 2.5×10^{-3} M DFP (Sigma Chemical Co., St. Louis, Mo.) in Mn^{2+}-free Hoskin buffer and 3.6 ml of Hoskin buffer, either Mn^{2+}-free or amended with 4×10^{-4} M $MnCl_2 \cdot 4H_2O$. A fluoride ion electrode (Orion Research, Inc., Cambridge, Mass.) was immersed in the solution, and the background rate of DFP hydrolysis was measured over a 2- to 3-min period. Subsequently, 0.4 ml of frozen cell sonicate or reconstituted acetone powder was added, and the reaction was followed for 10 min at 25°C.

Acetylcholinesterase assay. A modification of the procedure of Ellman et al. (5) was used to test for detoxification of DFP with respect to acetylcholinesterase inhibition. To 25 ml of PBS buffer, adjusted to pH 8.0, was added 0.25 ml of DFP (10^{-2} M), yielding a 10^{-4} M solution for the hydrolysis reaction. Frozen cell sonicate or reconstituted acetone powder (100 to 500 μl) was added to the solution, and the solution was kept at room temperature, ca. 25°C. At 20-min intervals, 3.0 ml of the reaction mixture was removed and 0.5 U of acetylcholinesterase (Sigma) was added. After 3 min, 100 μl of 5,5-dithiobis-(2-nitrobenzoic acid) (39.6 mg dissolved with 15 mg of $NaHCO_3$ in 10 ml of PBS) and 20 μl of acetylthiocholine (43 mg/2 ml) were added. The reaction was monitored by using a spectrophotometer (Spectronic 20; Bausch & Lomb, Inc., Rochester, N.Y.) set at 412 nm.

Controls were run with the identical solutions minus the cell extract. The spectrophotometer blank consisted of the reaction mixture without acetylcholinesterase. The extinction coefficient for the reaction was 1.36×10^4 M^{-1} cm^{-1}.

Parathion hydrolase assay. Parathion hydrolase (EC 3.1.3) activity of *Flavobacterium* sp. PAR^+ and *P. diminuta* MG was measured as described by Serdar and Gibson (17). The extinction coefficient for the reaction was 1.65×10^4 M^{-1} cm^{-1}.

RESULTS

Results of a comparison of DFPase activities for the bacterial strains examined in this study are shown in Table 2. The majority of bacteria tested demonstrated a low level of DFPase activity, which was enhanced by the addition of Mn^{2+} ions. Exceptions to this generalization were two plasmid-bearing, parathion-hydrolyzing organisms (MG and PAR^+), for which the DFPase was inhibited by Mn^{2+}, and selected acetone powder preparations of other organisms. A low level of DFPase was detected in the plasmid-cured (PAR^-) strain, similar to that detected in *Escherichia*, *Pseudomonas*, and *Vibrio* strains. It was obscured by high DFPase activity mediated by the parathion hydrolase of PAR^+. A higher activity of acetone powder-reconstituted material (DFPase) was detected upon removal of precipitated non-DFPase protein (Table 2).

For PAR^+ acetone powder preparations, activities of 243 and 312 μmol/min per g were obtained for the supernatant clarified by centrifugation; lower rates (57.4 and 72.0 μmol/min per g) were obtained for centrifuged supernatant that remained opaque. The opaque material contained more nonspecific protein, lowering the ratio of micromoles per minute per gram of protein. Acetone powder preparations of the non-parathion-hydrolyzing organisms were not significant in raising DFPase activity and, in some cases, resulted in the inhibition of DFPase activity.

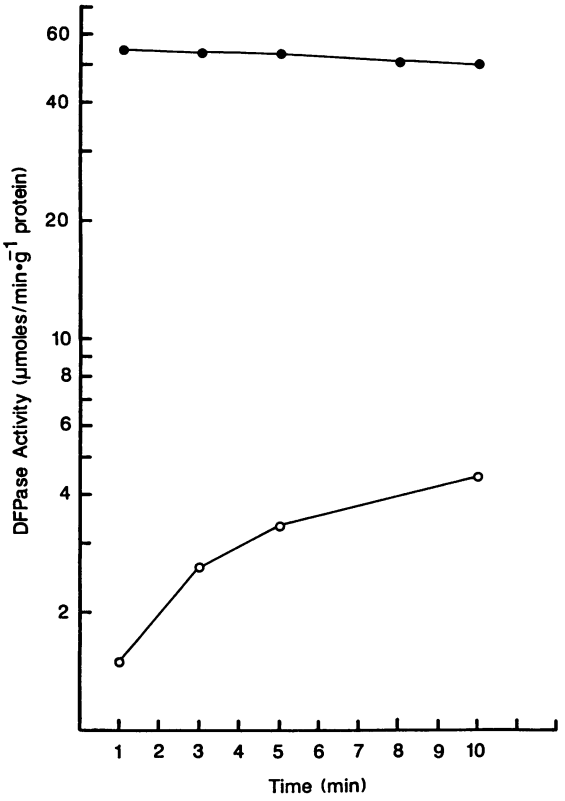

FIG. 1. Activities exhibited by low-level (○, *E. coli* ATCC 25922) and high-level (●, *Flavobacterium* sp. PAR plus DFPase) rates distributed with time.

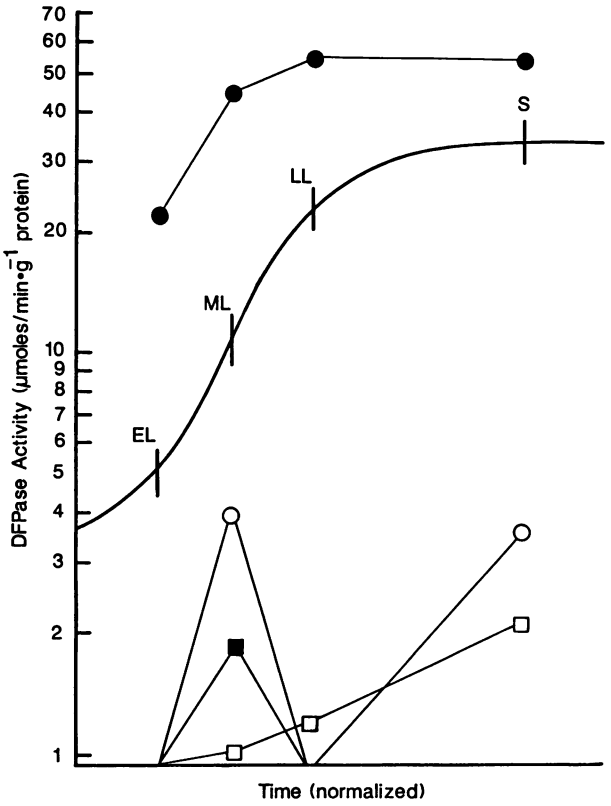

FIG. 2. DFPase activity exhibited by *Vibrio alginolyticus* F-46 (■), *E. coli* (□), *Flavobacterium* sp. PAR^- (○), and *Flavobacterium* sp. PAR^+ (●). Time is presented in arbitrary units, normalized to early-log (EL), mid-log (ML), late-log (LL), and stationary (S) phases of growth. Datum points for *E. coli* (□) represent an average of activities measured for strains K-12, ATCC 25922, HB101, K-12 CGSC 5346, K-12 N6399, and K-12 ML1410 (Table 1).

The low-level, non-plasmid-mediated DFPase activity in most strains tested could be differentiated from PAR^+ DFPase activity by rate increase with time. The time increase observed for low-level DFPase activity, as exemplified by *E. coli* ATCC 25922, and the slight decrease for PAR^+ DFPase activity are shown in Fig. 1.

The onset of DFPase activity with growth is shown in Fig. 2. For several isolates, both low-level and parathion hydrolase-mediated DFPase activities were highest in late-logarithmic and stationary phases, suggesting that the natural function of the enzymes may be for phosphate scavenging in nutrient-depleted environments. It should also be pointed out that growth of PAR^+ was very slow, typically requiring 48 h to achieve stationary phase and, thereby, optimum generation of DFPase activity.

Parathion hydrolase activity in frozen sonicate and in reconstituted acetone powder of both PAR^+ and MG preparations, adjusted to pH 8.0 and 7.2, respectively, is shown in Table 3. The rates for PAR^+ were consistently 1.5- to 2.4-fold higher than those measured for MG, whereas comparative results of their DFPase activities were less clear (Table 2).

TABLE 3. Parathion hydrolase activity

Strain	Parathion hydrolase activity (μmol/min per g of protein) ± SD at[a]:		
	pH 8.0		pH 7.2
	Frozen sonicate	Acetone powder	Frozen sonicate
Flavobacterium sp. PAR$^+$	0.57 ± 0.02	2.68 ± 0.07	0.64 ± 0.03
Pseudomonas diminuta MG	0.39 ± 0.05	1.13 ± 0.02	0.37 ± 0.04

[a] All tests were done in duplicate and, in some cases, triplicate.

Loss of acetylcholinesterase inhibition was also used to assay for detoxification of DFP. The increase in acetycholinesterase activity with time for the cell sonicate or reconstituted acetone powder to detoxify DFP is shown in Fig. 3. Data shown in Fig. 3 were normalized to equal the activity of 10 μg of parathion hydrolase per ml (17) added to 10^{-4} M DFP solution in buffer and measured relative to the time of DFP in the buffer and to the decrease with time of DFP acetylcholinesterase-inhibiting activity as hydrolysis proceeded. Although the datum points were normalized relative to 10 μg of parathion hydrolase per ml, it is obvious in the case of *Flavobacterium* sp. PAR$^+$ that the rates were not the same in 100 μl of frozen sonicate and in 500 μl of the same sonicates. The rates measured for *P. diminuta* MG were also not the same relative to the concentration of protein for 100 and 500 μl of sonicate. Excluding the discrepancies cited, there was a large increase in detoxification of DFP (Fig. 3), expressed as a reversal of acetylcholinesterase inhibition, particularly for the acetone powder preparation of PAR$^+$, in which case in the presence of 10 μg of parathion hydrolase per ml, acetylcholinesterase activity was 79% within 50 min, compared with unamended control, which showed less than 0.5% activity. At 1 μg of parathion hydrolase per ml, acetylcholinesterase activity would be expected to be 100% in less than 10 min.

DISCUSSION

The DFPase activities that were observed (Table 2) could be divided into two classes or levels. One level consisted of low enzyme activity, with specific activities between 0.1 and 7.0 μmol/min per g of protein in crude sonicates; these rates were observed for *Vibrio* spp., *Pseudomonas* spp., *E. coli*, and *Flavobacterium* spp. PAR$^-$. This low-level system has been previously described (8, 12, 21) and shown to be enhanced by Mn^{2+}. Our results correlated well with the published data (8, 12). It is possible that the low-level DFPase activity may be a nonenzymatic chemical reaction enhanced by Mn^{2+}, but data published by other investigators do not support such a conclusion (8, 12, 21).

A second system comprises the parathion hydrolase, a plasmid-mediated phosphotriesterase (2, 14, 17, 18) also exhibiting strong DFPase activity, viz. 47.21 μmol/min per g of protein in crude sonicates of the *Flavobacterium* sp. DFPase activity, in this system, was inhibited by Mn^{2+}, a significant difference from inhibition reported previously for PAR$^+$ parathion hydrolase activity. Brown (2) indicated that Mn^{2+} apparently has no effect on the activity of parathion hydrolase. DFPase rates also differ qualitatively, in that in the relatively weak system, the rate increased with the time of assay, whereas in the strong system (PAR$^+$), the rate decreased with time. Another significant difference is in the storage life of the enzymes. Enzymes of the weak system did not appear to decrease in activity following freezing and thawing, whereas the PAR$^+$ system showed a decrease of about 10% with freezing in this study and a 40% decrease in a previous study (2).

Rates for some of the *E. coli* strains examined in the present study were similar to those reported for *E. coli* K-12 (19) but lower than those reported for *E. coli* ATCC 25922 (8). Measurement of DFPase activity at low concentrations may not be precise.

Rates of parathion hydrolase activity measured in the present study were low compared with those reported for PAR$^+$ (7.15 μmol/min per mg of protein [2]) and for MG (2.1 μmol/min per mg of protein [17]). However, in the present study, the assays were done at near-neutral pH (8.0 for frozen sonicate and 7.2 for acetone powder) to minimize the corrosive effects of a high-pH liquid solution that might be applied to organophosphate-contaminated surfaces. Specifically, other reports have shown optimum activity between pH 8.5 and 9.5 (2, 15).

Results obtained when testing for detoxification of DFP by using reversal of acetylcholinesterase inhibition showed that the dilute enzyme solutions could decontaminate DFP and, very likely, more toxic agents, e.g., sarin and soman. This

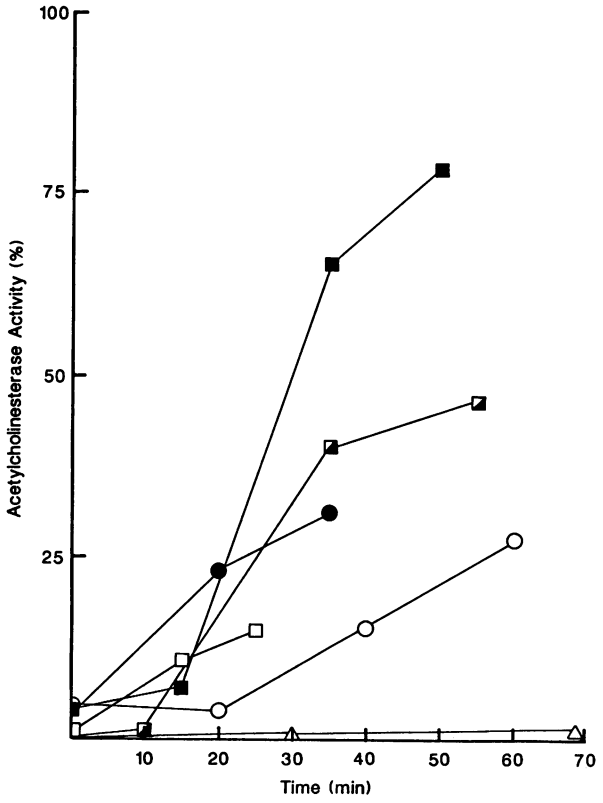

FIG. 3. Percent acetylcholinesterase activity remaining in reaction vessels containing DFP (the acetylcholinesterase inhibitor) and 100-μl frozen sonicates of *Flavobacterium* sp. PAR$^+$ (◪) or *P. diminuta* (○), 500-μl frozen sonicates of *Flavobacterium* sp. PAR$^+$ (□) or *P. diminuta* (●), or 100-μl acetone powder preparations of *Flavobacterium* PAR$^+$ (■) (the sources of DFPase) after exposure to DFPase. Control vessels contained DFP, acetylcholine, acetylcholinesterase, and appropriate buffers, but not DFPase (△). All datum points shown were normalized to 10 μg of parathion hydrolase activity per ml of reaction mixture (17). Base-line activity for 1.0 U of acetylcholinesterase was 0.441 μmol of acetylthiocholine hydrolyzed per min at pH 8.0 and 25°C.

suggests that parathion hydrolase is relatively nonspecific in its phosphoesterase activity, a phenomenon observed by others (2, 14).

In summary, an enzyme system with strong activity against DFP and possibly other neurotoxic organophosphates has been characterized and shown to function within a noncorrosive pH range of 7.2 to 8.0. The crude enzyme preparation, when dried in acetone to a powder, is very stable and can be maintained, if dry, at room temperature without significant loss of activity. Since the system is plasmid mediated, it can be readily cloned, and the gene products can be amplified or mutagenized site specifically to produce higher activity.

ACKNOWLEDGMENTS

We are grateful to A. M. Chakrabarty, David T. Gibson, and Walter W. Mulbry for providing strains used in this study. Robert Boethling provided a very useful review of the manuscript, and Walter Mulbry is also acknowledged for his helpful technical assistance.

This work was supported by U.S. Army Chemical Research, Development and Engineering Center contract DAAK11-84-0005.

LITERATURE CITED

1. **Boush, M., and F. Matsumura.** 1967. Insecticidal degradation by *Pseudemonas melophthora*, the bacterial symbiote of the apple maggot. J. Econ. Entomol. **60**:918–920.
2. **Brown, K. A.** 1980. Phosphotriesterases of *Flavobacterium sp.* Soil. Biol. Biochem. **12**:105–112.
3. **Cook, A. M., C. G. Daughton, and M. Alexander.** 1978. Phosphonate utilization by bacteria. J. Bacteriol. **133**:85–90.
4. **Cook, A. M., C. G. Daughton, and M. Alexander.** 1978. Phosphorus-containing pesticide breakdown products: quantitative utilization as phosphorus sources by bacteria. Appl. Environ. Microbiol. **36**:668–672.
5. **Ellman, G. L., K. D. Courtney, V. Andres, Jr., and R. M. Featherstone.** 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. **7**:88–95.
6. **Gay, D. D., and F. C. G. Hoskin.** 1979. Stereospecificity and active site requirements in a diisopropylphosphoroflouridate-hydrolyzing enzyme. Biochem. Pharmacol. **128**:1259–1261.
7. **Hoskin, F. C. G.** 1976. Distribution of diisopropylphosphorofluoridate-hydrolyzing enzyme between sheath and axoplasm of squid giant axon. J. Neurochem. **26**:1043–1045.
8. **Hoskin, F. C. G., M. A. Kirkish, and K. E. Steinmann.** 1984. Two enzymes for the detoxification of organophosphorus compounds: sources, similarities, and significance. Fundam. Appl. Toxicol. **4**:S165–S172.
9. **Hoskin, F. C. G., and R. D. Prusch.** 1983. Characterization of a DFP-hydrolyzing enzyme in squid posterior salivary gland by use of Soman, DFP and manganous ion. Comp. Biochem. Physiol. **75C**:17–20.
10. **Hoskin, F. C. G., and A. H. Roush.** 1982. Hydrolysis of nerve gas by squid-type diisopropyl phosphorofluoridate hydrolyzing enzyme on agarose resin. Science **215**:1255–1257.
11. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Moore, J. K., H. D. Braymer, and A. D. Larson.** 1983. Isolation of a *Pseudomonas* sp. which utilizes the phosphonate herbicide glyphosate. Appl. Environ. Microbiol. **46**:316–320.
13. **Mounter, L. A., R. F. Baxter, and A. Chanutin.** 1955. Dialkylfluorophosphatases of microorganisms. J. Biol. Chem. **215**: 699–704.
14. **Mulbry, W. W., J. S. Karns, P. C. Kearney, J. D. Nelson, C. S. McDaniel, and J. R. Wild.** 1986. Identification of a plasmid-borne parathion hydrolase gene from *Flavobacterium* sp. by Southern hybridization with *opd* from *Pseudomonas diminuta*. Appl. Environ. Microbiol. **51**:926–930.
15. **Munnecke, D. M.** 1976. Enzymatic hydrolysis of organophosphate insecticides, a possible pesticide disposal method. Appl. Environ. Microbiol. **32**:7–13.
16. **Rosenberg, A., and M. Alexander.** 1979. Microbial cleavage of organophosphorus insecticides. Appl. Environ. Microbiol. **37**: 886–891.
17. **Serdar, C. M., and D. T. Gibson.** 1985. Enzymatic hydrolysis of organophosphates: cloning and expression of a parathion hydrolase gene from *Pseudomonas diminuta*. Bio/Technology **3**:567–571.
18. **Serdar, C. M., D. T. Gibson, D. M. Munnecke, and J. H. Lancaster.** 1982. Plasmid involvement in parathion hydrolysis by *Pseudomonas diminuta*. Appl. Environ. Microbiol. **44**:246–249.
19. **Tanaka, T., M. Tsunoda, and S. Mitsuhashi.** 1973. Distribution of R factors among *Shigella* strains isolated in Japan (II). Jpn. J. Microbiol. **17**:291–295.
20. **Wheelis, M. L., and L. N. Ornston.** 1972. Genetic control of enzyme induction in the β-ketoadipate pathway of *Pseudomonas putida*: deletion mapping of *cat* mutations. J. Bacteriol. **109**:790–795.
21. **Zech, R., and K. O. Wigand.** 1975. Organophosphate-detoxicating enzymes in *E. coli*: gel filtration and isoelectric focusing of DFPase, paraoxonase and unspecific phosphohydrolase. Experientia **31/2**:157–158.