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Enhancement of Diazinon Degradation, Isolation of Organophosphorus Hydrolase and Chemical Analysis of Metabolites

Nivien A. Abosereih[1*], Ghada M. El-sayed[1], S. A. Ibrahim[2], A. B. Abd El-Razik[2], M.A. Hammad[3] and F. M. Hafez[1]

[1]*Department of Microbial Genetics, National Research Centre, 33 El-Bohouth St., Dokki, Giza 12622, Egypt.*

[2]*Department of Genetics, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.*

[3]*Department of Plant Protection, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.*

IN THIS study, Pseudomonas aeruginosa strain GH2NO8 showed effective activity in diazinon degradation. This bacterial strain was able to degrade 38.19 of diazinon as compared to 1.83% in control media after 5 days. oph gene that encodes a protein involved in diazinon hydrolysis was isolated and sequenced. It is a member of the MBL-fold metallo hydrolase superfamily and has beta lactamase fold. Moreover, it has 99% similarity with that of Pseudomonas aeruginosa strain FDAARGOS. Partial DNA sequence of oph gene was deposited in Genbank database under accession number MF443870. PAM8 and PAMS9 were the best mutants in diazinon biodegradation after ethylmethane sulphonate (EMS) mutation induction for Pseudomonas aeruginosa strain GH2NO8 resulted from first step and second step EMS mutation induction, respectively. PAM8 was able to degrade 62.19 of diazinon as compared to 38.19 % in wild type after 5 days. However PAMS9 exhibited the ability to degrade 86.21% %. 2-Iso-4-methyl-6- hydroxypyrimidine (IMHP) was detected as the main degradation product of diazinon through GC/MS analysis after 10 days of incubation which was further metabolized to unknown polar metabolites. High diazinon tolerance and degradation capability of the Pseudomonas aeruginosa strain GH2NO8 and its higher mutants make these strains suitable for decontamination and bioremediation of pesticides contaminated sites.

Keywords: OPs pesticides biodegradation, Diazinon, EMS, GC/MS analysis.

Introduction

Pesticides use is considered the most important factor contributing to the massive increase in food production worldwide and their applications have been an environmental concern for the past several decades[1] but their widespread use has caused environmental pollution ecology[2,3]. Organophosphate (OPs) compounds are highly potent neurotoxins that are commonly used as pesticides. These compounds inhibit acetylcholine esterase and disrupt the normal function of central nervous system followed by severe muscle paralysis and death [4]. OPs are much more toxic to vertebrates compared to other of insecticides classes [5].

Diazinon (DZ) (O,O-diethyl-O-[2-isopropyl-6-methyl-4-pyrimidinyl] phosphorothioate) is an organophosphate pesticide which is extensively used to control household insects and vegetable crops. The exposure to this pesticide has been

*Corresponding author e-mail: nivienabdelrahman@yahoo.com
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linked to the development of the serious problem in several experimental animals; moreover, the contamination of food by DZ may increase its danger to human so that elimination and degradation of these pesticides is urgent issue. Chemical treatments, incineration and landfills are the current methods in OPs detoxification [6]. Chemical methods are problematic due to production of large volumes of acids and alkali subsequently must be disposed. Incineration although is considered the most reliable method for OPs destructions has involved a serious public opposition due to the potentially toxic emissions [7]. Landfills function adequately but leaching of pesticides into ground water supplies and surrounding soil is a big issue of concern. One of the main methods to resolve the problems of (OPs) residues accumulation is to bioremediate these compounds [8,9]. Many bacterial isolates have been reported to be potent in pesticides biodegradation such as *Flavobacterium* sp. [10], *Arthrobacter* sp. [11] and *Pseudomonas diminuta* [12]. Mutations induction using chemical and physical mutagens still have an effective role in improvement of bacterial production improvement. The advantages of mutagenesis using UV irradiation and chemical mutagens such as ethylmethane sulfonate (EMS) are still preferable due to their simplicity and low cost procedure [13]. EMS was widely used by the following studies for different enzymes improvement from different microorganisms for example Ho and Chor[13] used improved xylanase production from *Bacillus subtilis*. Afifi et al. [14] improved alkaline protease production by *Penicillium chrysogenum*, Adsul et al. [15] increased cellulase production from *Penicillium janthinellum*. Jayaraman and Ilyas[16]improved lipase production from *Pseudomonas* sp. and Suribabu et al. [17] used EMS to enhance α-amylase production from *Brevibacillusborostelensis*.

In this study, it is the first time to improve the organophosphates bacterial degradation by random mutation using EMS. Organophosphorus hydrolase (OPH) enzyme involved in organophosphates degradation is encoded by *oph* gene, *mpd* gene and/or *opd* gene in different unrelated bacterial strains. These genes have no DNA sequence similarity [18].

The objectives of this study are to identify and characterize *oph* gene responsible for diazinon biodegradation in *Pseudomonas aeruginosa* strain GH2NO8 and its higher mutant. Improving the ability of this strain for diazinon biodegradation by chemical mutation induction using ethylmethane sulphonate (EMS) was the second goal.

Materials and Methods

Reagents and chemicals

Analytical grade diazinon (60%) emulsifiable concentrate was purchased from (Sinochem Agro.Co.Ltd, China). All reagents and solvents used in the present study were of analytical grade. Diazinon was used as organophosphorus insecticide model due to its high residues in Egyptian soils [19].

Evaluation of diazinon biodegradation by Pseudomonas aeruginosa strain GH2NO8

Pseudomonas aeruginosa strain GH2NO8 was isolated, molecularly identified in microbial genetic department, national research Centre by Ghada et al. [20] and was inoculated in broth minimal salt media [21]containing diazinon with a concentration of 60 mg/l as a sole carbon source and incubated on an orbital shaker (Thermoscientific, UK) at 30^{O}C for 5, 10 and 15 days at 150 rpm and samples were done in triplet [22]. At the same time Non-inoculated media were also run in parallel to the other cultures as control. Extraction of diazinon residues was done as follow; a known volume of a mineral salt liquid media (MSL), 100 ml was transferred into 500 ml separatory funnel and partitioned successively three times with 50 ml dichloromethane each and 40 ml of sodium chloride solution (20%). The combined extracts were filtered through a pad of cotton and anhydrous sodium sulfate then evaporated at 30^{O}C to dryness using a rotary evaporator at 30^{O}C, then the residue was quantitatively transferred to standard glass stopper test tube with ethyl acetate, and the solvent was evaporated to dryness [19]. Diazinon residue and its metabolites were determined using Gas Chromatography analysis in the Central Agriculture Pesticides Lab (CAPL), Agriculture Research Center, Giza, Egypt. At the same time, 1 ml of bacterial culture was used to make serial dilutions 10^{-4} to 10^{-6} and plated on LB agar plate. Plate was incubated at 30^{O}C for 18 h and a single colony was subjected to DNA extraction and EMS mutation induction.

Data calculation

$$\text{Degradation (\%)} = \left(\frac{\text{Residual amount in blank control} - \text{Residual amount in sample}}{\text{Residual amount in blank control}}\right) \times 100$$

Ethylmethane sulfonate (EMS) mutation induction of Pseudomonas aeruginosa strain GH2NO8 and mutant selection

First step EMS mutation induction was done as follow; one ml of 18 h old culture was centrifuged at 12,000 rpm for 5 min. The pellet was washed with and dissolved in 1ml of 100 mM sodium phosphate buffer pH 7. 100 µl of sample content was withdrawn to determine the initial population (cfu/mL). 20 µl/mL of EMS stock solution 1gm/ml (Merck) was added to the samples in falcon tubes for different times 20, 40 and 60 minutes then incubated at 30^{O}C. The reaction was stopped by addition of 4 ml of sodium thiosulfate (5%). The reaction was centrifuged; pellets were washed and resuspended in sodium phosphste buffer. Portions of 0.1 mL of suitable dilutions were spread on LB agar plates and incubated at 30^{O}C for 48 h. Bacterial colonies developed after incubation were counted and the survival percentages were estimated for each treatment [23]. Second step EMS mutation induction was employed for the best mutant resulted from first step EMS mutation induction in the same manner mentioned earlier. In this study, the results of improved mutants are directed primarily based on their potential in diazinon biodegradation compared to their wild strain.

Extraction of genomic DNA from Pseudomonas aeruginosa strain GH2NO8 best mutant

A Single colony was cultured in conical flask (Pyrex, USA) containing 20 ml LB medium by shaking in an orbital shaker (Thermo fisher scientific, UK) at 150 rpm for 18 h. The culture was centrifuged at 13,000 rpm for 5 min at 4^{O}C. The pellet was subjected to genomic DNA extraction using the (QIAamp DNA Mini Kit, QIAGEN, Germany). The extracted DNA was used as a template for PCR to amplify *oph* gene in *Pseudomonas aeruginosa* strain GH2NO8 and its best mutants.

Identification and partial amplification of oph gene; the gene that might be responsible for diazinon degradation

DNA sequences of *oph* gene in *Pseudomonas aeruginosa* strain GH2NO8 was predicted based on the conserved domain database (CDD) of NCBI (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi. Fan et al. [24] succeeded to identify a novel gene, the *cpd* gene of putative esterase involved in chlorpyrifos biodegradation. It was identified and cloned by analysis of genomic sequence of *Paracoccus* sp. TRP and the prediction of ORFs was estimated based on the conserved domain of deduced protein CPD predicted by conserved domain database (CDD) of NCBI. The *oph* gene of putative organophosphorus hydrolase (OPH) enzyme was detected by analysis of genomic sequence of *Pseudomonas aeruginosa* isolate B10W, complete genome genbank CP017969, region (556862 to 557728) was used to design the forward primer GH2NO8-F (5´CAACAGCCAGAAACTCGACG´3) and the reverse primer GH2NO8-R (5´GGTGTCGTAGAGGCTGTAGC´3).

Results and Discussion

Evaluation of diazinon biodegradation by Pseudomonas aeruginosa strain GH2NO8

Pseudomonas aeruginosa strain GH2NO8 showed an effective activity in diazinon degradation. Figure 1 shows that this bacterial strain was able to degrade 38.19% after 5 d and 91.82% after 20 d of diazinon as compared 1.03% after 5 and 3.05% after 15 d in control media (Fig. 2).

First Step EMS Mutation Induction in Pseudomonas aeruginosa strain GH2NO8.

Despite genetic engineering has made a significant contribution to the improvement of bacterial enzymes, but random mutagenesis is still a cost-effective procedure for reliable short-term strain development and is frequently preferred as the method of choice [25]. It is the first time to improve the organophosphorus hydrolase enzyme responsible for diazinon biodegradation by random mutation using ethyl methanesulfonate (EMS) as a chemical mutagen. After induction of EMS mutation for *Pseudomonas aeruginosa* strain GH2NO8 for different treatment periods, viable colonies were counted from appropriate dilution, survival percentage was calculated and the results were depicted in Fig. 3. It was noticed that survival percentage decreased as the treatment time increased due to the lethal effect of EMS. The least survival percentage was recorded at an exposure period of 40 min. The exposure

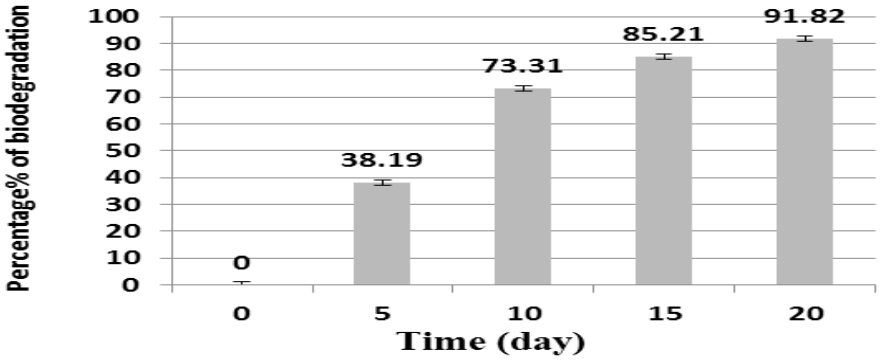

Fig. 1. Biodegradation percentage of diazinon (600 mg/l) by *Pseudomonas aeruginosa* strain GH2NO8.

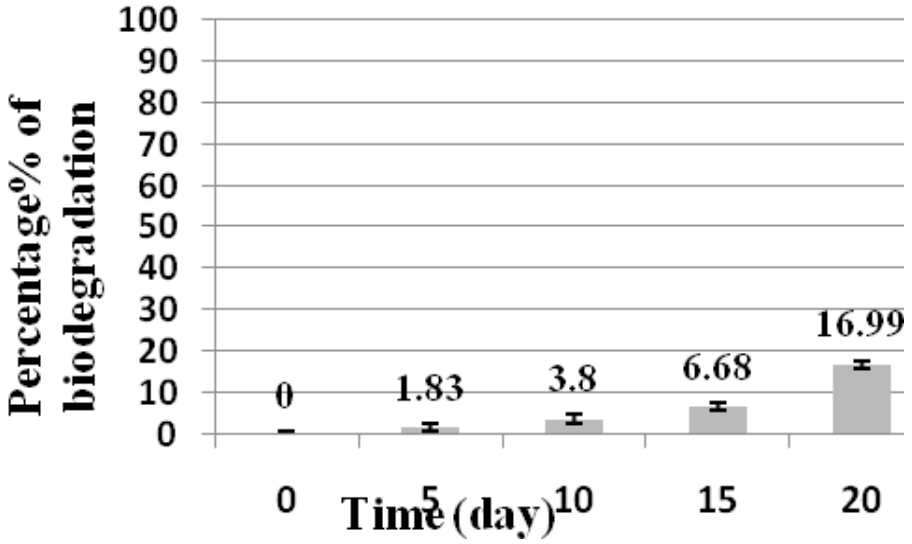

Fig. 2. Biodegradation percentage of diazinon (600 mg/l) in control media.

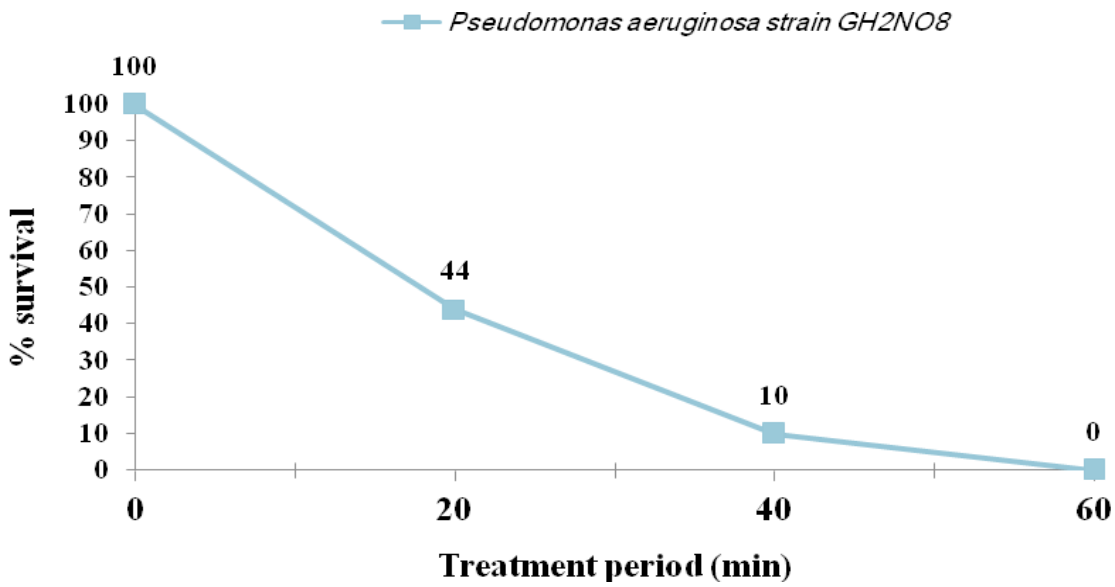

Fig. 3. Survival percentage of *Pseudomonas aeruginosa* strain GH2NO8 after EMS (20 µl/ml) treatment.

period of 60 minutes was lethal for wild strain.

After EMS treatment of *P. aeruginosa* strain GH2NO8, single colonies which showed a good growth after 48 h on LB agar plates were picked. Thirty two single colonies were picked, each selected mutant took a number, a symbol M for mutant, PA for *P. aeruginosa.* These selected mutants were allowed to grow on MSM containing diazinon as carbon source with the concentration 600 mg/l for five days and the biodegradation activity was calculated. From EMS treatment for 20 min, 21 mutants were selected, no mutant recorded lower activity than wild type, 15 mutants exhibited approximately equal activity to wild type and 6 mutants recorded biodegradation activity higher than wild type especially PAM8 which degraded 62.19% of diazinon as compared with wild type that degraded 38.19%. In the highest mutants, the increase in OPs biodegradation may be due to several reasons such as the overexpression of the gene encodes the enzyme (organophosphorus hydrolase enzyme) responsible for OPs biodegradation, increasing the efficiency of secretion system or changes in one or more amino acids of enzyme resulting in more binding efficiency between produced enzymes and their substrates and that may lead to more biodegradation activity.

Eleven mutants were selected after EMS treatment for 40 min, 7 mutants showed slightly equal activity to wild type and 4 mutants exhibited biodegradation activity less than wild type (Table 1). Indeed, mutation changes the protein structure and most probably results in the deterioration of function [26]**,** therefore, structural components changes by mutation are rarely cause improvements. Ho and Chor [13] obtained a mutant exhibited the highest xylanase overproduction reached the maximum peak of 1.638±0.027 U/mL after treatment of *B. subtilis* with 100 µg/mL of EMS for 30 min. Results of Kotchoni et al. [27] showed that cellulase yielded by certain selected mutant was four times higher than *Bacillus pumilus* wild type after EMS treatment.

Second step EMS mutation induction

PAM8, the highest mutant in diazinon biodegradation resulted from *P. aeruginosa* strain GH2NO8 first step EMS treatment for 20 min was then mutated by EMS and their biodegradation potentials were measured as the same conditions employed in wild types.

Due to the second step of EMS treatment of PAM8 for 60 min, PAMS9 was selected as the highest mutant. It degraded 86.21% of diazinon as compared with 62.19% in case of PAM8. It included that PAM8 has been more resistant to EMS than its wild type and that because EMS mutation of 60 min was lethal for *P. aeruginosa* strain GH2NO8, on the contrast, this dose did not behave the same effect on mutant PAM8. The increase in biodegradation activity might be due to

TABLE 1. Effect of EMS Induction on Biodegradation of diazinon by *Pseudomonas aeruginosa* strain GH2NO8.

Treatment exposure time (min)	Bacterial isolates	*dry weight g/100 ml	*% diazinon biodegradation	Bacterial isolates	*dry weight g/100 ml	*% diazinon biodegradation
20	wild type	0.281	34.23	PAM11	0.190	33.99
	PAM1	0.163	33.90	PAM12	0.170	33.98
	PAM2	0.160	33.90	PAM13	0.187	34.09
	PAM3	0.159	34.50	PAM14	0.180	34.08
	PAM4	0.165	36.89	PAM15	0.180	63.20
	PAM5	0.156	33.99	PAM16	0.175	33.89
	PAM6	0.158	36.07	PAM17	0.170	33.44
	PAM7	0.190	37.13	PAM18	0.180	38.87
	PAM8	0.179	62.19	PAM19	0.174	37.08
	PAM9	0.179	53.33	PAM20	0.170	37.97
	PAM10	0.189	49.80	PAM21	0.177	38.98
40	PAM22	0.170	38.58	PAM28	0.170	39.80
	PAM23	0.170	37.43	PAM29	0.164	37.81
	PAM24	0.169	39.00	PAM30	0.167	15.90
	PAM25	0.170	38.75	PAM31	0.171	39.03
	PAM26	0.093	21.09	PAM32	0.170	19.08
	PAM27	0.173	20.60			

*Mean of three replicates.

stabilizing the structure of the enzyme and promote the interactions between enzyme and substrate.

This study generated efficient OPs-degrading enzymes contributing to increasing in OPS detoxification. Xie et al. [28] used (error-prone PCR), which is by far the most popular random mutagenesis method to evolve MPH (methyl parathion hydrolase) and improve its efficiency in chlorpyrifos hydrolysis for decontamination of OP compounds. Single, double and triple mutants of BjMPH (methyl parathion hydrolase) in *Burkholderia jiangsuensis* MP-1 were constructed by site-directed mutagenesis [29]; they identified a new mutant, BjMPHT64N, exhibiting 3.78-fold higher catalytic efficiency (k_{cat}/K_M) towards MP (methyl parathion) than its wild-type, reaching $4.20 \times 106M^{-1}s^{-1}$.

This study achieved improved OPH (organophosphorus hydrolase) variants with increased activity towards poorly hydrolysed substrate such as diazinon by random mutation using EMS treatment approach which leads to an enzymatic solution. Figure 4 shows the growth of *Pseudomonas aeruginosa* strain GH2NO8 and its higher mutants

Evaluation the behavior of Pseudomonas aeruginosa strain GH2NO8 and its higher mutants in OPs degradation through time shift

Biodegradation of diazinon by wild *P. aeruginosa* strain GH2NO8and their selected higher mutants, PAM8 and PAMS9 were identified after 5, 10 and 15 days (Fig. 5). This procedure was done to determine the most effective time at which the highest biodegradation occurred compared with other time shift. There was noticeable increasing in diazinon biodegradation after 5 days in PAM8 and PAMS9 which degraded 62.19% and 86.21% of diazinon, respectively as compared with 39.03% in case of *P. aeruginosa* strain GH2NO8. It was noticed that the biodegradation rate of wild strains and their mutants decreased after 10 days, in the other words, the highest degradation occurred through the first 10 days of incubation, this may be due to the accumulation of diazinon metabolites which have antimicrobial activity [30,31]. It included that biodegradation achieved by wild strains into 20 days could be achieved into 10 days by their higher mutants so that these mutants are suggested to be candidates for OPs detoxification in Ops contaminated environmental niches.

Identification of the degradation products of diazinon by PAMS9 using GC/MS

In this study, it is important to identify the end product of diazinon degradation by higher mutant, PAMS9. It was used as a model strain to detect diazinon metabolites. Diazinon insecticide is a phosphorothionate moiety which belongs to the main chemical group of organophosphorus [30]. 2-Iso-4-methyl-6- hydroxypyrimidine (IMHP) was detected as the main degradation product of diazinon through GC/MS analysis after 5 days of incubation (Fig.6). Retention time for IMHP as the main degradation product was detected at Rt 20.3200 min with molecular formula of C8H12 N2O and molecular weight of 152.1 kDa. The base peak at m/z 137 in the mass spectrum of IMHP corresponded to the pyrimidine species produced following the loss of a methyl radical from the molecular ion of m/z 152. Similarly diazinon was detected at Rt 26.449 min and it had molecular formula C12H21N2O3PS with molecular weight of 304.3 kDa these peaks disappeared concomitantly with formation of other new peaks with a retention time of around 41.38. Subsequently, the hydrolysis product, IMHP was further transformed by ring breakage, resulting in its detoxification. The degradation pathway for diazinon by PAMS9 was proposed in Fig. 7. Each peak was identified on the basis of its

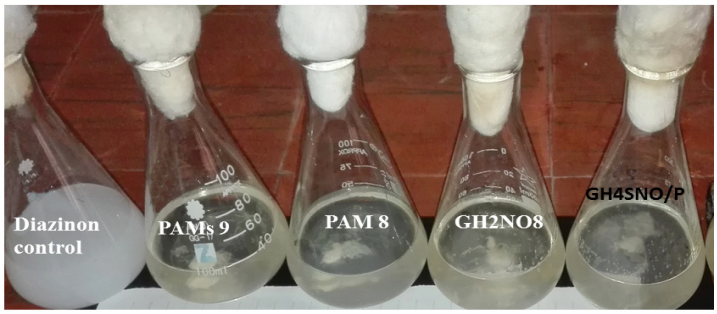

Fig. 4. Bacterial growth of *Pseudomonas aeruginosa* strain GH2NO8 and its higher mutants on liquid MSM containing diazinon (600 mg/l) after 5 days. GH2NO8: wild strain of *Pseudomonas aeruginosa*. PAM8: The best mutant from first step EMS mutation induction. PAMS9: The best mutant from second step EMS mutation induction.

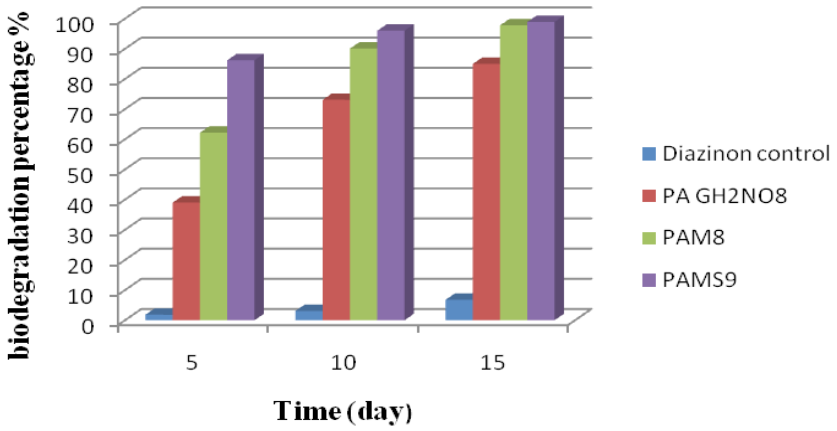

Fig. 5. Diazinon biodegradation by *Pseudomonas aeruginosa* strain GH2NO8 and its higher mutants.

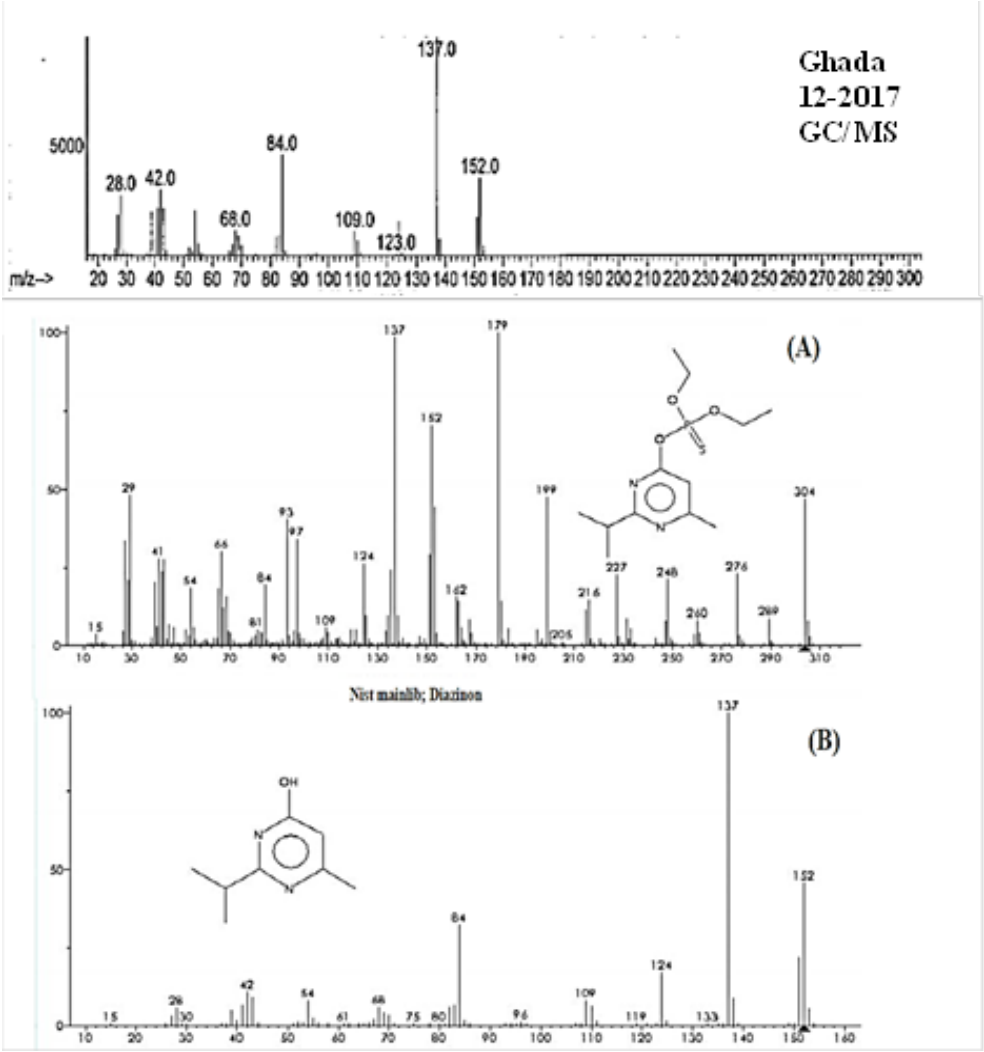

Fig. 6. Mass spectra of 2-Iso-4-methyl-6- hydroxypyrimidine (IMHP) produced from diazinon degradation byPAMS9; Ghada sample. A: authentic standard diazinon from the National Institute of Standards and Technology (NIST, USA) library database. B: authentic standard IMHP from the National Institute of Standards and Technology (NIST, USA) library database.

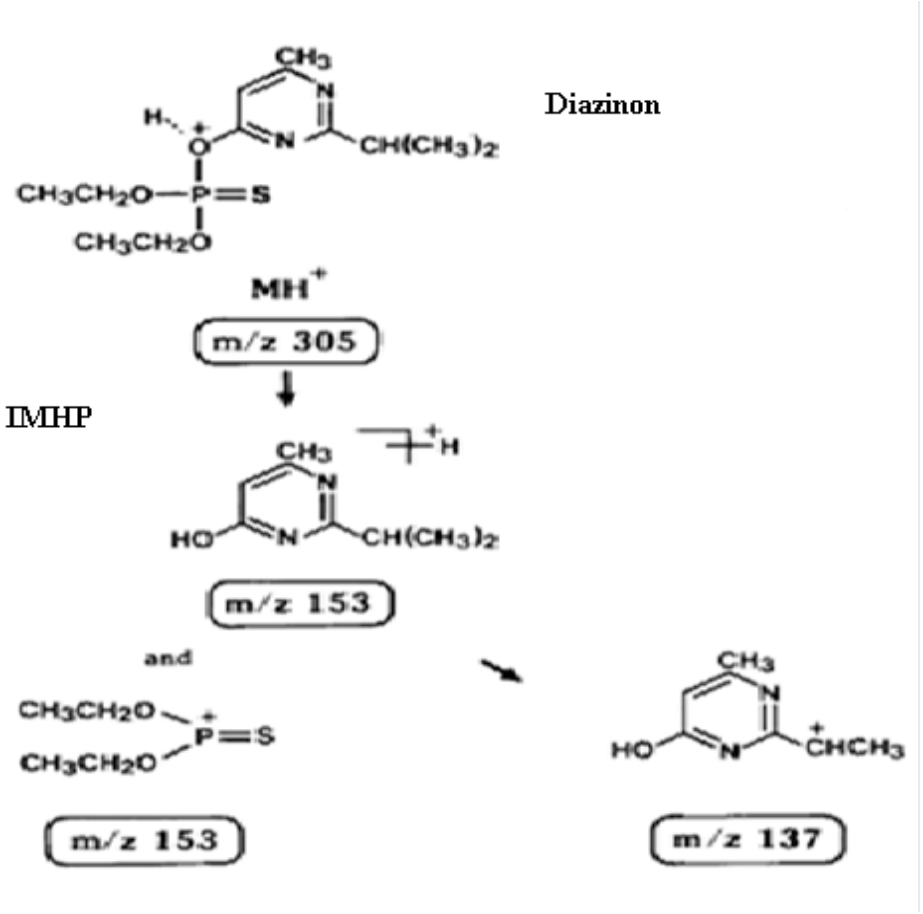

Fig. 7. The proposed pathway for the diazinon degradation by PAMS9.

mass spectra and the NIST library identification program.

Previously, scientists have reported that 2-isoprophyl-4-methyl-6 hydroxypyrimidine as the degradation product of diazinon is less toxic than diazinon [30]. He succeeded in detection of diazinon end product biodegradation by *Bacillus licheniformis* by GC/MS analysis, Similarly IMHP, the main degradation product was detected at Rt 10.06 min. with molecular formula of C8H12N2O and molecular weight of 152.19 kDa. It is known that diazinon released into the environment is moderately persistent and mobile [32, 33] and its application to soils showed that it is not likely to adsorb on soils. It was found to be mobile in 80% of the tested soil samples. This can enhance the leaching of diazinon, especially in light-textured soils with low organic matter content [34]. Microbial degradation in soils is the primary route of diazinon dissipation from the environment. Diazoxon is the primary product of degradation in case of diazinonhydrolysis. However, diazoxon is rapidly hydrolyzed to oxypyrimidine which is more mobile in the environment than the parent compound [34].

Identification and partial sequencing of oph gene; the gene that might be responsible for OPs degradation in PAMS9

Firstly, identification and partial sequencing of *oph* gene in PAMS9 as the higher mutant were done to compare it with that of *Pseudomonas aeruginosa* strain GH2NO8 as a wild type. *Oph* gene in *Pseudomonas aeruginosa* strain GH2NO8 was detected and partially sequenced [20]. The same molecular length,~810 bp and partial sequence of *oph* gene in both *P. aeruginosa* strain GH2NO8 and PAMS9 were found (Fig. 8) and were deposited in Genbank database under the accession numbers MF443870.

Analysis of oph gene partial sequences using bioinformatics tools

It was analyzed by conserved domain database (CDD) of NCBI (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi), the result is shown in Fig. 9 which illustrates that conserved domains

is a member of the metallo hydrolase super family (MBL-fold) and has beta lactamase fold which is mainly hydrolytic enzyme involved in organophosphates hydrolysis. Organophosphorus hydrolase (*oph)* and methyl parathion degrading (*mpd*) genes have β-lactamase fold rather than TIM-barrel fold which is found in organophosphorus degrading (*opd*) genes [35]. Moreover (OPH and MPH) enzymes encoded by *oph* and *mpd* genes respectively are a homodimer; each monomer interacts as a αβ/βα sandwich and atypical of the β-lactamase fold is involved. Although these proteins have dissimilar sequences and protein folds, they share common features with PTE (phosphotriesterase) in that the active site is made up of three hydrophobic pockets [18]. The conserved domain of these isolated genes is the same one present in *Pseudomonas pseudoalcaligenes* OPHC2 which is a thermostable organophosphorus hydrolase and hydrolyzes various phosphotriester, esters, and lactone. This subgroup also includes *Pseudomonas oleovorans* PoOPH which exhibits high lactonase and esterase activities and latent PTE (Phosphotriesterase) activity. No doubt that Prediction of specific gene function based on analysis of the conserved domain present in the deduced protein by using database (CDD) of NCBI that is an effective bioinformatics tool [24]. He succeeded to identify a novel gene, the *cpd* gene of putative esterase involved in chlorpyrifos biodegradation. It was identified and cloned by analysis of genomic sequence of *Paracoccus* sp.TRP and the prediction of ORFs was estimated

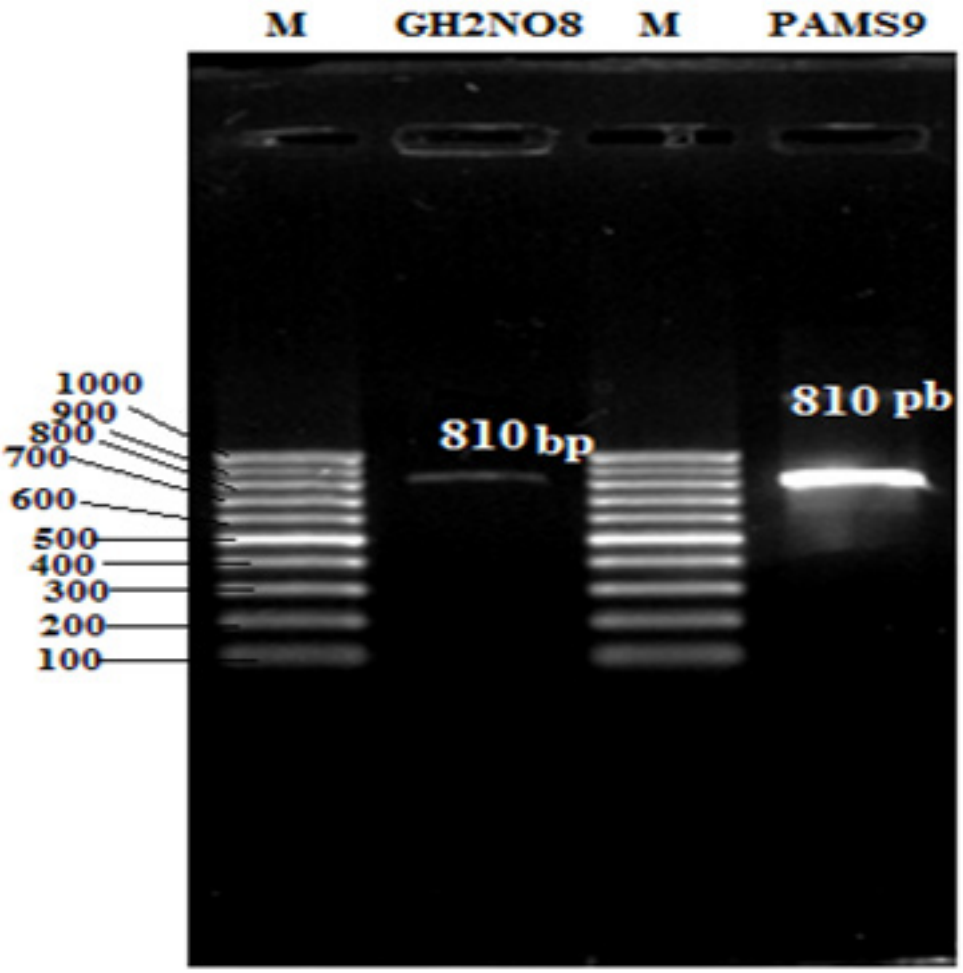

Fig. 8. Agarose gel electrophoresis for PCR product of oph gene, M: 100 bp DNA ladder (Jenabio). GH2NO8: *Pseudomonas aeruginosa*. PAMS9: the best mutant in diazinon biodegradation.

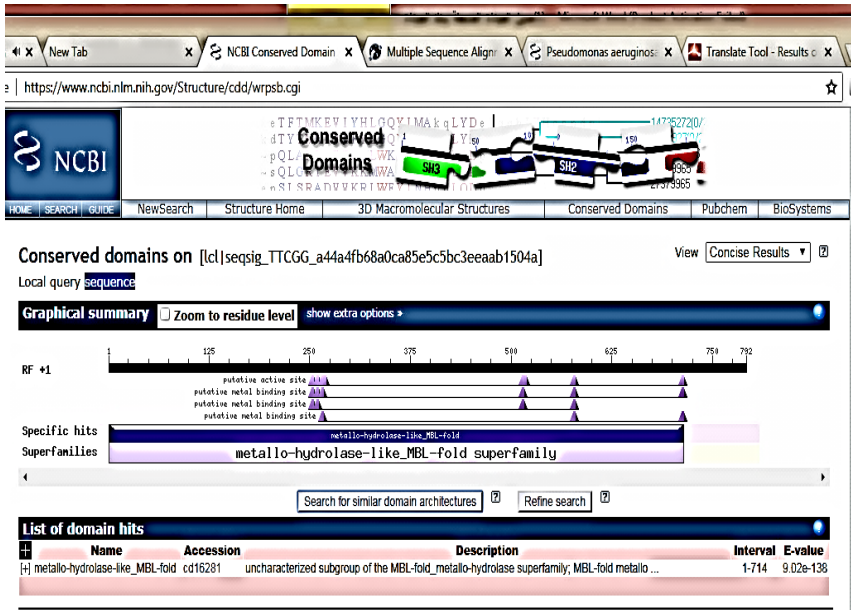

Fig. 9. Predicted conserved domain in PAMS9 *oph* gene based on conserved domain database (CDD) of NCBI.

based on the conserved domain of deduced protein CPD predicted by conserved domain database (CDD) of NCBI.

Conclusion

This study demonstrated that an effective activity in diazinon degradation was shown by *Pseudomonas aeruginosa* strain GH2NO8. As the EMS treatment time increased, Survival percentage decreasedand that due to the lethal effect of it. PAM8 as the highest mutant resulted from the first EMS mutation induction degraded 62.19% of diazinon as compared with wild type that degraded 38.19%. Due to the second step of EMS treatment of PAM8 for 60 min, PAMS9 as the highest mutant was selected, it degraded 86.21% of diazinon, thus generated efficient OPs-degrading enzymes contributing to increasing in OPS detoxification. The highest diazinon biodegradation occurred through the first 10 days of incubation. 2-Iso-4-methyl-6-hydroxypyrimidine (IMHP) was detected as the main degradation product of diazinon through GC/MS analysis after 5 days of incubation in PAMS9.*Oph*gene with ~ 810 bp was isolated in *P. aeruginosa* strain GH2NO8 and PAMS9 and the partial sequence was deposited in Genbank database under the accession numbers MF443870. *Oph*enzymeis a homodimer; each monomer interacts as a αβ/βα sandwich and atypical of the β-lactamase fold is included.

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تعزيزتحلل الديازينون، عزل الجين المشفر لانزيم الفسفور العضوى الهيدروليزى والتحليل الكيميائى لنواتج الأيض

نيفين عبدالرحمن أبوسريع[1]، غادة مصطفي السيد[1]، سمير عبد العزيز إبراهيم[2]، أشرف بكرى عبد الرازق[2]، ماهر عبد العليم حماد[3]، فاطمة ممدوح حافظ[1]

[1]قسم الوراثة الميكروبية - المركزالقومى للبحوث - 3 شارع البحوث - 12622 الدقى - الجيزة - مصر.

[2]قسم الوراثة - كلية الزراعة - جامعة عين شمس - القاهرة - مصر.

[3]قسم وقاية النبات - كلية الزراعة - جامعة عين شمس - القاهرة - مصر

فى هذه الدراسة تم تحسين قدرة *Pseudomonas aeruginosa* strain GH2NO8 عن طريق تعريضها للمطفر الكيميائى (EMS) لفترات مختلفة (٢٠، ٤٠، ٦٠) دقيقة وهذه هى الخطوة الأولى من مرحلة التطفير حيث لوحظ انخفاض نسبة البقاء للمستعمرات البكتيرية النامية بعد تعريضها للمطفر مع زيادة مدة التعرض. بعد تعرض سلالة *Pseudomonas aeruginosa* لمدة ٢٠ دقيقة تم الحصول على PAM8 كأفضل طفرة تحلل ٦٢,١٩ ٪ من الديازينون مقارنة بالطراز الاصلى الذي يحلل ٣٤,٢٣ ٪ بعد خمسة أيام من التحضين. بنفس الطريقة تم تطفير سلالة PAM8 ،حيث تم الحصول على PAMs9 كأفضل طفرة بعد التعريض لمدة ٦٠ دقيقة ، حيث قامت بتحليل ٨٦,٢١ ٪ من الديازينون بالمقارنة مع ٦٢,١٩ ٪ من في حالة PAM8 بعد خمسة أيام من التحضين. تم عزل، تحديد، ومعرفة التتابع النيوكليوتيدى الجزئى لجين (oph) فى سلالات *Pseudomonas aeruginosa* وسلالة PAMs9 كأفضل طفرة، حيث سجل طول الجين 810 نيوكلبوتيدة فى كلا السلالتين وبنفس التسلسل. تم الكشف عن ٢-Iso-٤-methyl-٦- hydroxypyrimidine (IMHP) كناتج التدهورالرئيسي للديازينون من خلال التحليل GC / MS بعد ١٠ أيام من التحضين للطفرة PAMs9. حيث ينهار هذا الركب إلى مركبات غير قطبية أقل سمية. وبذلك يمكن القول بأن استخدام (EMS) كعامل مطفر طريقة فعالة للحصول على طفرات لها قدرة أعلى فى التحليل الحيوي للديازينون على النقيض من التقارير الأخرى عن التأثيرات السمية لتلك المبيدات على الكائنات الحية الدقيقة الأخرى المتنوعة.