Full Length Research paper

Assessment of detoxification of malathion by *Pseudomonas* isolates

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Malathion degrading bacteria were isolated from a soil sample. Growth of mono and co-cultured strains of *Pseudomonas* in media containing the organophosphate insecticide, malathion as sole source of carbon was employed to assess degradability of the bacteria. On the seventh day of inoculation, bacteria free culture fluids as well as un-inoculated fluids were analyzed by gas chromatography and mass spectrometry (GC-MS). Malathion was not detected in the cell free culture fluids, instead four degradation products namely phoratoxon sulfone, malaaxon, butanedioic acid and ethyl methyl-methyl phosphonate made their appearance in the cultivation culture. All of the three strains used in this study, appeared efficient in degrading the insecticide especially in co-culturing conditions. These bacteria are promising for detoxifying the insecticide contaminated soils.

Key words: *Pseudomonas*, detoxification, malathion.

INTRODUCTION

Malathion [diethyl 2-((dimethoxyphosphorothioyl) sulfanyl) butanedioate] is a ten carbon compound used widely in agriculture and in public health pest control programs (US EPA, 2013), while in US, it is the most commonly used organophosphate insecticide (Bonner et al., 2007).

Following application, malathion may be detected on plant surfaces up to 9 weeks (Delmore and Appelhans, 1991), yet with low persistence (Extoxnet, 2000) and rapid degradation in soil (Howard, 1991; Brenner, 1992). It is readily metabolized into even more toxic malaaxon in human body following absorption or injection. Long-term oral exposure of malaaxon in rats has been found to be 61 times more toxic than malathion (Edwards, 2006). Despite lack of reliable information on adverse health effects of malathion (ATSDR, 2008), it has been found to induce oxidative stress in erythrocytes and alteration of the cellular antioxidant defense system (Durak et al.,

2009). However, it is cleared from the body in three to five days (Maugh, 2010).

Bacteria capable of malathion degradation have been well documented by various workers (Shan et al., 2009; Hamouda et al., 2013). This paper reports degradability of *Pseudomonas* isolates from chronically insecticide influenced soil.

MATERIALS AND METHODS

Three strains of *Pseudomonas* were isolated from soil in the vicinity of an insecticide formulating unit on a selective medium. This medium contained commercial grade malathion (50%) as sole source of carbon as well as K₂HPO₄ (dipotassium hydrogen phosphate), MgSO₄ (magnesium sulfate), NH₄NO₃ (ammonium nitrate), FeSO₄·7H₂O (hydrated ferrous sulfate), CaCl₂ (calcium chloride), copper nitrate, MnCl₂ (manganese chloride),

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Figure 1. Chromatogram showing the presence of some molecules (a) Malathion (b) Butanedioic acid (c) Phoratoxon Sulfone and (d) Ethylmethyl methyl phosphonate.

zinc powder and agar (Andleeb et al., 2013). This medium was inoculated by mono and co-cultures of bacterial isolates at their optimum growth conditions with 5% inoculum size for *Pseudomonas aeruginosa* strain MY 06 and 10% inocula size for *P. aeruginosa* and *P. aeruginosa* strain SWD. Cultured fluids as well as un-inoculated control were sampled on seventh day of inoculation filtered through 0.2 μ m Millipore filters (Sartorius). The filters were analyzed by using technique GC-MS, available in HEJ (Hussain Ebrahim Jamal), Research Institute of Chemistry, University of Karachi. The column DB-5(optima-5) 30 m x 0.25 mm was used with injection volume of 1.00 μ l and flow rate of 1.00 ml/min. Start temperature was 60°C for 5 min and end temperature was 280°C for 1 min. Retention time was fixed at 15-35 min for detection of different compounds. An Agilent 6890 NGC was interfaced with a VG Analytical 79-250s double focusing mass

spectrometer. MS operating conditions were ionization voltage 70 eV and ion source 250°C. GC was fitted with a 30 m x 0.32 mm fused capillary silica column coated with DB-5. Data were acquired by means of GC solution software (Shimadzu). Detection and identification of the insecticide and its metabolites produced as a result of bacterial metabolism was accomplished by consulting and comparing data with Standard Molecule library available at Department of Chemistry, Government College University, Lahore (Figure 1).

RESULTS

The GC/MS analysis detected the presence of malathion

Figure 1. Contd.

in untreated samples (Figures 2 and 3), whereas malaoxon, butanedioic acid, phoratoxon sulfone and ethyl-methyl phosphonate were detectable metabolites in the fluids obtained after bacterial growth (Table 1; Figures 4 to 6).

As can be seen from the Table 1 the isolate *P. aeruginosa* yielded the four metabolites while the isolates designated as *P. aeruginosa* MY06 detoxified malathion into ethyl methyl methyl phosphonate. The isolate *P. aeruginosa* SWD also yielded only one metabolite, that

Figure 2. Chromatogram (GC-MS) showing detection of malathion in technical grade used in the study.

Figure 3. Chromatogram showing GC-MS of 0.5% malathion un-inoculated (control).

Table 1. Metabolites produced by bacterial isolates while growing for 7 days in selective medium containing 0.5% malathion.

Bacterial Isolate	Metabolites			
	Malaoxon (Mol. wt. 314)	Butanedioic acid (Mol. wt. 302)	Phoratoxon sulfone (Mol. wt. 276)	Ethyl methyl methyl- phosphonate (Mol. wt.138)
<i>P. aeruginosa</i>	+ve	+ve	+ve	+ve
<i>P. aeruginosa</i> MY06	-ve	-ve	-ve	+ve
<i>P. aeruginosa</i> SWD	-ve	+ve	-ve	-ve
Poly-culture of above three isolates	+ve	-ve	-ve	+ve

Figure 4. Chromatogram (GC-MS) presenting analysis of a cell-free sample of malathion (concentration 0.5%) inoculated with *P. aeruginosa* strain MY06, indicating absence of malathion and presence of ethyl methyl methyl phosphonate.

is, butanedioic acid. Under co-culture conditions (Figure 6), only malaoxon and ethyl methyl methyl phosphonate were produced.

DISCUSSION

In this study, degradation of malathion has been observed by three isolates of *Pseudomonas*. Various degradation products of malathion, malaoxon, butanedioic acid, phoratoxon sulfone and ethylmethylmethyl phosphonate

were detectable. The differential ability of the isolates to yield different sets of the four metabolites reflects their genomic diversity that appeared either in a metabolic scarcity or complete consumption of the non-detectable product by the bacterial isolate. Very interesting observation came from the results of co-culturing experiments, that is, butanedioic acid and phoratoxon sulfone were non-detectable by the analytical techniques employed. This result suggests the change in gene activity of *P. aeruginosa* and *P. aeruginosa* SWD involved in detoxification of malathion. This might have been possible due to

Figure 5. Chromatogram (GC-MS) presenting analysis of cell free sample of malathion (0.5%) inoculated with *P. aeruginosa* strain SWD indicating absence of malathion and presence of butanedioic acid.

Figure 6. Chromatogram (GC-MS) of cell-free fluid from a co-culture of strains *P. aeruginosa* MY06, *P. aeruginosa* and *P. aeruginosa* SWD.

accumulative metabolic/enzymatic potentials of the three isolates.

The present results suggest that *Pseudomonas* has consistent genetic potential that immediately becomes responsive for detoxification of varied nature of pollutants such as detoxification potential of *Pseudomonas* for naphthalene, nylon oligomer, 4-chlorophenol and toluene, respectively (Guerin and Boyd, 1995; Prijambada et al., 1995; McLaughlin et al., 2006; Duetz et al., 1996).

Yield of malaoxon by *Pseudomonas* sp. was paradoxically different from those of Bennett et al. (1984). However, other workers have reported the formation of malathion mono acid (MMA), malathion diacid (MDA) (Talebi and Shibamoto, 2007), malaoxon (Bavcon et al., 2003), diethyl thiosuccinate, O,O-dimethyl phosphorothionic acid (Pehkonen and Zhang, 2002). Varying reports on the degradation products of malathion are suggestive for considering metabolic differences of the same species' isolates and their biodegradative potential for varying situations.

Numerous studies have suggested mechanism(s) involved in metabolism of malathion by *Pseudomonas*. Matsumura and Boush (1966) and Singh et al. (1989) isolated *Pseudomonas* sp. from a contaminated soil, which rapidly degrades malathion through carboxylesteratic hydrolysis as well as desmethylation processes.

Regarding the pathway of biodegradation of malathion, hydrolysis has been found as the most common one (Kim et al., 2005). A number of metabolites of malathion formed in plants, soil and water have been formed as a result of various chemical reactions like de-esterification, oxidation and hydrolysis (Kaur et al., 1997). Presently, the detection of malathion and its degradative metabolites is done routinely and successfully with the advanced techniques like GC/FID (Bavcon et al., 2003) as well as GC/MS (Talebi and Shibamoto, 2007).

As far as the enzyme for malathion degradation is concerned, dicarboxylesterase has been found responsible both *in vivo* (Talebi and Shibamoto, 2007) and *in vitro* (Sun et al., 1992). The enzyme Carboxylesterase (Goda et al., 2010) hydrolase (OP hydrolase) has been isolated from several bacteria but with slower rate (Guha et al., 1997; Horne et al., 2002).

Further work on such lines may yield the identification of bacterial ecological relationship that might prove beneficial in designing biotransformation model with complete removal of a pollutant with no consequent generation of metabolites that may bear toxicity even greater than the original substrate.

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