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Full Length Research Paper

Isolation and characterization of five chlorpyrifos degrading bacteria

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Several strains of bacteria were successfully isolated from effluent storage pools of factories producing pesticides and from soil moisture around them. The isolates were capable of utilizing chlorpyrifos (Cp) as the sole source of carbon, phosphorus and energy. Isolates were identified based on16SrRNA sequence analysis and were named IRLM.1, IRLM.2, IRLM.3, IRLM.4, and IRLM.5. IRLM.1 was able to grow at concentrations of chlorpyrifos up to 2000 mg/L and was selected as a preferable isolate for further analysis. The amount of the degraded Cp and the amount of metabolite 3,5,6-trichloropyridinol (TCP) produced were assessed in IRLM.1 by using high performance liquid chromatography (HPLC) techniques. Additionally, the location of the chlorpyrifos-degrading enzyme was determined by comparing the activity of intact bacteria to cytoplasm activity. Our study reveals that Cp-degrading enzyme of IRLM.1 is cytoplasmic and 10 µl cytoplasm isolated from 0.05 g dry-weight bacteria can degrade 50% of 2 mM Cp in 2 min. Furthermore, the HPLC analysis showed accumulation of TCP in the medium, revealing that IRLM.1 was able to degrade Cp without being affected by the antimicrobial activity of TCP. Moreover, results show that the IRLM.1 isolate could grow and utilize diazinon and malathion as the sole source of carbon, phosphorus and energy. Thus IRLM.1 can successfully participate in efficient degradation of organophosphorus compounds (OPs).

Key words: Biodegradation, organophosphorus pesticides, chlorpyrifos, isolation.

INTRODUCTION

The term pesticide covers a wide range of compounds including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators and others (Aktar et al., 2009). Organophosphorus compounds (OP), which are a group of highly toxic agricultural chemicals widely used for plant protection, have generated a number of environmental problems such as contamination of air, water and terrestrial ecosystems, they have harmful effects on different biota, and disrupt biogeochemical cycling (Zeinat et al., 2008; Horne et al., 2002; Cisar and Snyder, 2000). Diazinon (Di), malathion (Mt) and chlorpyrifos (Cp) as insecticides and acaricides, are the most commonly used OPs. Diazinon (O, O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate) acts as a contact to stomach and respiratory poison, and has been identified as a potential chemical mutagen (Bolognesi and Morasso, 2000). It is used throughout the world to control a wide range of sucking and chewing insects and mites on a number of crops and is applied as a sheep dip to control ectoparasites such as sheep scab and blow fly strike (Tomlin, 2003; Cycon et al., 2009). Chlorpyrifos (O, O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate), has a high soil absorption co-efficient, but low water solubility (Cycon et al., 2009). Reports from the Environmental Protection Agency (EPA) suggest that a wide range of water and terrestrial ecosystems may be contaminated with chlorpyrifos (Singh and Seth, 1989). Malathion, S-(1,2-dicarbethoxyethyl)-O,O-dimethyl dithiophosphate, is used extensively for the control of sucking and chewing insects on field crops, fruits, vegetable, livestock, and is also used as substitute for dichlorodiphenyltrichloroethane (DDT) to kill mosquitoes, flies,

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household insects, animal parasites, and head body lice (Barlas, 1996; Ningfeng et al., 2004). Several bacterial and fungal species have been isolated and characterized that can degrade malathion (Singh and Seth, 1989; Ningfeng et al., 2004; Goda et al., 2010; Zeinat et al., 2008). Current methods to detoxify contamination by OP pesticides mainly rely on chemical treatment, incineration and landfills (Serdar and Gibson, 1985).

A reliable cost-effective technique for pesticide removal is to biodegrade the organophosphate compounds. In general, microorganisms demonstrate considerable capacity to metabolize many pesticides. They possess the unique ability to completely mineralize many aliphatic, aromatic, and heterocyclic compounds (Bhagobaty, 2007). The bacteria, *Flavobacterium* sp. strain ATCC 27551 and *Pseudomonas* diminuta strain MG, with the capability of hydrolyzing OPs such as diazinon and parathion, were isolated from soils in the Philippines and United States, respectively (McDaniel et al., 1988; Harper et al., 1988). Many microorganisms can specifically hydrolyze the phosphoester bonds of OPs and thus reduce the toxicity of OP pesticides and OP chemical warfare agents (e.g. sarin). The study of Munnecke showed that the rate of enzymatic hydrolysis was two to 450 times faster than that of chemical hydrolysis, when parathion was used as a substrate (Munnecke et al., 1974). Considering that chlorpyrifos is one of the most commonly applied insecticides for control of pests and insects, the purpose of this experiment was to isolate and characterize chlorpyrifos degrading-bacteria, to investigate their degradation potential, to assess their adaptation to high concentrations of chlorpyrifos and to determine their usefulness in biodegradation of contaminated sources.

MATERIALS AND METHODS

Growth media and culture conditions

Mineral salts medium (MSM) enriched with chlorpyrifos was used for isolation of chlorpyrifos-degrading bacteria. The carbon source in MSM was replaced with chlorpyrifos. The MSM has the following composition in (g/L): NaCl, 0.1; KCl, 0.2; $(NH_4)_2SO_4$, 0.5; $CaCl_2.H_2O$, 0.05; $MgSO_4.7H_2O$, 0.2; $FeCl_3.6H_2O$, 0.02; nutrient agar (NA) and mineral agar (MSM agar) were used to assess growth of the bacteria and for their macroscopic study on solid medium.

Sample collection, screening and isolation of chlorpyrifos-degrading bacteria

The samples were collected from the effluent storage ponds and moist soil around a few factories producing pesticides in Iran. Five isolates were screened from the collected samples as follows: the samples (10 g soil, or 15 ml effluent) were suspended in 250 ml Erlenmeyer flasks containing 50 ml MSM and were incubated on a rotary shaker at 260 rpm for 72 h. Then the samples were inoculated into 50 ml of MSM supplemented with chlorpyrifos (50 mg/L) as a sole source of carbon, energy and phosphorus for their growth. Technical grade Cp with 95% purity was procured from Iran's chemical production company (Certification No 127). The flasks were incubated on a rotary shaker at 150 rpm for seven days at 30°C. Then the samples were cultured on MSM agar supplemented with chlorpyrifos (50 mg/L) and NA (without pesticide) at 30°C for 24 h. The isolates that could grow were designated as chlorpyrifos-degrading bacteria and subjected to 16S rRNA studies. In order to find the optimal isolate, a second screening was performed as follows: the isolates (obtained from individual colonies) were inoculated into flasks containing MSM supplemented with (50, 80, 110, 140,170, 200, 300, 400, 600, 100, 2000 and 3000 mg/L) chlorpyrifos and were incubated on a rotary shaker at 150 rpm for seven days at 30°C. Growth of the isolate was detected on NA (without chlorpyrifos) to find the most capable isolate.

Growth kinetic detection in presence of chlorpyrifos

Isolate IRLM.1 was inoculated in MSM supplemented with 140 mg/L chlorpyrifos (as the optimum concentration for growth, data not shown). The growth kinetics was followed by monitoring the optical density of the medium for 10 days using a UV/VIS spectrophotometer at 600 nm wavelength. The MSM containing 140 mg/L chlorpyrifos and Escherichia coli BL21, which is not able to degrade OPs, was used as the negative control.

Biodegradation assay of chlorpyrifos and TCP

Isolate IRLM.1 was inoculated in MSM supplemented with 140 mg/L chlorpyrifos (as the optimum concentration for growth, data not shown). Remaining chlorpyrifos after 2, 5, 8,, 10 days and TCP after 10 days were analyzed by high-performance liquid chromatography (HPLC Cecil 1100) using a Zorbax SB-C18 column (250 × 4.6 mm^2, 5Rm). The mobile phase was acetonitrile: water (80:20, v:v), and the flow rate was 1.0 ml min-1. Chlorpyrifos and TCP were detected at 230 and 320 nm, respectively.

Substrate range

Degradation of other organophosphate pesticides was carried out using commercial-type diazinon and malathion. Liquid MSM medium, supplemented with diazinon or malathion, was inoculated with IRLM.1. Growth of the isolate was detected after seven days on NA. The MSM supplemented with diazinon or malathion and inoculated by E. coli BL21, which is not able to degrade OPs, and the MSM without Di, Mt or glucose and inoculated with the bacterial cells, were used as control samples.

Assays for Cp-degrading enzyme activity and its location

Fractionation of the bacteria was used to trace the location of OP-degrading enzyme as follows; cells harvested, with dry weight of 0.05 g, were resuspended in PBS buffer (pH = 6.8) containing 1 mM EDTA and lysozyme at 10 µg/ml to set as unit cell density (OD_{600}=1) and incubated for 2 h at room temperature. The cell suspension was treated with an ultrasound sonication at 30 s × 2 cycles. To obtain total membrane fraction, whole cell lysate was pelleted by centrifugation at 14000 rpm for 2 h using an ultracentrifuge. For further outer-membrane fractionation, the pellet (total membrane fraction) was resuspended with PBS buffer containing 0.01 mM $MgCl_2$ and 2% Triton X-100 and was incubated for 30 min at room temperature for solubilizing the inner membrane, and then the outer-membrane fraction was repelleted after 2 h centrifugation in 14000 rpm. The isolated components were used in

Table 1. Growth of the isolates in different concentrations of chlorpyrifos.

Strain	50	60	80	140	180	240	360	600	1000	2000	3000
IRLM1	+	+	+	+	+	+	+	+	+	+	-
IRLM2	+	+	+	-	-	-	-	-	-	-	-
IRLM3	+	+	+	+	+	+	+	+	+	-	-
IRLM4	+	+	+	+	-	-	-	-	-	-	-
IRLM5	+	+	+	+	-	-	-	-	-	-	-

+, Utilizable, -, non-utilizable.

next stages (Massoud et al., 2007). Equal volume of whole cell and cytoplasm fraction was used for enzyme activity and location study. Enzyme activity was detected by recording the decrease in absorbance of chlorpyrifos at 215 nm (after 2 and 3 min), based on a method by Chao Yang et al. (Yang et al., 2006). Enzyme location was studied by comparing the activities of whole cell and cytoplasmic fraction. For this purpose, 0.5 ml of samples transferred to 2 ml HPLC vials containing 1.5 ml of acetonitrile and analyzing by HPLC (Cecil 1100) using a Zorbax SB-C18 column (250 × 4.6 mm^2, 5Rm), changes in absorbance were measured after 3 min at 37°C. For each assay, 10 µl of cytoplasmic fraction and intact bacterial suspension were separately added to 890 µl of citrate-phosphate buffer (pH = 7.4) and 100 µl of 20 mM Cp (Sigma) in 50% acetonitrile.

Identification of chlorpyrifos-degrading bacteria

Isolated chlorpyrifos-degrading bacteria were characterized based on 16S rRNA gene analysis. The genomic DNA was extracted as described previously (CTAB DNA extraction and purification protocol, Murray and Thompson 1980), where CTAB is cetyltrimethyl ammonium bromide. The 16S rRNA gene was amplified by PCR using the universal primers 27f (5-AGAGTTTGATCMTGGCTCAG- 3- , forward) and 1492r (5' TACGGYTACCTTGTTACGAC TT 3', reverse). Sequencing was carried out with an automated sequencer (Genetic analyzer 31030, Accessories Applied Biosystems).

16S rRNA sequences were compared to other 16S rRNA sequences available in the National Center for Biotechnology Information (NCBI) public database by basic local alignment search tool (BLAST) searching. Selected sequences from the database with the greatest sequence similarity to isolated bacterial sequence were aligned and compared.

Phylogenetic analysis was performed using the software package MEGA4 (Kumar et al., 2004) after multiple alignment of data available from public databases by CLUSTALW (Thompson et al., 1997). Pair wise evolutionary distances were computed using the correction method (Jukes and Cantor, 1969) and clustering was performed using the unweighted pair-group method with arithmetic averages (UPGMA) method (Backeljau et al., 1996). Bootstrap analysis was used to evaluate the tree topology by means of 1000 resembling (Felsenstein, 1993).

RESULTS

Isolation of chlorpyrifos-degrading strain

During primary screening five strains were isolated that were capable of utilizing chlorpyrifos (50 mg/L) as the sole source of carbon. The isolates, designed IRLM.1, IRLM.2, IRLM.3, IRLM.4, IRLM.5, were grown in different concentrations of chlorpyrifos (50, 60, 80, 100, 140, 180, 240, 360, 600 1000, 2000 and 3000 mg/L). According to the results in Table 1, although 140 mg/L is the optimum concentration for growth of most isolates, IRLM.1 could grow in up to 2000 mg/L chlorpyrifos. Therefore, IRLM.1 was selected as the isolate for further analysis.

It is notable to indicate that in comparison to an en masse addition of a high chlorpyrifos concentration, gradually increasing the concentration of chlorpyrifos allowed IRLM.1 to become compatible and increased the growth rate of the isolate at high concentrations (data not shown).

Growth kinetics of IRLM.1 in the presence of chlorpyrifos

Bacterial growth in the presence of chlorpyrifos is shown in figure 1. As compared to control sample, the growth of Cp-exposed bacteria was significantly stimulated and approximately two to three times faster at the beginning of incubation period (one to four days). Maximum bacterial growth was obtained by day eight. The growth curve reached a stasis on day nine to 10, and then decreased (data not shown). In contrast, the control sample inoculated with *E. coli* Bl21 showed no change at 600 nm for 10 days incubation.

Substrate range

The results show that the IRLM.1 isolate could grow and utilize diazinon and malathion for growth. In contrast, in the control samples, no growth was observed. It was proved that the isolate was not able to use carbon dioxide (CO_2) from the air as a carbon source.

Biodegradation assay for chlorpyrifos and TCP

The amount of chlorpyrifos remaining in the medium and the amount of the metabolite TCP that was produced from degradation of Cp was analyzed by HPLC analysis. It was observed that chlorpyrifos gradually decreased over a 10 day period, whereas TCP accumulated. This

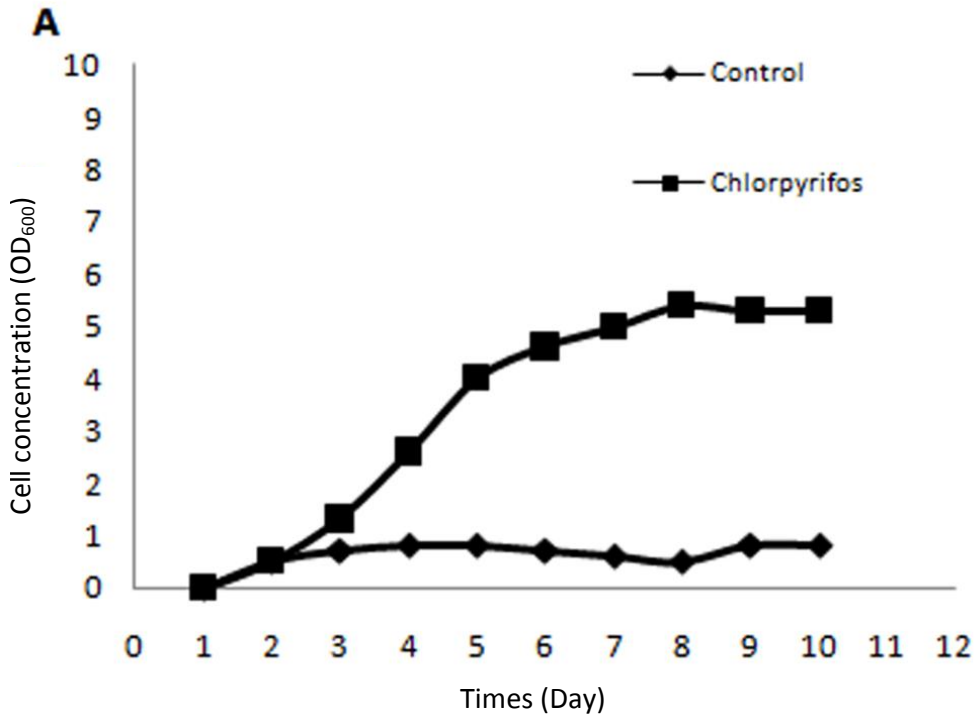

Figure 1. Growth of IRLM.1 in Cp 140 mg/L. Maximum bacterial growth in chlorpyrifos was obtained by day 8. The growth curve reached a stasis by day 9 to 10, and then decreased.

result shows that the bacteria degrade chlorpyrifos by hydrolysis of the phosphoester bond to trichloropyridinol. The enzyme responsible for this esterase activity is a soluble chlorpyrifos hydrolase.

Assays for Cp-degrading enzyme activity and its location

Comparison of the chlorpyrifos hydrolase activity of the whole bacterium and the cytoplasmic fraction showed that the highest enzyme activity was in the cytoplasm. The 10 µl cytoplasmic fraction of the IRLM.1 isolated from bacterial cells with dry weight of 0.05 g could degrade 50% of 2 mM chlorpyrifos in 2 min (Table 2).

Characterization of isolates

Isolates were identified based on their 16S rRNA and BLAST analysis. The results of BLAST analysis of bacterial strains IRLM.1, IRLM.2, IRLM.3, IRLM.4, IRLM.5, reveal that these strains have the greatest similarity to *Pseudomonas aeruginosa* AF137358, *P. aeruginosa* AF531099, *P. aeruginosa* AY264292, *Pseudomonas nitroreducens* EF107515 and *Pseudomonas putida* AF291048, respectively. A dendrogram illustrates the results of 16S rRNA analysis using PHYLIP (Figure 2).

DISCUSSION

In this research, among the dozens of isolates examined, strain IRLM.1 had the strongest ability to grow in MSM supplemented with chlorpyrifos and to utilize chlorpyrifos as the sole energy source.

It is notable that although IRLM.1 had maximum growth in 140 mg/L, it was able to grow in medium supplemented with 2000 mg/L and could achieve maximum growth in chlorpyrifos concentrations higher than 140 mg/L when the bacteria were adapted to gradually increasing concentrations of chlorpyrifos.

Previous studies have shown that *Serratia* sp. and *Pseudomonas* sp. isolates completely degraded diazinon and malathion initial concentration (50 mg/L) within 14 days (Cycon et al., 2009; Massoud et al., 2007).

Various bacterial and fungal species have been reported to be able to grow on diazinon, chlorpyrifos or malathion such as *Serratia* sp. and *Pseudomonas* sp. (Cycon et al., 2009), *Providencia stuartii* MS09 (Rani et al., 2008), *Agrobacterium* sp. (Horne et al., 2002), *Paracoccus* sp. strain TRP (Xu et al., 2008), *Entrobacter* strain B-14 (Singh et al., 2004), *Arthrobacter* sp. (Racke, 1993), *P. putida* (Goda et al., 2010), *Aspergillus* sp. and *Penicillium* sp. (Ningfeng et al., 2004). *P. stuartii*, *Sphingomonas* sp. strain Dsp-2, *Pseudomonas* sp., *Paracoccus* sp. and *Entrobacter* strain B-14 isolates were identified that could utilize chlorpyrifos (Rani et al., 2008; Xu et al., 2008; Singh et al., 2004; Li et al., 2007;

Table 2. Optical density of Cp after exposure to bacterial samples.

Bacteria	OD_{215} [a] T=0	OD_{215} T=2	OD_{215} T=3
Negative control	~1.1 [b]	~1.1	~1.1
P. aeruginosa IRLM1 Whole cell	~1.1	~1	~1
P. aeruginosa IRLM1 cytoplasm	~1.1	0.537	0.323

[a]Optical density of Cp at 215 nm after exposure of the bacterial sample to 2 mM Cp for 3 min; [b]Tilde symbol (~) imply estimation.

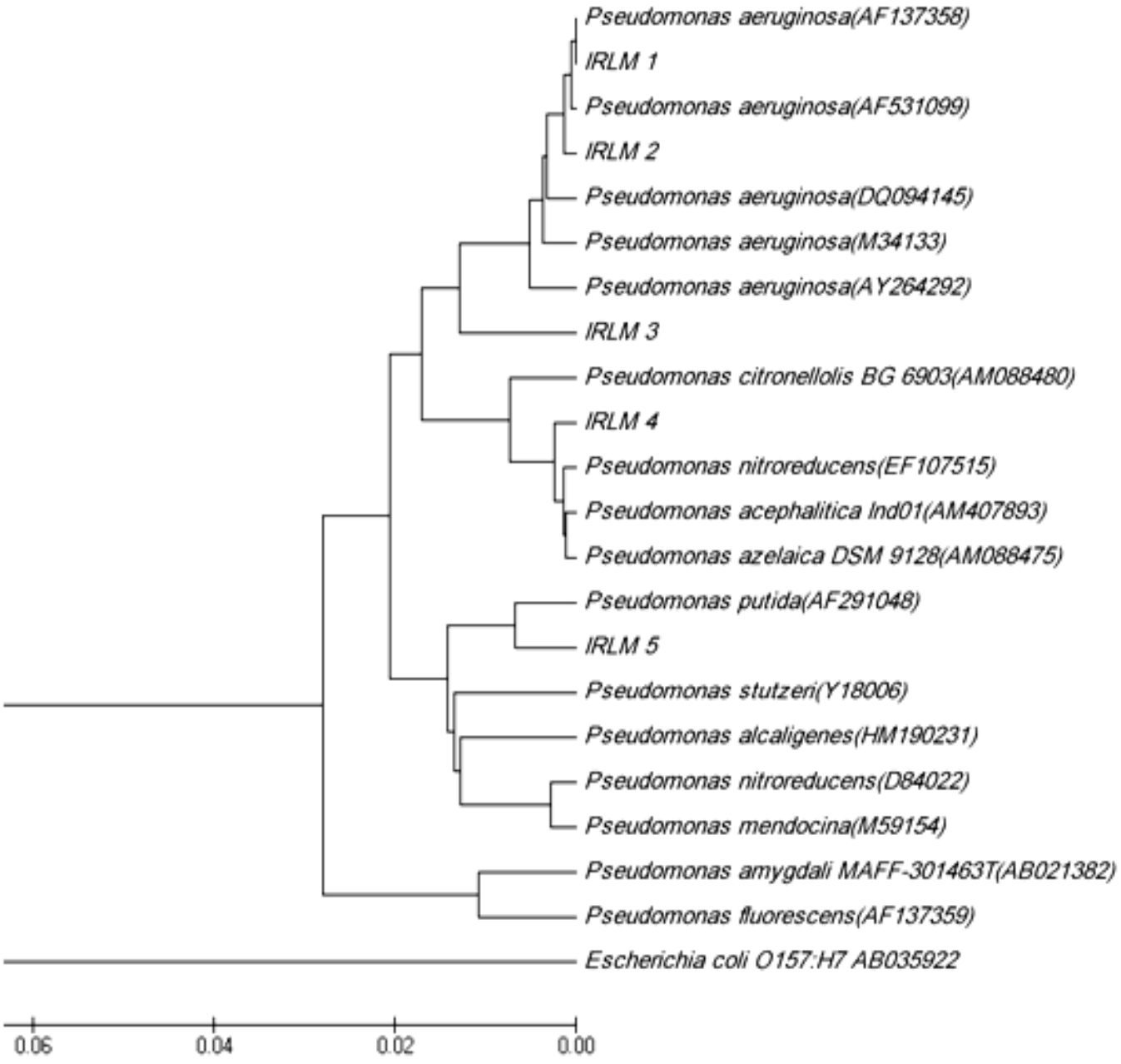

Figure 2. Dendrogram to illustrate the similarity of the pesticide-degrading bacteria to members of the *pseudomonas* genus that had highest sequence similarity (RDP analysis and Fasta). Phylogenetic tree was prepared using the maximum composite likelihood algorithm and the UPGMA linking method. A distance bar is illustrated.

Bhagobaty and Malik, 2008). A *Paracoccus* sp. isolate was the first report of a bacterial strain reported to be able to completely mineralize chlorpyrifos with no accumulation of TCP or diethyl thiophosphate (DETP) (Xu et al., 2008). *Entrobacter* strain B-14 was observed to hydrolyze chlorpyrifos to DETP and TCP, and utilized DETP for growth and energy (Singh et al., 2004). Bhagobaty and Malik, (2008) isolated four bacteria from the soil that were able to grow in 1600 mg/L Cp. Morphological and biochemical tests indicate that they might belong to *Pseudomonas* sp. (Bhagobaty and Malik, 2008). Bacteria from *P. putida* strain are known to be capable of degrading different OP compounds (Goda et al., 2010).

In our research, among the dozens of isolates examined, strain IRLM.1 had the strongest ability to grow in MSM supplemented with chlorpyrifos, utilizing it as the sole energy source. According to our results, 140 mg/L chlorpyrifos was completed degraded by isolate IRLM.1 in eight to nine days. Growth of this isolate on chlorpyrifos is comparable with that of other isolates in previous studies, for example, *P. aeruginosa* isolated in India could degrade 80% of chlorpyrifos (50 mg/L) in liquid medium after 20 days (Lakshmi et al., 2009).

Antimicrobial activity of TCP normally prevents the proliferation of Cp-degrading microorganisms therefore identification of Cp-degrading bacteria is noteworthy. The accumulation of TCP after 10 days growth of IRLM.1 in 140 mg/L chlorpyrifos and the gradual decrease of chlorpyrifos during that 10 days period showed that IRLM.1is able to degrade DETP-containing organo-phosphates while not being affected by the antimicrobial activities of TCP. It is notable that although IRLM.1 had maximum growth in 140 mg/L, it was able to grow in medium supplemented with 2000 mg/L chlor-pyrifos by gradually increasing the chlorpyrifos concentration.

For the first time, adaptation to increasing concentrations of pesticides was achieved in this study. It was concluded that if the bacteria were initially exposed to a low concentration of pesticides, followed by gradual higher concentrations noteworthy increase in their degrading power could be observed. For instance before adaptation, 140 mg/L was the optimum concentration for growth, while gradually increasing the concentration showed that IRLM1 could grow in 2000 mg/L chlorpyrifos which is the highest OP concentration ever reported to support growth of bacteria. This makes the IRLM.1 isolate the strongest microorganism yet found for degradation of OP compounds. Additionally, HPLC analysis showed that the OP-degrading enzyme of *P. aeruginosa* IRLM.1 is cytoplasmic rather than on the surface of the bacterium and 10 µl cytoplasm isolated from 0.05 g dry-weight bacteria is able to degrade 2 mM Cp in 2 min and can tolerate toxicity effect of TCP. 16S rRNA analysis revealed that IRLM.1 is related to *P. aeruginosa*, which is able to participate in efficient degradation of OP compounds. The results of the present study suggest that the bacteria isolated are able to grow in the presence of added pesticide as a sole energy source and may therefore be used for bioremediation of pesticide-contaminated soil.

REFERENCES

Aktar MW, Sengupa D, Chowdhury A (2009). Impact of pesticides use in agriculture: their benefits and hazard. Interdiscp. Toxicol. 2: 1-12.

Backeljau T, Bruyn LD, Wolf HD, Jordaens K (1996). Multiple UPGMA and neighbor-joining trees and the performance of some computer. Mol. Biol. Evol. 13: 309-313.

Barlas NE (1996). Toxicological assessment of biodegraded Malathion in albino mice. Bull. Environ. Contamin. Toxicol. 57: 705-712.

Bhagobaty K, Joshi SR, A. Malik A (2007). Microbial Degradation of Organophosphorous Pesticide: Chlorpyrifos. Internet J. Microbiol. p. 4.

Bhagobaty RK, Malik A (2008). Utilization of chlorpyrifos as a sole source of carbon by bacteria isolated from wastewater irrigated agricultural soils in an industrial area of western Uttar Pradesh. India. Res. J. Microbiol. 3: 230-293.

Bolognesi C, Morasso G (2000). Genotoxicity of pesticides: potential risk for consumers. Trend Food. Sci. Tech. 11: 182-187.

Cisar JL, Snyder GH (2000). Fate and management of turfgrass chemicals. ACS. Symp. Ser. 743: 106-126.

Cycon M, Wojcik M, Piotrowska-seget Z (2009). Biodegradation of the organophosphorus insecticide diazinon by *serratia* sp. and *Pseudomonas* sp. and their use in bioremediation of contaminated soil. Chemosphere, 76: 494-501.

Felsenstein J (1993). PHYLIP (Phylogeny Inference Package) version 3. 5C, Distributed by the author, Department of Genetics University of Washington, Seattle.

Goda SK, Elsayed IE, Khodair TA, El-Sayed W, Mohamed ME (2010). Screening for and isolation and identification of malathion-degrading bacteria: cloning and sequencing a gene that potentially encodes the malathion-degrading enzyme, carboxylestrase in soil bacteria. Biodegradation, 21: 903-913.

Harper LL, McDaniel CS, Miller CE, Wild J (1988). Dissimilar plasmids isolated from *Pseudomonas diminuta* MG and a *Flavobacterium* sp. (ATCC 27551) contain identical opd genes. Appl. Environ. Microbiol. 54: 2586-2589.

Horne I, Sutherland TD, Harcourt LR, Russell RJ, Oakeshott JG (2002). Identification of an opd (organophosphate degradation) gene in an Agrobacterium isolate. Appl. Environ. Microbiol. 68: 3371-3376.

Jukes TH, Cantor CR (1969). Evolution of protein molecules. In: Mammalian protein Metabolism: Munro HN. 3: 21-32.

Kumar S, Nei M, Dudley J, Tamura K (2004). Mega4: Molecular Evolutionary Genetics Analysis Mega software version 4.0. Mol. Biol. Evol. 24: 1596-1599.

Lakshmi CV, Kumar M, Khanna S (2009). Biodegradation of Chlorpyrifos in Soil by Enriched Cultures. Curr. Microbiol. 58: 35-38.

Li X, He J, Li S (2007). Isolation of a chlorpyrifos-degrading bacterium, *Sphingomonas* sp. strain Dsp-2, and cloning of the mpd gene. Res. Microbiol. 158: 143-149.

Massoud AH, Derbalah AS, Belal B, El-Fakharai I (2007). Bioremediation of Malathion in aquatic system by different microbial isolates. Pest. Cont. Environ. Sci. 15: 13-28.

McDaniel CS, Harper LL, Wild JR (1988). Cloning and sequencing of a plasmid-borne gene (opd) encoding a phosphotriesterase. J. Bacteriol. 170: 2306-2311.

Ningfeng W, Minjie D, Guoyi L, Xiaoyu C, Bin Y, Yunliu F (2004). Cloning and expression of ophc2, a new organphosphorus hydrolase gene. Chin. Sci. Bull. 49: 1245-1249.

Racke KD (1993). Environmental Fate of Chlorpyrifos. Rev. Environ. Contamin. Toxicol. 131: 1-150.

Rani MS, Lakshmi KV, Devi PS, Madhuri RJ, Aruna S, Jyothi K, Narasimha G, Venkateswarlu K (2008). Isolation and characterization of chlorpyrifos degrading bacterium from agricultural soil and its growth response. AFR J. Microbiol. Res. 2: 026-031.

Serdar CM, Gibson DT (1985). Enzymatic hydrolysis of organophosphates: cloning and expression of a parathion hydrolase gene from *Pseudomonas* diminuta. Biotechnology, 3: 567-571.
Singh BK, Allan S, Walker A, Alun J, Morgan W, Denis J (2004). Biodegradation of Chlorpyrifos by Enterobacter Strain B-14 and Its Use in Bioremediation of Contaminated Soils. Appl. Environ. Microbiol. 70: 4855-4863.
Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DJ (1997). The clustral X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acid Res. 24: 4876-4882.
Tomlin CDS (2003). The Pesticide Manual. 13th Ed. British Crop Protection Council Surrey UK. pp. 135-136.
Xu G, Zheng W, Li Y, Wang S, Zhang J, Yan Y (2008). Biodegradation of chlorpyrifos and 3,5,6-trichloro-2-pyridinol by a newly isolated *Paracoccus* sp. strain TRP. Int Biodeterior. Biodegr. 62: 51-56.
Yang C, Liu N, Guo X, Qiao C (2006). Cloning of mpd gene from a chlorpyrifos-degrading bacterium and use of this strain in bioremediation of contaminated soil. FEMS. Microbiol. Lett. 265: 118-125.
Zeinat KM, Nashwa AH, Fetyan A, Ibrahim AM, El-Nagdy S (2008). Biodegradation and Detoxification of Malathion by of Bacillus Thuringiensis MOS-5. Aust J. Basic. Appl. Sci. 2: 724-732.