Biotech Health Sci. 2014 May; 1(1): e19193.

Published online 2014 April 27. **Research Article**

Cleaning From the Inside: Biodegradation of Organophosphate Pesticides by *Pseudomonas plecoglossicida*

Abasali Borji [1]; Ghazal Naserpour Farivar [2]; Pouran Johari [3]; Taghi Naserpour Farivar [3, *]; Saeideh Senemari [2]; Gholamrea Karimi [2]

[1]Department of Microbiology, Neyshabour University of Medical Sciences, Neyshabur, IR Iran
[2]Department of Mining and Oil Engineering, Faculty of Engineering, Imam Khomeini International University, Qazvin, IR Iran
[3]Cellular and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin, IR Iran

*Corresponding author: Abasali Borji, Department of Microbiology, Neyshabour University of Medical Sciences, Neyshabur, IR Iran. Tel: +98-2813324971, Fax: +98-2813324971, E-mail: t.naserpour@qums.ac.ir

Received: February 8, 2014; **Revised:** February 22, 2014; **Accepted:** March 09, 2014

Background: Diazinon is one of the most widely used organophosphrous pesticides in the world may affect animals and human.
Objectives: This study was done to assess whether *Pseudomonas plecoglossicida* can grow on diazinon as the sole carbon metabolic source in laboratory and agricultural soil.
Materials and Methods: Serial dilutions of pure and commercial diazinon in minimal salt medium were made and 2.5 micro liters of these liquid cultures before and after inoculation with *Pseudomonas plecoglossicida* were injected to a high performance liquid chromatography column, and subsequently the bacterial growth along with the concentration of diazinon were measured by high performance liquid chromatography (HPLC).
Results: Results of this study indicated that *Pseudomonas plecoglossicida* can grow not only in Minimal salt Medium but also in the diazinon contaminated soil.
Conclusions: Our findings showed that *Pseudomonas plecoglossicida* grows in Minimal salt Medium supplemented with serial dilutions of diazinon. Furthermore, this bacterium is capable to biodegrade the residual diazinon in the agricultural soil.

Keywords: Diazinon; Pseudomonas; Biodegradation

1. Background

Organophosphate pesticides are heterogeneous compounds, containing a phosphoric acid derivative. Diazinon (0,0–dimethyl-0-2-isopropyl-6-milhylpryimidin-4-yl-phosphthionat) is one of the organophosphate pesticides which is used widely in the world (1). Diazinon and other pesticide, applied directly to the soil or gross, can be washed off into storm drains and ditches, which typically transport water to streams and lakes. Once the pesticides are distributed in the environment, they become pollutant, hence requiring remediation (2). Chemicals are broken down in soil and are degraded through the biotic and abiotic pathways. Microbial biodegradation is the primary mechanism of pesticide break down and detoxification (3, 4). Previous studies have been showed that Pseudomonas species are attractive microorganisms for bioremediation and biodegradation (5-10) and within this genus, relatively recently recognized *Pseudomonas plecoglossicida* specie has been attracted many interests (11, 12). *Pseudomonas plecoglossicida* is a soil habitant, motile, oxidative, and Gram positive bacterium which is able to grow in the presence of 0-5% (W/V) NaCl at 10-30°C (13). *Pseudomonas plecoglossicida* is also able to produce catalase and cytochromoxidase. This specie can grow on different substance as a source of carbon including many carbohydrates such as D-fructose, D-malate and glucose, many amino acids such as D-alanine and D-aspartate and many other chemicals (6).

2. Objectives

In this study, for the first time, we investigate whether *Pseudomonas plecoglossicida* can grow on diazinon as the sole carbon metabolic source.

3. Materials and Methods

Diazinon as a pesticide is used in a 4 mM concentration (Parto Nar Co. Ltd/Iran). We used a 2 mM, 4 mM and 8 mM dilutions of pure (Wenzhou Co, Zhejiang, China) and commercial diazinon in minimal salt medium (14) and 2.5 μL of these solutions were injected in High Per-

Implication for health policy/practice/research/medical education:
This study s useful for laboratory and agricultural soil.

formance Liquid Chromatography and concentrations of diazinon were measured. For a control of Minimal Salt Medium and its effect of survival of *Pseudomonas plecoglossicida*, we supplemented a minimal salt medium with 1.5% of dextrose. Also as a control of viability of bacteria, we inoculated a Lauri Bertani broth medium with *Pseudomonas plecoglossicida*. Bacterial inoculums. One loop of *Pseudomonas plecoglossicida* which was provided kindly by Dr. Faramarzi (15) was added to two 50 mL Falcon tube containing 10 mL of Minimal Salt Medium. One of these tubes was used for checking the growth curve and the other one was used for inoculation purpose. Five hundred μL from mL of above mentioned bacterial inoculating tube was added to each dilutions of Minimal Salt Medium which were containing related concentrations of pure and commercial diazinon and these tubes incubated in a shaker incubator at 37°C with 250 RPM and after 24 hours of inoculation, bacterial growth was measured by colony counting and diazinon concentrations were measured by HPLC.

High Performance Liquid chromatography conditions: HPLC conditions was previously described (16). Briefly, column: Hichrom C18 with 25 × 14 mm dimension; Mobile phase: Acetonitril (80%) _DDW(19.5%)_ Acetic Acid (0.5%); Flow rate: 1.5 mL/min; Absorbance 246 nm. We used a Cecil 1100 HPLC apparatus equipped with Power Stream Software (Waters, UK); All tests were repeated three times.

3.1. Soil

Soil was collected from a field at Ashnestan, Qazvin, Iran for environmental studies. Collected soils were ground, dried in air and sieved. Soil Spiking: In the treatment procedure, 25 mL of acetone containing diazinon was added to 250 g of sample soil and containing flasks were closed for 5 minutes to disperse the solvent. Then the flasks maintained in room temperature overnight and by this way the solvent was evaporated and the sample was mixed with remaining 750 g of soil. All samples were thoroughly mixed.Soil was spiked to reach final concentrations of diazinon at 25, 50 and 100 mg/kg dry soil.

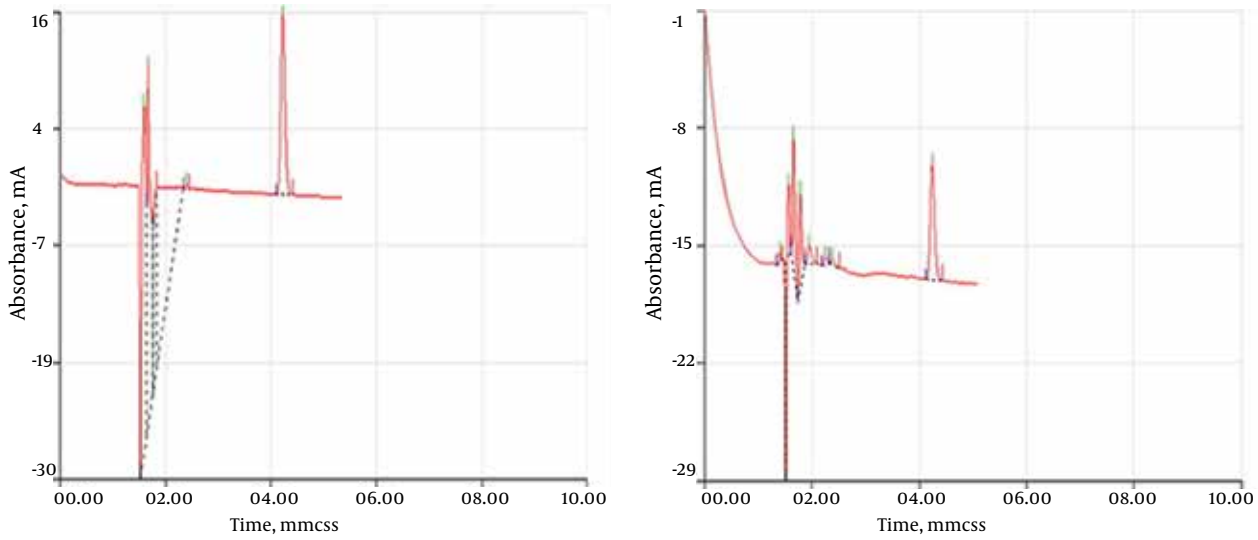
3.2. Statistical Analysis

Statistical analysis was performed with SPSS 17 software. Continues variables were compared using the Students t-test.

4. Results

Inoculation of Lauri Bertani (LB) broth with *Pseudomonas plecoglossicida* was an indication of viability of bacteria and supplementation of Minimal Salt Medium with 1.5% of dextrose and growth of *Pseudomonas plecoglossicida* on this medium showed that this minimal salt medium has not negative effect on the growth of these bacteria (data not shown). Our study showed that *Pseudomonas plecoglossicida* has grown on 4 mM concentration of pure diazinon in Minimal Salt Medium inoculated with 500 μL of bacterial suspension equal to McFarland's 0.5 standard after 24 hours of incubation at 37 degrees centigrade as the diazinon peak decrease in its heights and area (Figure 1). Results of inoculation of Minimal Salt Medium supplemented with and without 4 mM concentration of commercial diazinon as the sole carbon source inoculated with 0.5 mL of *P. plecoglossicida* of standard 0.5 McFarland suspension in growth culture has showed in Figure 2.

Figure 1. Pure Dazinion With 4 mM Concentration in Minimal Salt Medium After 24 Hours of Incubation With 0.5 mL of *Pseudomonas plecoglossicida* Suspension (0.5 McFarland)



A) Peak area = 39.1 ± 5.2 and without incubation with *Pseudomonas plecoglossicida*; *B) Peak area* peak area = 100.4 ± 7.1. All tests were reported three times.

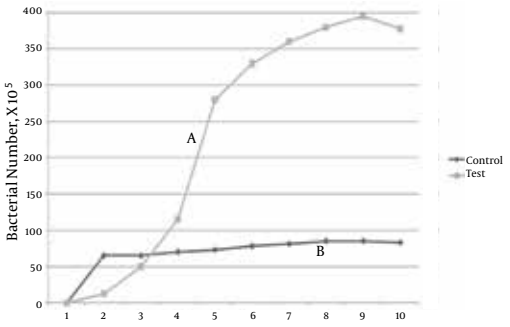


Figure 2. Growth of *Pseudomonas plecoglossicida* in Minimum Salt Medium Enriched Which (A) and Without (B) Diazinion After During First 24 Hours

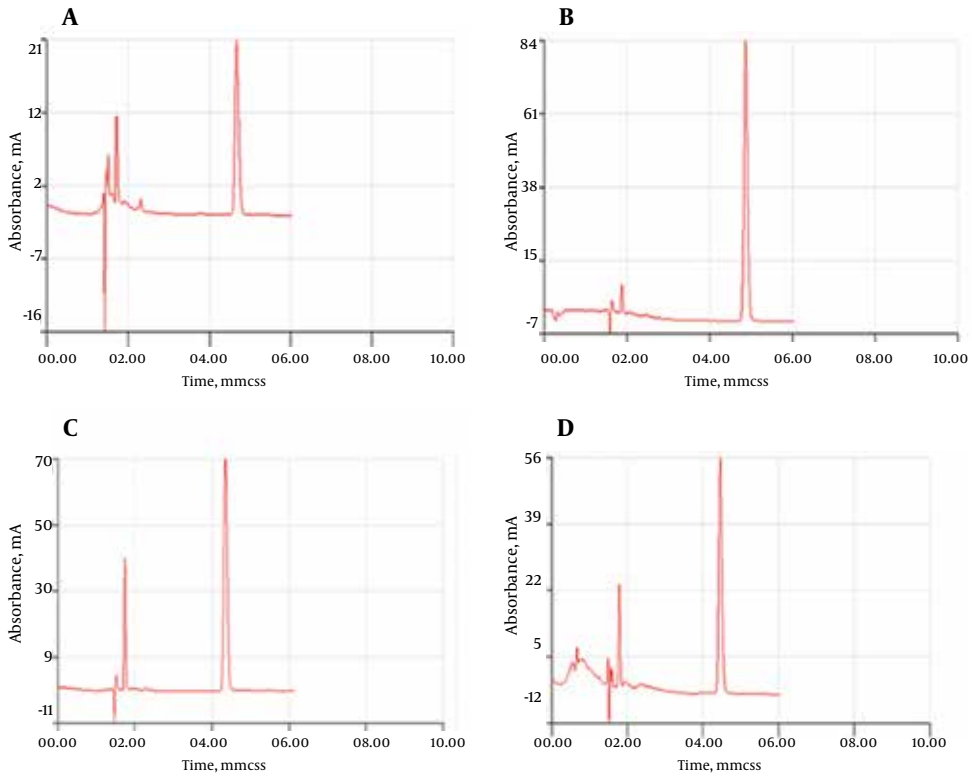
Chromatograms of diazinon after 24 hours of inoculation of Minimal Salt Medium containing 4 mM of pure diazinon with 0.5 mL of *Pseudomonas plecoglossicida* suspension showed that concentrations of diazinon decreased after incubation with *Pseudomonas plecoglossicida* in mentioned conditions (data not shown). Peak area and peak height of 4 mM diazinon inoculated with *Pseudomonas plecoglossicida* after 2, 4 and 7 days of incubation in Minimal Salt Medium has shown in Table 1. These chromatograms showed that by increasing incubation time, concentration of diazinon in this culture tubes has been decreased (Figure 3).

Table 1. Retention Time, Peak Area and Peak Height of 4 mM Dilution of Diazinon After Incubation With *Pseudomonas plecoglossicida* in Minimal Salt Medium [a, b]

	Time of Inoculation, d			
	0	2	4	7
Retention time	04:20.3 ± 0:3.7	04:51.4 ± 0:4.5	04:20.9 ± 0:1.7	04:27.4 ± 0:5.5
Peak areas	1006.9 ± 30.4	543.0 ± 12.9	415.9 ± 9.5	361.3 ± 10.2
Peak height	170.7 ± 5.9	87.4 ± 3.8	71.5 ± 2.6	60.3 ± 4.2

[a] All tests were repeated three times.
[b] Data are presented as Mean ± SD.

Figure 3. Commercial Diazinon With Different Concentration in Minimal Salt Medium



A) Commercial diazinon with 4 mM concentration in minimal salt medium, peak area = 1006.9 ± 30.4; B) Commercial diazinon with 4 mM concentration in minimal salt medium after 2 days of inoculation with 0.5 mL of *Pseudomonas plecoglossicida* solution, peak area = 543.0 ± 12.9; C) Commercial diazinon with 4 mM concentration in minimal salt medium after 4 days of incubation with 0.5 mL of *Pseudomonas plecoglossicida* solution, peak area = 415.9 ± 9.5; D) Commercial diazinon with 4 mM concentration in minimal salt medium after one week of inoculation with 0.5 mL of *Pseudomonas plecoglossicida* solution; peak area = 361.3 ± 10.2. Hichrom C18 column; flow rate: 1.5 mL/min; wave length: 246; mobile phase: acetonitril 80%- deionized distilled water 19.5%-acetic acid 0.5%; Injection: 2.5 μL. all tests were repeated three times.

5. Discussion

Study of bacterial growth on biohazard substances is a good clue for their effectiveness in biodegradation of these biohazard substances. Some studies lead to finding and isolating suitable microorganisms for this purpose (3, 15-18). Application of synthetic pesticides in agriculture has been routine and present information suggested that notable amounts of these pesticide have been distributed in soil and water and they found the first priority in decontamination of the environment (11, 19, 20). In this study diazinon was the only source of carbon in our Minimal Salt Medium and *Pseudomonas plecoglossicida* was grown on theses media and concentration of diazinon has decreased by increasing time of inoculation, it is obvious that *Pseudomonas plecoglossicida* can utilize diazinon as a source of carbon. There are very few studies related to use of organophosphoric compounds as a source of carbon and phosphorous in growth of bacteria but our study showed that *Pseudomonas plecoglossicida* can use diazinon in its growth medium as a carbon and phosphorous source.

Results of this study is in accordance with Soruri and his colleague study (14) on the bacteria which were isolated from industrial sludge. They found a strain of Pseudomonas which was able to grow on organophosphoric compounds as a source of carbon and phosphorous but they were not able to identified it. Also Boricha studies (11) showed that *Pseudomonas plecoglossicida* can degrade cypermethrin pesticide. These findings along with results of this study showed that *Pseudomonas plecoglossicida* can be used as a possible bioremediation agent in biodegradation of pesticides in agricultural soil.

Acknowledgements

Authors would like to thanks Mrs. Afsaneh Mandokht for providing pure diazinon and Dr. Faramarzi, School of Pharmacology, Tehran University of Medical Sciences for providing *Pseudomonas plecoglossicida* strain.

Author's Contribution

Abasalt Borji, designing the research, performing the experiment and analysis the data; Ghazal Naserpour Farivar, performing the experiment and analysis the data; Pouran Johari, performing the experiment and analysis the data; Taghi Naserpour Farivar, designing the research, performing the experiment and analysis the data; Sepideh Senemari, designing the research and analysis the data; Gholamrea Karimi, designing the research and analysis the data.

Financial Disclosure

There was not conflict of interest.

Funding/Support

This study was performed by Qazvin and Neyshabur University of Medical Sciences grant.

References

1. Singh BK, Walker A, Morgan JA, Wright DJ. Biodegradation of chlorpyrifos by enterobacter strain B-14 and its use in bioremediation of contaminated soils. *Appl Environ Microbiol.* 2004;**70**(8):4855–63.
2. Abo-Amer A. Biodegradation of diazinon by Serratia marcescens DI101 and its use in bioremediation of contaminated environment. *J Microbiol Biotechnol.* 2011;**21**(1):71–80.
3. Arbeli Z, Fuentes CL. Accelerated biodegradation of pesticides: An overview of the phenomenon, its basis and possible solutions; and a discussion on the tropical dimension. *Crop Prot.* 2007;**26**(12):1733–46.
4. Geetha M, Fulekar MH. Bioremediation of pesticides in surface soil treatment unit using microbial consortia. *African J Envir Sci Tech.* 2008;**2**(2):36–45.
5. Park SC, Shimamura I, Fukunaga M, Mori KI, Nakai T. Isolation of bacteriophages specific to a fish pathogen, Pseudomonas plecoglossicida, as a candidate for disease control. *Appl Environ Microbiol.* 2000;**66**(4):1416–22.
6. Fulekar MH, Geetha M. Bioremediation of Chlorpyrifos by Pseudomonas aeruginosa using scale up technique. *J of Ap Biosci.* 2008;**12**:657–60.
7. Otenio MH, Silva MTLd, Marques MLO, Roseiro JC, Bidoia ED. Benzene, toluene and xylene biodegradation by Pseudomonas putida CCMI 852. *Brazilian J Microbiol.* 2005;**36**(3):258–61.
8. Raghavan PUM, Vivekanandan M. Bioremediation of oil-spilled sites through seeding of naturally adapted Pseudomonas putida. *Int Biodeter Biodegr.* 1999;**44**(1):29–32.
9. Silva RMP, Rodríguez AÁ, de Oca JMGM, Moreno DC. Biodegradation of crude oil by Pseudomonas aeruginosa AT18 strain. *Tecnología Química.* 2010;**26**(1).
10. Zhang GL, Wu YT, Qian XP, Meng Q. Biodegradation of crude oil by Pseudomonas aeruginosa in the presence of rhamnolipids. *J Zhejiang Univ Sci B.* 2005;**6**(8):725–30.
11. Balali-Mood M, Balali-Mood K. Neurotoxic disorders of organophosphorus compounds and their managements. *Arch Iran Med.* 2008;**11**(1):65–89.
12. Cetın AK, Gur N, Fırat Z. Growth rate of Scenedesmus acutus in laboratory cultures exposed to diazinon. *Afr J Biotechnol.* 2013;**10**(34):6540–3.
13. Boricha H, Fulekar MH. Pseudomonas plecoglossicida as a novel organism for the bioremediation of cypermethrin. *Biol Med.* 2009;**1**(4).
14. Soruri Z, Miresmaieli R, S M, Latifi AM. Isolation of organophosphorus acid anhydrase producing bacteria and identification of strains with most hydrolysis activity. *J Maz Sch Med.* 2008;**68**:19–26.
15. Faramarzi MA, Brandl H. Formation of water-soluble metal cyanide complexes from solid minerals by Pseudomonas plecoglossicida. *FEMS Microbiol Lett.* 2006;**259**(1):47–52.
16. Jadhav M, Kalme S, Tamboli D, Govindwar S. Rhamnolipid from Pseudomonas desmolyticum NCIM-2112 and its role in the degradation of Brown 3REL. *J Basic Microbiol.* 2011;**51**(4):385–96.
17. Kang DG, Choi SS, Cha HJ. Enhanced biodegradation of toxic organophosphate compounds using recombinant Escherichia coli with sec pathway-driven periplasmic secretion of organophosphorus hydrolase. *Biotechnol Prog.* 2006;**22**(2):406–10.
18. Pandey BV, Upadhyay RS. Pseudomonas fluorescens can be used for bioremediation of textile effluent Direct Orange-102. *Trop Ecol.* 2010;**51**(2):397–403.
19. Fulekar MH. Bioremediation of fenvalerate by Pseudomonas aeruginosa in a scale up bioreactor. *Rom Biotechnol Lett.* 2009;**14**:4900–5.
20. Kanekar PP, Bhadbhade BJ, Deshpande NM, Sarnaik SS. Biodegradation of organophosphorus pesticides. *Proce Indian Natl Sci Acad B.* 2004;**70**(1):57–70.