Biodegradation and Detoxification of Malathion by of Bacillus Thuringiensis MOS-5

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Abstract: Efficiency of a strain of *B. thuringiensis* MOS-5 (Bt), isolated from agricultural waste water near Berket El-Sabaa Egypt contaminated with organophosphorus insecticide, for degradation of malathion was investigated. It was able to utilize malathion as a sole carbon and energy source and to degrade it cometabolically. In a minimal salt medium supplied with 250 mg l^{-1} malathion ether alone or incombination with glucose or yeast extract MOS-5 caused 99.32% reduction in malathion after 30 days. Addition of glucose (5 g l^{-1}) and yeast extract (0.5 g l^{-1}) increased the growth rate 10^4 and 10^5 fold, respectively, compared to malathion alone. Results of HPLC, gas chromatography/ mass spectrometry (GC/MS) and infrared spectroscopic analysis revealed that one malathion-derived compound mal-monocarboxylic acid (MMA) was produced after three days. Two additional malathion derivatives, mal-dicarboxylic acid (MDA) and unidentified mal-x were detected after 7 days. MMA and MDA were the major degrading compounds. Esterase activity involved in malathion degradation was also determined in culture filtrate of MOS-5. Results indicated that esterase activity was two folds more in the presence of yeast extract compared to glucose. These results indicate that Bt MOS-5 may consider as highly potential candidate in the biodegradation of organophosphorus in contaminated soil.

Key words: Degradation of malathion, cometabolism, esrase

INTRODUCTION

Synthetic organophosphorus compounds are a group of highly toxic agricultural chemicals widely used for plant protection. Up to the present, organophosphate pesticides such as parathion and malathion (O, O-dimethyl-S (1, 2 dicarbethoxyethyl) di-thiophosphate), are still extensively used worldwide despite their high toxicity (Kumar *et al*, 1996). Due to environmental concerns associated with the accumulation of these pesticides in food products and water supplies, efforts are currently underway to develop safe, convenient and economically feasible methods for pesticides detoxification.

Current methods to detoxify organophosphate pesticides mainly rely on chemical treatment, incineration and -landfills (Richins *et al*., 1997). Chemical methods, although feasible, are problematic due to production of large volume of acids and alkali that subsequently must be disposed. Landfills function adequately, but leaching of pesticides into surrounding soil and ground water supplies is a big concern. Incineration, which is the most reliable methods for destruction of these compounds, has met serious public opposition because of the potentially toxic emissions (Richins *et al*., 1997). Soil micro flora is another potential candidate for detoxification of pesticides. Some investigators found that soil contaminated with pesticides could be possibly decontaminated by inoculation with specifically adapted microorganisms (Cho *et al.,* 2000). Moreover, microbial attack on wide ranges of organophosphorus insecticides have been reported (Kamel and Al-Awadi, 1987, Boldrin *et al*., 1993, Cheng *et al*., 1993; Richins *et al*., 1997 and Zhongli *et al*., 2001). Enzymatic detoxification of organophosphorus insecticides by some bacterial species have also received considerable attention (Cheng *et al*., 1993; Richins *et al*., 1997; Di Sloudi *et al.,* 1999; Chen-Goodspeed; 2001 and Gilbert *et al*., 2003, Kim *et al*, 2005).

In this study the efficiency of a local bacterial isolate for hydrolyzing malathion in minimal or enriched media was evaluated. Metabolites resulting from biodegradation were also estimated and identified.

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MATERIALS AND METHODS

Microorganism:

The local bacterial isolate MOS-5 was originally isolated from agricultural waste water that was taken from Berket El-Sabaa Lake near Cairo, Egypt, as malathion-degrading bacteria. Isolate MOS-5 was identified as a strain of *Bacillus thuringiensis*. This isolate was capable of growing and hydrolyzing malathion in minimal salt medium as the sole carbon and energy source. Malathion was dissolved in acetone and added to M9 medium (250 mg/300 ml).

Malathion:

Malathion diethyl (dimethoxy thiophosphorylthio) succinate was obtained from Kafr Elzeiat Company, Egypt, water solubility 130 mg/L soluble in most organic solvents.

Degradation and Residual Determination of Malathion by the Local Isolate Bacillus Thuringiensis (Mos-5): Residual Determination of Malathion in Mos-5 Inoculated Media:

The non-degraded residual malathion was monitored in liquid culture of MOS-5 through GC/MS analysis. In this assay conical flasks containing M9 minimal salt medium and malathion (250 ppm) was inoculated with 5.6×10^8 cfu/ml of MOS-5 and incubated at 30°C for 30 days. Percentage of residual malathion was determined at 0, 3, 7, 10, 15, 21 and 30 days post inoculation. M9 minimal salt medium containing 0.64% $Na_2 HPO_4, 7H_2O$, 0.15% KH_2PO_4, 0.025% NaCl, 0.05% NH_4Cl. To 800 ml sterile deionized water, 200 ml of M9 salts was added.

Growth of B. Thuringiensis (Mos-5) in Liquid Culture Supplied with Malathion:

Viable bacterial cell counts in minimal media M9 lacking carbon source and supplied with malathion (250 ppm) either alone or in combination with glucose (5000 ppm) and/or yeast extract (500 ppm) were determined on days 12 & 15 post inoculation. Four different media (M_1, M_2, M_3 and M_4) were prepared and inoculated with MOS-5 (5.6×10^8 cfu/ml). M_1 was minimal media supplied with 250, 500 and 5000 ppm malathion, yeast extract and glucose, respectively. M_2 was minimal media supplied with 250 and 5000 ppm malathion and glucose, respectively. M_3 was minimal media supplied with 250 and 500 ppm malathion and yeast extract, respectively. M_4 was minimal media supplied with 250 ppm malathion for determinate of bacterial growth serial dilutions of cultures was inoculated.

Determination of Growth Rate:

MOS-5 was inoculated onto M9 minimal medium supplied with 250 ppm malathion as the sole carbon source. Malathion was dissolved in acetone (250 mg / 300 ml) and added to 100 ml M9 media. In other assays, glucose (5000 ppm) or yeast extract (500 ppm) were added to M9 supplied with 250 ppm malathion. Rate of bacterial growth was estimated based on determination of viable cell count per ml (CFU ml^{-1}).

Determination of Residual Malathion and its Derivatives:

Malathion and its metabolites were extracted and separated according to Singh and Seth (1989). Residual malathion was determined in the culture filtrate of MOS-5 using Gas Liquid Chromatography and High Performance Liquid Chromatography according to Chukwudebe *et al.*, (1989). Malathion derivatives were also analyzed by HPLC (Chukwudebe *et al.*, 1989). HPLC analysis was performed using water R CM-100 HPLC system. Compounds were eluted with a gradient consisting of methanol and ethyl acetate (8:2 V/V, respectively) isocratically delivered by a water 600 pump at rate of 5mllmin to 8 mm-i.d. stainless steel radial pack-silica gel column. Malathion derivatives were identified using Infra Red Spectroscopy (IR) unit in the Central Lab of Micro-analysis, Cairo University. Parallel samples of metabolites were transferred to test tubes and methylated using the method of Muan and Skare (1989) and analyzed using finnigan SSQ 700 Mass Spectrometer for which the data acquisition parameters were: Ionizing voltage 70 eV, cyclic time 0.5 s decade^{-1}, resolution 25000 at (10% valley level) and accelerating voltage 3 KV. Samples were methylated by drop-wise addition of ethereal solution of diazomethane until a yellow color persisted in the mixture. After about 1 min, extra diazomethane was removed by evaporation using N_2 gas. One ml of acetone and 3 ml cyclohexane were added, and the samples were mixed for 30s on a whirl mixer. The organic layer was separated and the final volume adjusted to 5 ml with cyclohexane. Products were identified by comparing their retention times and mass spectral fragmentation patterns with instrument library.

Esterase Activity:

Esterase activity in the culture filtrate of MOS-5 was determined according to the method described by Gomori (1953). The reaction mixture was prepared by mixing 2 ml of 1.5 mM a-Naphthyl acetate and 0.1 ml sample. After incubation at 25°C for 30 min, 0.5 ml of Fast Blue B salt, prepared in water, was added and the tubes were incubated for additional 15 min. Change in colour due to formation of a-naphthol was measured at 490 nm.

RESULTS AND DISCUSSION

Results:
Monitoring of Degradation of Malathion by Local Isolate of B. Thuringiensis (Mos-5):
    The non-degraded residual morlathion was monitored in M9 minimal salt medium containing malathion (250 ppm) and inoculated with MOS-5 through GC/MS analysis. Strain MOS-5 was able to use malathion as a sole source of carbon and energy.

    The obtained results showed considerable removal of malathion with elapsed time in inoculated media (Table 1). More the 50% of initial malathion was degraded *B. thuringiensis* strain (MOS-5) during the first three days. Four days later, however, malathion was reduced to 26% in inoculated media. After thirty days, the amount of malathion in inoculated media was 0.68%.

Table 1: Percentage of residual amount of malathion in M9 media inoculated with *B. thuringiensis* (MOS-5)

Incubation time (days)	Residual malathion (%)
0	100
3	49.4
7	26.1
10	17.0
15	9
21	4
30	0.7

Growth of B. Thuringiensis (Mos-5) in Liquid Culture Supplied with Malathion:
    Data in table (2) revealed that malathion supported growth of ***B**. thuringiensis as sole sources of carbon and energy (Fig. 1). After 12 days* incubation, bacterial growth reached 7.87×10^{11} cfu/ml. Longer incubation didn't increase bacterial growth. Cometabolic degradation of malathion was also found with other growth substrates. In the presence of either glucose (M3) or yeast extract (M2) in culture media, supplied with malathion, an increase in bacterial growth was observed after 12 days incubation compared to growth in media containing only malathion (M4) as the sole carbon source. With prolonged incubation (15 days), this stimulatory effect increased in the presence of yeast extract (5.72×10^{14}) and glucose (8×10^{13}) while decreased in the presence of malathion (1.97×10^{11}).

Table 2: Growth of Bt strain MOS-5 growth in liquid M9 minimal media supplied with 250 ppm malathion.

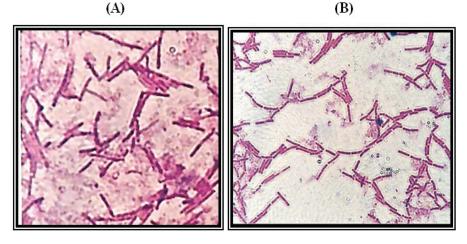| Culture type	Days	Bacterial count (cfu/ml)
(M1)	12	1.05×10^{13}
Malathion + MOS-5 + 0.5% Glucose + 0.05 % Yeast Extract	15	1.6×10^{21}
(M2)	12	5.25×10^{13}
Malathion + MOS-5 + 0.05% Yeast Extract	15	5.72×10^{14}
(M3)	12	6.5×10^{12}
Malathion + MOS-5 + 0.5% Glucose	15	8×10^{9}
(M4)	12	7.87×10^{11}
Malathion + MOS-5	15	1.97×1011

(A)   **(B)**

Fig. 1: Photomicrograph of (A) *B. thuringiensis* (MOS-5) (growth in minimal salt media (M9) + malathion + yeast extract (500 ppm) after 12 days and (B) *B. thuringiensis* (MOS-5) (grown in minimal salt media (M9) + malathion) after 12 days.

Analysis and Identification of Malathion Degradation Products:

During degradation of malathion by Bt MSO-5 we analysed the formation of malathion - derived degradation products using GC/MS , HPLC and GLC.

Results of HPLC revealed that biodegradation of malathion in MOS-5 liquid culture began during the first three days. One decomposition product at R value 24, t3, was obtained and given the name mal-der-1 (Fig. 2a). after seven days, another two degradation products at R values of 4 & 6.65 were observed and given the names mal-dar-2 and mal-der-3, respectively, (Fig. 2b). Mal-der-1 and mal-der-2 representing two major products and were subjected to identification. Mal-der-3, on the other hand, was found in trace amount and no attempt was made to identify it. No metabolites were detected in the organic fraction of the culture media (data not shown).
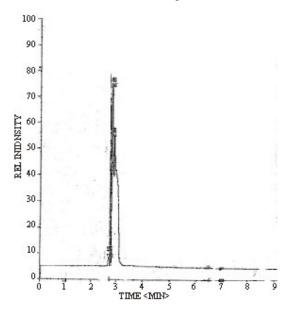

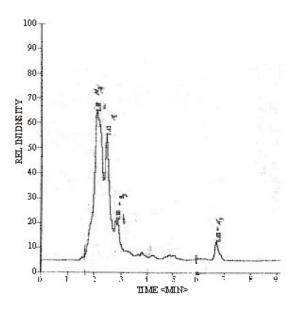**Fig. 2a:** HPLC chart of Standard malathion

Fig. 2b: HPLC chart showing analysis of the metabolites extracted from MOS-5 liquid culture after 7 days incubation.

IR spectrum of mal-der-1 showed main characteristic bands at 665, 1380, 1730, 3440 and reduced adsorption at 1100, and 1170 [cm^{-1}] (data not shown).

Mass spectrum of mal-der-1 (Fig. 3) showed Mt at M/Z 302 for the molecular formula $C_8H_{15}O_6PS_2$ These characteristics were found to be identical with those of malathion monocarboxylic acid reported by Walker and Stojanovic (1973), Singh and Seth (1989) and Muan and Skare (1989). Accordingly, mal-der-1 was identified as malathion mono-carboxylic acid (MMA). The IR spectrum of mal-der-2 showed the main characteristic band of 3420, 1730 confirmed the presence of O=C-OH, 1220 for O-CH methoxy group and reduced adsorption at 1100 and 1170. These characteristics were identical with those of malathion dicarboxylic acid suggested by Walker and Stojanovic (1973). Mass spectrum of mal-der-2 (Fig 3) showed M/Z 274 for the molecular formula $C_6H_{11}O_6PS_2$. Based on these data, the structure of mal-der-1 was concluded to be malathion dicarboxylic acid (MDA) both MMA and MDA were major degradation products in Bt MOS-5 culture.
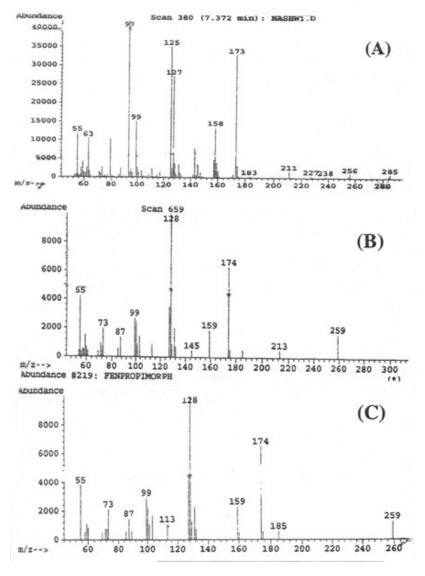

Fig. 3: (A) Mass spectrum chart of malathion, (B) Mass spectrum of malathion monoacid and (C) Mass spectrum of malathion diacid

The obtained results indicate that *B. thuringiensis* (MOS-5) is very active in degrading malathion probably through the action of carboxylester hydrolysis. The fact, however, that the carboxylic acid derivatives of
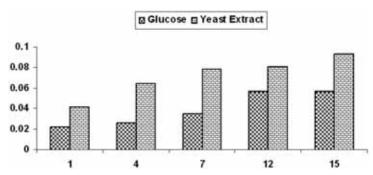
malathion constitute the major portion of malathion metabolites strongly suggest the presence of powerful carboxylesterase in this organism.

Iii.4. Esterase Activity in Culture Filtrate of B. Thuringiensis Mos-5:

The obtained results indicated that malathion biodegradation to malathion mono-acid and malathion di-acid may occur through the action of esterase. Subsequently, the presence of esterase activity was examined in culture of the bacterial isolate Bt MOS-5. Two types of media were used, M2 (minimal media supplied with 250 ppm malathion and 500 ppm yeast extract) and M3 (minimal media supplied with 250 ppm malathion and 5000 ppm glucose). Both yeast extract and glucose were used as growth initiators. Both cultures were incubated for 15 days and aliquots were taken after 1,4,7,12 and 15 days (Table3). The results revealed that there is an increase in the esterase enzyme with increasing incubation time as shown in table (3) and Fig. (4). There were slight differences in enzyme activity between M2 and M3 media esterase activity was slightly lower in the presence of glucose compared to yeast extract at all time intervals. At day one, for instance, esterase activity was 0.042 in M2 compared to 0.022 in M1. Similarly, esterase activity at day 12 was 0.08 in cultures containing yeast extract compared to 0.05 in culture containing glucose.

Table 3: Esterase activity (OD) in culture filtrate of *B. thuringiensis* (MOS-5) containing supplied with two different growth substrates.

Day	Glucose	Yeast extract
1	0.0226	0.0420
4	0.0264	0.0639
7	0.0349	0.0787
12	0.0564	0.0807
15	0.0569	0.0930

Fig. 4: Esterase activity (OD) in culture filtrate of *B. thuringiensis* (MOS-5) containing malathion and supplied with two different growth substrates.

Discussion:

Organophosphorus compounds are a group of highly toxic agricultural chemicals widely used for plant protection. Up to the present these pesticides such as parathion and malathion, are still extensively used world wide despite their high toxicity (Kumar *et al*., 1996). Due to environmental concerns associated with the accumulation of these pesticides in food products and water supplies, there is a great need to develop safe, convenient and economically feasible methods for pesticide detoxification in environment.

In the present study, we investigated the efficiency of a strain   of *B. thuringiensis* (MOS-5) previously isolated from an agriculture waste water in Egypt in degradation of malathion . The isolate MOS-5 was capable of growing on minimal salt medium containing 1000 ppm malathion as the sole carbon source.

The persistence rate of malathion in liquid culture off

B. thuringiensis isolate (MOS-5) was estimated during 30 days incubation time. Considerable removal of malathion after three days incubation was observed. In inoculated salt media for instance, more than 50% of malathion was degraded to other compounds. After one week of incubation, residual malathion decreased to 26.5% and reached 0.68% after 30 days incubation. On the contrary, low concentration of malathion degradation in non-inoculated salt media incubated for 30 days was observed due to spontaneous degradation.

Similar results have been observed. Horne *et al*. (2002) found that *Agrabacterium r*a*diobate*r has the ability to hydrolyze a wide range of organophosphorus insecticides. Further, a methyl parathion-degrading bacterial strain was isolated and identified as *plesiomonas* sp.

In accordance with the above results Roy *et al*. (1995) isolated five malathion degrading bacterial strains, identified as *Pseudomonas* and *Micrococcus* from soil during their search for facultative anaerobes capable of degrading malathion. The same authors (Kumari *et al*., 1998) used mixed cultures of the five bacterial strains

for degradation of malathion. A 61% degradation of malathion could be achieved. The results indicated that malathion supported the growth of MOS-5 as a sole source of carbon. Consistent with these results, different bacteria have shown to grow on parathion and methyl parathion as the sole carbon source (Rani and Lalithakumari, 1994; Shimazu *et al.*, 2001; Zhongli *et al.*, 2001). The growth of MOS-5 in this poor media was due to the biodegradation of this insecticide by the enzyme machinery that already exists inside the cell. These enzymes are largely hydrolytic to such compounds and plays major role in utilization of these materials as
 nutrient sources. This finding was also confirmed by those obtained by Bhaskaran *et al.* (1973), Rosenberg and Alexander (1979), Nelson (1982) and Kamel and Al-Awadi (1987) who found that some soil micro-organisms especially *Streptomyces* sp. *Pseudomonas* sp*., Streptomyces rimosus, Fusarium moniliform* can utilize malathion and other the organophosphorus insecticides under the in-vitro conditions.

Longer incubation of MOS-5 did not significantly affect growth rate in media containing only malathion as the sole source of carbon. This observation might be explained by the disappearance of this insecticides due to its complete utilization by the tested organism.

Cometabolic degradation of malathion by MOS-5 was also observed with either glucose or yeast extract. An increase in bacterial growth was observed after 12 days incubation compared to growth in salt media containing only malathion as sole carbon source.

Consistent with these results, Singh and Seth (1989), who studied the growth rate of *Pseudomonas* sp. On various co substrates like glucose, ethanol and succinate in addition to malathion (150 ppm), found that among these co substrate, ethanol was the best to support the growth of this strain. On the contrary, little growth of the bacterium was observed in the absence of co substrates.

Presence of yeast extract and glucose led to an increase in MOS-5 growth rate compared to growth in a media containing only malathion. However, glucose didn't significantly increase growth rate compared to yeast extract. This non significant effect of glucose on growth rate might be attributed to the inhibition of glucose uptake by MOS-5 in the presence of organophosphorus insecticides as previously reported, (Rahmatullah *et al.*, 1978) and Kamel and Al-Awadi (1987). These groups authers have observed a decrease in glucose uptake by some fungal and *Streptomyces* species in presence of dichlovos and metadathion insecticides. Maleszewsk (1974), on the other hand, showed differences in sugar catabolism in the presence of malathion. He observed that malathion accelerated starch and sucrose catabolism but delayed the break down of sorbitol. Rahmatullah *et al.* (1979), observed that malathion inhibit carbohydrate metabolism in *A. niger* and reduced cellular ATP. Malathion also affected enzymes involved in carbohydrate metabolism in *B. subtilus* (Maleszewsk, 1974).

As a matter of fact, one of the major biochemical mechanisms responsible for the development of malathion resistant insects is the enhanced activity of the detoxifying enzymes carboxylesterase. On the contrary, little information is available on mechanisms of microbial biodegradation and mineralization of malathion. Masumura and Boush (1968) found that certain trichodema viride had very marked ability to breakdown malathion through the action of carboxylesterase. Others (Khalil and Mostafa, 1987) showed that the blue green algae *Anabaena oryzme* and *Phormidium fragile* were able to break down malathion into malaxon and five additional metabolites.

Identification of the main metabolites of malathion biodegradation in aqueous fraction of culture filtrate of MOS-5 was investigated using HPLC, IR and GC/Ms.

Data revealed that the two major metabolites produced during the first 7 days from biodegradion of malathion is malathion monoacid (MMA) and malathion di-acids (MDA), formed by removal of one or two elcohol, respectively from diethyl succinate portion of malathion molecule (Fig. 5). The molecular formulation of MMA and MDA was $C_8H_5O_6PS_2$ and $C_6H_{11}O_6PS$ respectively.

Fig. 5: The degradation products of malathion (I) into malathion di-acid (II) and malathion mono (III) acids.

The identification of malathion mono-and di-acids as intermediates of malathion biodegradation agree well with findings obtained by Walker and Stojanovic (1974), who noticed that *Arthrobacter* sp. Was able to degrade malathion to malathion mono and di-carboxylic acids. Similarly, Singh and Seth (1989) reported that a strain of *Pseudomonas* isolated from peppermill effluents was capable of degrading malathion cometabollically up to malathion mono carboxylic acid. All these data were agreed well with our findings. Bt-MOS-5 was able to degrade malathion and convert it to mono and di-carboxylic acids derivatives of malathion.

Similarly, Muan and Skare (1989) found that biodegration of malathion to inactive metabolites results from hydrolytic cleavage of one or two of the carboxyl groups, giving rise to mono-or di- acid respectively.

The obtained results indicate that MOS-5 is very active in degrading malathion probably through the action of carboxylester hydrolysis. The fact that the carboxylic acid derivatives of malathion constitute the major portion of malathion metabolites strongly suggest the presence of powerful carboxylestererases in this organism.

Organophosphates contain three phosphoester linkages and are hence termed phosphotriesters. The phosphorus is also linked by a double bond to either an oxygen (P=O) on oxon organophosphates or a sulfur (P+S) in thion organophosphates. In general, hydrolysis of one of the phosphoester bonds reduces the toxicity of organophosphates (Serdar, 1996; Horne *et al*., 2002).

Enzymatic detoxification of organophosphates has become the focus of many studies because other means of removing organophosphates residues are either impractical, costly or are themselves environmentally hazardous.

Detoxification of several organophosphates pesticides in the environment is carried out by carboxy esterase (EC 3.1.1.1). different groups of these enzymes are found in bacteria (Dumas *et al*., 1989) and Shimazu et al., 2001). The most widely studied bacterial enzyme is the organophosphorus hydrolase (OPH) enzyme. OPH catalyzes the hydrolysis of wide range of organophosphates pesticides (Dumas *et al*., 1989; Rogers *et al*., 1999; Shimazu *et al*., 2001). Malathion degradation by fungal cutinase and yeast estrase to MMA and MDA was reported by Kim *et al.* (2005).

Bacterial enzymes have received considerable attention and may have advantages in terms of broader substrate specificities (both oxon and thion organophosphates) and superior kinetics (Dumas, 1989 and Cheng, 1993). Therefore OPH from bacteria is better suited to bioremediation of insecticidal organophosphorus pesticides. Horne *et al*. (2002) isolated a bacterial strain *Agrobacterium radiobacter* P230, which can hydrolyze wide range of organophosphates. In addition, the gene encoding an organophosphorous (OP) hydrolase was cloned and sequenced.

In the present study, estimation of esterase activity in the minimal salt media culture of MOS-5 containing either glucose or yeast extract in addition to malathion, was determined during 15 days incubation period. The results indicated an increase in esterase activity in the bacterial culture by increasing incubation period, a phenomenon that was more pronounced in presence of yeast extract. The increase of esterase activity by increasing culture age is consistent with the increase of esterase activity by increasing culture age is consistent with the increase in the bacterial growth (as indicated from Table 2) and biodegradation rate (Table 1). In conclusion organophosphorus hydrolase play important roles in the decontamination of organophosphorus pesticides and will be useful in the bioremediation of pollution caused by these pesticides (Cho *et al*., 2000). Malathion is often present in contaminated soils cometabolic degradation might be of importance for bioremediation.

REFERENCES

Bhaskaran, R., Kandsasamy, G. Oblisantiand and T.R. Subramania, 1973. Utilization of disyston as carbon and phosphorus sources by soil microflora. Curr. Sd., 42: 835-836.

Boldrin, B., A. Tiehm and C. Fritzsche, 1993. Lehrstuhl fur Mikrobiologie, Universitat Karlsruhe, Kaiserstrasse 12, D-76128 Karlsruhe, Germany, Applied and Environmental Microbiology, 1927-1930

Cheng, T.C., S.P. Harvey, and A.N. Stroup, 1993. Purification and properties of a highly active organophosphorus acid anhydrolase from *Alteromonas undina*. Appl. Environ. Microbiol., 59: 3138-3140.

Chen-Goodspeed, M., M.A. Sogorb, F. Wu, S.B. Hong and F.M. Raushel, 2001. Structural determinants of the substrate and stereochemnical specificity of phosphotriesterase. Biochemistry, 40: 1325-1331.

Cho, T.H., J.B. Wild and K.C. Donnelly, 2000. Utility of organophosphorus hydrolase for the remediation of mutagenicity of methyl parathion. Environ. Toxicol. Chem., 19: 2022-2028.

Chukwudebe, A., R.B. March, M. Othman and T.R. Fukuto, 1989. Formation of trialkyl phosphorothioate esters from organophosphorus insecticides after exposure to either ultraviolet light or sunlight. J. Agric. Food Chem., 3: 340-345.

DiSloudi, B., J.K. Grimsley, K. Lai and J.H. Wild, 1999. Modification of near active site residues in organophosphorus hydrolase reduces metal stoichiometry and alters substrate specificity. Biochemistry. 38: 2866-2872.

Dumas, D.P., S.R. Caldwell, J.R. Wild and F.M. Raushel, 1989. Purification and properties of the phosphotriesterase from *Pseudomonas diminuta*. I. Biol. Chem., 26(1): 19659-19665.

Gilbert, E.S., A.W. Walker and J.D. Keasling, 2003. A constructed microbial consortium for biodegradation of the organophosphorus insecticide parathion. Appl. Microbiol. Biotechnol., 61: 77-81.

Gomori, 1953. Human esterase. J. Lab. Clinical Med., 42: 445-453 (c.f. Hashem, M., 1971).

Horne, I., Tara D. Sutherland, Rebecca L. Harcourt, Robyn J. Russell and John G. Oakeshott, 2002. Identification of an opd (organophosphate degradation) gene in an *Agrobacterium* isolate. Appl. Environ. Microbiol., 3371-3376

Kamel, Z. and Al-Awadi, 1987. Some metabolic activities of *Streptomyces rimosus* and *Fusarium moniliforme* as affected by two organophosphorus insecticides. Proc. Conf. of Agric. Science on Food Deficiency. Mansoura University. 3: 316-324.

Khalil, Z. and I.Y. Mostafa, 1987. Interaction of pesticides with fresh water algae III. Degradation of C^{14} labeled malathion by *Anabaena oryzae* and *Phormidium fragile*. Isotope and Radiation Research, 19: 123-129.

Kim, Y.H., J.Y. Ahng, S. Hyeonmoon and J. Lee, 2005. Biodegradation and detoxification of organophosphate insecticide, malathion by Fusariumoxisporum F.sp pisi cutinase. Chemosphere, 60: 1349-1355.

Kumar, S., K.G. Mukerji, R. Lai, 1996. Molecular aspects of pesticide degradation by microorganisms. Crit Rev. Microbiol., 22: 1-26.

Kumari, B., A. Guha, M.G. Pathak, T.C. Bora and M.K. Roy, 1998. Experimental biofilm and its application in malathion degradation. Folia Microbiol., 43: 27-30.

Maleszewsk, J., 1974. Degradation of methyl parathion by microorganisms occurring in surface water and sewage. Pol. Arch. Hydrobiolog II, 163-171.

Masumura, F. and G.M. Boush, 1968. Degradation of insecticides by soil fungus *Trichoderma viride*. J. Econ. Entom., 61: 610-612.

Muan, B. and J.U. Skare, 1989. A method for determination of the main metabolites of malathion in biological samples. J. Agric. Food Chem., 37: 1081-1085.

Nelson, L.M., 1982. Biologically- induced hydrolysis of parathion in soil. Isolation of hydrolyzing bacteria. Soil Biol. Biochem., 14: 219-222.

Rahmatullah, M., M.S. Ali, H.K.M. Yusuf and J. Rownak, 1979. ATP-induced reversal of malathion inhibition of citric acid fermentation with *Aspergillus niger*. J. Ferment. Technol., 57: 379-381.

Rani, N.L. and D. Lalithakumari, 1994. Degradation of nmethyl parathion by *Pseudomonas putida*. Can J. Microbiol., 40(12): 1000-6.

Richins, R.D., I. Kaneva, A. Mulchandani and W. Chen, 1997. Biodegradation of organophosphorus pesticides by surface-expressed organophosphorus hydrolase. Nat. Biotechnol., 15: 984-7.

Rogers, K.R., Y. Wang, A. Mulchandani, P. Mu1chandani and W. Chen., 1999. Organophosphorus Hydrolase-Based Assay for Organophosphate Pesticides. Biotechnol. Prog., 15: 517-521.

Rosenberg, A., M. Alexander, 1979. Microbial cleavage of various organophosphorus insecticides. Appl. Environ. Microbiol., 37: 886-91.

Roy, M.K., A. Guha, B. Kumari, T.C and Borta, 1995. Pesticide degradation characteristics, plasmid profile and degradative efficiency of facultative anaerobes. Intern at.Symp in Microbiology, Calcutta (India), 62.

Serdar, C.M., 1996. Hydrolysis of cholinesterase inhibitors using parathion hydrolase. U.S. patent, 5: 589-386.

Shimazu, M., A. Mulchandani and W. Chen, 2001. Simultaneous degradation of organophosphorus pesticides and p-nitrophenol by a genetically engineered *Moraxella* sp. with surface-expressed organophosphorus hydrolase. Biotechnol. Bioeng., 76: 318-324.

Singh, A.K. and P.K. Seth, 1989. Degradation of malathion by microorganisms isolated from industrial effluents. Bull. Environ. Contam. Toxicol., 43: 28-35.

Walker, W.W. and B.J. Stojanovic, 1973. Microbial versus chemical degradation of malathion in soil. J. Environ. Qual., 2: 229- 232.

Walker, W.W. and B.J. Stojanovic, 1974. malathion degradation by *an Arthrobacter sp*. J. Environ. Qual., 3: 4-10.

Zhongli, C., L. Shunpeng and F. Guoping, 2001. Isolation of methyl parathion-degrading strain M6 and cloning of the methyl parathion hydrolase gene. Appl. Environ. Microbiol., 4922-4925.