Isolation, characterization and fingerprinting of some chlorpyrifos- degrading bacterial strains isolated from Egyptian pesticides-polluted soils

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Five bacterial isolates (B-CP5- B-CP6 - B-CP7- B-CP8- B-CP9) were isolated from pesticides-contaminated soil in Egypt. The capability of these isolates to degrade chlorpyrifos was investigated using enrichment mineral salt (MS) medium containing chlorpyrifos. Two different PCR-based techniques, RAPD-PCR and PCR-RFLP for amplified16S rRNA fragment were used to conduct genetic fingerprinting and obtain specific molecular markers for the studied isolates. The isolates exhibited substantial growth in mineral salt medium supplemented with 100-300 mg/L chlorpyrifos as a sole source of carbon and energy. Based on their morphological, cultural and biochemical characters, the isolates have been identified as *Pseudomonas stuzeri*, *Enterobacter aerogenes*, *Pseudomonas pseudoalcaligenes*, *Pseudomonas maltophilia* and *Pseudomonas vesicularis* respectively. *Pseudomonas stuzeri* was the most potent degrader strain. Five specific markers for this strain were determined. The highest genetic similarity was observed between CP8 and CP7 (66%), while the lowest genetic similarity was detected between CP8 and CP6 (37%). All isolates had the same pattern after digestion of 16S rRNA amplified fragment with two restriction enzymes (EcoRI and AluI) except *Enterobacter aerogenes*, which generate two monomorphic bands at 420 and 130 bp, respectively. In conclusion, the strain *Pseudomonas stuzeri* could be used to clean up the areas contaminated with Chlorpyrifos. Obtained molecular markers might be used for identifying and tracking the most potent bacterial isolate. The used PCR techniques represent a powerful tool and could be used for rapid typing of this strain.

Key words: Pesticide, chlorpyrifos, biodegradation, 16S rRNA, RAPD-PCR, *Pseudomonas* sp.

INTRODUCTION

Recently the use of microbes for effective detoxify, degrades and removal of toxic chlorinated compounds from contaminated soils has emerged as an efficient and cheap biotechnological approach to clean up polluted environments (Strong and Burgess, 2008). Chlorpyrifos (CP) is one of the most widely used organophosphorous insecticide. Chlorpyrifos-oxon and 3,5,6-trichloro-2-pyridinol (TCP) are the two potent transformation products of chlorpyrifos (Bhagobaty et al., 2007). Unlike other organophosphorous compounds, CP has been reported to be resistant to enhanced degradation since its first use in 1965. Although CP has been reported to be degraded co-metabolically by bacteria in liquid media, several attempts to isolate CP-degrading bacteria from agricultural soil have not been successful (Mallick et al., 1999; Racke et al., 1990). Singh et al. (2004) isolated the first CP-degrading bacterium, it was Enterobacter B-14, which hydrolyzed CP to diethylthiophosphate DETP and TCP, and utilized DETP for growth and energy. Two recently isolated CP-degrading bacteria, *Stenotrophomonas* sp. and *Sphingomonas* sp., could also utilize CP as the sole source of carbon and phosphorous, but they did not degrade TCP (Yang et al.,

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2006; Li et al., 2007). DNA-based analyses can contribute significantly to the characterization of bacteria that have been successfully isolated from the polluted environments. RAPD-PCR approach has been shown to be useful in fingerprinting and distinguish between microbial strains within a species (Nowrouzian et al., 2001). An excellent target for bacterial identification and phylogenetic characterization is 16S rRNA gene, the 16S rRNA gene is universally distributed and highly conserved (Woese, 1987). Due to its conserved nature and ease of manipulation, it has been extensively used to establish accurate identification of bacterial isolates. Cleavage of PCR-generated 16S rRNA gene amplicons by a restriction enzyme(s) (RE) results in differentiation by 16S rRNA PCR-RFLP technique. This procedure has been used extensively as a method for bacterial species identification (Wilson et al., 1995; Marshall et al., 1999; Conville et al., 2000; Steinhausserova et al., 2001; Bayoumi et al., 2010). The present study aims to isolate, characterize and fingerprinting of chlorpyrifos degrading bacteria from Egyptian pesticides-polluted soils as well as obtain specific molecular markers of the most potent bacteria from Egyptian pesticides-polluted soils as well as characterize and fingerprinting of chlorpyrifos degrading bacterial isolates. In addition examine the effects of various environmental factors which affect the bacterial biodegradation potentiality of chlorpyrifos.

MATERIALS AND METHODS

Pesticide used

Chlorpyrifos (CP) (95%purity) was obtained from Dow-agroscience Co.

Media

Mineral salts (MS) medium enriched with Chlorpyrifos as a sole source of carbon and energy was used as a routine medium for isolation and characterization of different species of chlorpyrifos utilizing bacteria and was prepared as the followings: Solution (A) contained in (g/L): KNO₃, 0.5; KH₂PO₄, 0.68; NaH₂PO₄, 1.79; NH₄NO₃, 1.o; MgSO₄.7H₂O, 0.35; CaCl₂.2H₂O, 0.4; and agar-agar, 15.0 for solid medium and distilled water up to 1000 ml. Chlorpyrifos was added by 100 to 300 mg/L. Solution (B) trace salt solution (100g / ml): FeSO₄.7H₂O, 0.1; ZnSO₄, 0.1; MnSO₄, 0.1; CuSO₄, 0.1, CaCl₂.2H₂O, 0.1; H₃PO₄, 0.1, and distilled water up to 100 ml/L. The solid and liquid mineral salts media were supplemented with yeast extract and glycerol (3.0 g/ml) or (3.0 ml/l) respectively for obtaining enrichment media.

Study site

Four Chlorpyrifos polluted soil samples were collected from various agricultural fields belong to Cairo and Giza Egyptian governorates.

Reagent used

The 4-aminoantipyrene was used to measure the production of phenolic compounds in culture solution. It was prepared as following: (1) 5 mM Fe (NH₄)₂(SO₄)₂ solution, (2) 100 mM α-ketoglutarate solution (3) 40 mM 4-aminoantipyrine solution. This reagent used for detection of phenolic products of the enzymatic cleavage of 2,4-D and related compounds. 2,4-D α-ketoglutarate deoxygenase, cleaves the phenoxy ether bond of o,o-diethyl o-(3,5,6-trichloro-2-pyridyl) phosphonothionate (Chlorpyrifos), to form 2,4-trichlorophenol TCP. The red color resulting from a 2 mg/l of TCP solution is capable of being visually distinguished from the control (remains bright yellow).

Isolation of cp-degrading bacteria by enrichment and screening

One gram of each soil sample was added to 99 ml MS medium supplemented with yeast extract and glycerol containing 0.1 ml CP in 250 ml flasks. The suspension was incubated at 30°C with shaking at 100 rpm for 10 days; 10 ml of the culture broth was transferred after 10 days to 90 ml of the fresh media. After the fourth enrichment transfer to fresh medium containing CP as sole source of carbon and energy, the media was supplemented with 1.5% agar and incubated at 30°C for 7 days. Bacterial isolates grown on chlorpyrifos-containing agar were subjected to morphological, cultural and biochemical studies by the aid of Berge’s Manual of Determinative Bacteriology (Holt et al., 1994). All bacterial isolates were screened based on the formation of degrading haloes around the bacterial colonies and potential isolates were obtained and tested for their potential degrading ability of CP.

Parameters controlling cp biodegradation by CP5

Eight parameters were investigated for studying CP biodegradation by CP5 on MS medium supplemented with CP as sole source of carbon and energy viz. incubation period (1, 2, 3, 4, 5, 6 and 7 days), different CP concentrations (1, 2, 4, 6, 8, 10, 20, 40, 60, 80 and 100 ml/L); inoculum size (0.5, 1, 2, 4, 8, 10 ml/100ml); incubation temperatures (25, 30, 35, 40 and 45°C); different carbon sources (D-Glucose, D-Fructose, D-Mannose, D-Ribose, Maltose, Maltose, Arabinose, L-Rhamnose, Sucrose, Lactose, and Starch). All different tested carbon sources were applied as equimolecular weight located in 1% (w/v) sucrose except poly-sorbit and starch which were applied by 1%; different pH values (5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5 and 9) using 1 N HCl and 1 N NaOH; different nitrogen sources (sodium nitrate, magnesium nitrate, potassium nitrate, ammonium chloride, ammonium nitrate. EDTA, urea and peptone); under static and shaking conditions. After each parameter, 4-aminoantipyrine reagent was used to measure the production of phenolic compounds.

Molecular genetics fingerprinting

Genomic DNA extraction

Genomic DNA was extracted from five studied isolates using Easy Quick DNA extraction kit (Genomix) following the manufacturer’s instructions.

Random amplified polymorphic DNA (RAPD)

RAPD-PCR

PCR reactions were carried out using sixe arbitrary 10-mer primers (Operon Tech., Inc.). PCR reactions were conducted using 2x superhot PCR Master Mix (Bioron; Germany) with 10 Pmol of each primer.
Table 1. List of used primers, their nucleotide sequences, total number of bands, monomorphic and polymorphic bands produced by six primers.

| Primer code | Prime sequence | Isolate | Total bands | Amplified bands | Monomorphic bands | Polymorphic bands |
|-------------|----------------|---------|-------------|-----------------|-------------------|-------------------|
| A2 5'-TGCCGAGCTG-3' | 11 | 11 | 11 | 14 | 13 | 60 | 17 | 7 | 10 |
| A3 5'-AGTCAGCCAC-3' | 4 | 11 | 7 | 8 | 7 | 37 | 13 | 6 | 7 |
| B3 5'-CATCCCCCTG-3' | 12 | 9 | 13 | 10 | 12 | 56 | 15 | 8 | 7 |
| B5 5'-TGCGCCCTTC-3' | 7 | 10 | 8 | 7 | 8 | 40 | 18 | 4 | 14 |
| C3 5'-GGGGGTCTTT-3' | 8 | 7 | 6 | 8 | 6 | 35 | 10 | 8 | 2 |
| C5 5'-TGCGCCCTTC-3' | 11 | 7 | 9 | 11 | 11 | 47 | 11 | 6 | 5 |
| **Total** | **53** | **55** | **54** | **56** | **57** | **275** | **84** | **39** | **45** |

6 different arbitrary 10-mer primers. The codes and sequences of these primers are listed in Table 1. The 25 µl reaction mixture was (10 Pmol. of each primer, 30-50 ng of DNA template and 12.5 µl of 2x superhot PCR Master Mix). The PCR protocol was initial denaturation, 94°C for 2.5 min and 35 cycles of subsequent denaturation, 94°C for 45 s; annealing temperature, 37°C for 30 s; extension temperature, 72°C for 2 min and final extension, 72°C for 10 min. PCR products were analyzed on 10 x 14 cm 1.5% agarose gel electrophoresis with DNA ladder standard 100 bp (Jena Bioscience, Germany) for 30 min using Tris-borate-EDTA Buffer and visualized by ultraviolet illumination after staining with 0.5 µg/ml ethidium bromide.

16S RNA PCR-RFLP analysis

The amplification of 16s rRNA gene was performed with universal primer 968F AAC GCGAAGAACCCTAC and 1401R GCGTGTGTACAACCC. The reaction mixture was (10 Pmol. of each primer, 50 ng of DNA template and 12.5 µl of 2x superhot PCR Master Mix). The PCR program was as follows: 1 cycle at 95°C, 5 min; 35 additional cycles consisting of 95°C 1 min, 63°C 1 min, and 72°C 1 min and 72°C 10 min as post PCR reaction time. The amplified DNA fragments were digested with two restriction enzymes (EcoRI and. AluI) separately as described by the manufacturer (Jena Bioscience, Germany). The digested DNA fragments were separated on 2.5% agarose gel electrophoresis with DNA ladder standard 100 bp (Jena Bioscience, Germany), stained with 0.5 µg/ml ethidium bromide, visualized on a UV Transilluminator, photographed by Gel Doc. System and analyzed with software data analysis for Bio-Rad Model 620 USA.

Statistical analysis

The presence / absence RAPD and 16S rRNA PCR-RFLP data were analyzed using the SPSS-PC programs of (Nie et al., 1975). Pair-wise comparisons between strains were used to calculate the genetic similarity values (F) derived from Dice similarity coefficient.

RESULTS

Five different bacterial isolates were selected on the basis of production of halo zone around the bacterial colonies and growth in high concentrations of CP (100-300 mg /L). Morphological, cultural and biochemical parameters were determined and illustrated in Table 2. Degrading bacterial isolates were characterized and identified as Pseudomonas stutzeri (B-CP5), Enterobacter aerogenes (B-CP6), Pseudomonas pseudoalcaligenes (B-CP7), Pseudomonas maltophilia (B-CP8) and Pseudomonas vesicularis (B-CP9), by consulting Bergey’s Manual of Systematic Bacteriology (Krieg and Holt, 1984) and Bergey’s Manual of Determinative Bacteriology (Holt et al., 1994). Pseudomonas stutzeri (B-CP5) was selected as the most potent CP-utilizing bacterial isolate.

Parameters controlling the Chlorpyrifos biodegradation by Pseudomonas stutzeri B-CP5

Data recorded in Table 3 showed that, when the Pseudomonas stutzeri (B-CP5) was grown on MS medium supplemented with 0.3 ml/L Chlorpyrifos as sole source of carbon and energy proved to be capable of grown with 7 days as optimum incubation period, preferred Chlorpyrifos concentration 0.1-0.35 ml/L, maximum inoculum size (0.5 ml), optimum temperature (30° C), optimum pH (7), favorite sugar (fructose) and preferred nitrogen source (ammonium nitrate) under shaking rotation of (100 rpm). Below and above optimal incubation period, Chlorpyrifos concentrations, inoculum size, temperature, pH, the phenolic compounds production decreased gradually. Fructose only was induced in biodegradation of Chlorpyrifos whereas all tested carbon sources failed to induce the production of phenolic compounds. Ammonium nitrate was exhibited the most preferred nitrogen source whereas other tested nitrogen sources failed in biodegradation of Chlorpyrifos.

Molecular genetics characterization

RAPD and 16S rRNA PCR-RFLP

RAPD and 16S rRNA PCR-RFLP techniques were used to conduct the genetic fingerprinting, construct the genetic relationship and determine genetic distance between the studied isolates and identify specific molecular markers for most potent isolate. An informative profile was obtained (Figures 1 and 2). The six used primers produced multiple band profiles with a variable number
Table 2. Characteristics of Chlorpyrifos-degrading strains isolated from contaminated Egyptian soils.

| Test                          | CP5  | CP6  | CP7  | CP8  | CP9  |
|-------------------------------|------|------|------|------|------|
| Gram’s reaction              | _    | _    | _    | _    | _    |
| Cell shape                    | Cocccobacilli | Short rods | Short rods | Short rods | Short rods |
| Methyl red                    | _    | _    | _    | _    | _    |
| Motility                      | _    | _    | _    | _    | _    |
| Spores                        | _    | _    | _    | _    | _    |
| Catalase                      | _    | _    | _    | _    | _    |
| KoH (3%)                      | _    | _    | _    | _    | _    |
| Oxidase                       | _    | _    | _    | _    | _    |
| Methyl red                    | +    | +    | +    | +    | +    |
| Levan formation               | _    | _    | _    | _    | _    |
| Citrate utilization           | _    | _    | _    | _    | _    |
| Voges-Proskauer test          | _    | _    | _    | _    | _    |
| Nitrite reduction             | _    | _    | _    | _    | _    |
| Fermentation of glucose       | _    | _    | _    | _    | _    |
| Fermentation of mannose       | _    | _    | _    | _    | _    |
| Fermentation of starch        | _    | _    | _    | _    | _    |
| Fermentation of arabinose     | _    | _    | _    | _    | _    |
| Fermentation of fructose      | _    | _    | _    | _    | _    |
| Fermentation of lactose       | _    | _    | _    | _    | _    |
| Fermentation of mannitol      | _    | _    | _    | _    | _    |
| Fermentation of rhamnose      | _    | _    | _    | _    | _    |
| Fermentation of sucrose       | _    | _    | _    | _    | _    |
| Production of amylase         | _    | _    | _    | _    | _    |
| Production of pectinase        | _    | _    | _    | _    | _    |
| Production of cellulose       | _    | _    | _    | _    | _    |
| Production of gelatinase       | _    | _    | _    | _    | _    |
| Production of H\(_2\)S         | _    | _    | _    | _    | _    |
| KCN resistance                | _    | _    | _    | _    | _    |

Table 3. Parameters controlling the biodegradation of Chlorpyrifos by *Pseudomonas stutzeri* –B-CP5.

| Incubation periods (days) | Phenolic compounds µg/ml | CP Concentration (mL/L) | Phenolic compounds µg/ml | Inoculum size (mL/l) | Phenolic compounds µg/ml | Temperature (°C) | Phenolic compounds µg/ml | pH   | Phenolic compounds µg/ml |
|---------------------------|--------------------------|------------------------|--------------------------|---------------------|--------------------------|-------------------|--------------------------|------|--------------------------|
| 1                         | 0.024                    | 0.1                    | 0.256                    | 0.1                 | 0.199                    | 20                | 0.123                    | 5    | 0.0                      |
| 2                         | 0.045                    | 0.15                   | 0.233                    | 0.2                 | 0.298                    | 25                | 0.199                    | 5.5  | 0.0                      |
| 3                         | 0.084                    | 0.2                    | 0.21                     | 0.2                 | 0.244                    | 30                | 0.229                    | 6    | 0.199                    |
| 4                         | 0.122                    | 0.25                   | 0.188                    | 0.5                 | 0.20                     | 35                | 0.21                     | 6.5  | 0.25                     |
| 5                         | 0.157                    | 0.3                    | 0.132                    | 1                   | 0.142                    | 40                | 0.12                     | 7    | 0.299                    |
| 6                         | 0.190                    | 0.35                   | 0.122                    | 2                   | 0.121                    | 45                | 0.11                     | 7.5  | 0.312                    |
| 7                         | 0.234                    | 0.4                    | 0.02                     | 2.5                 | 0.122                    | 50                | 0.11                     | 8    | 0.12                     |
| 8                         | 0.20                     | 0.45                   | 0.01                     | 5                   | 0.1                      | 8.5               | 0.12                     |      |                          |
| 9                         | 0.14                     | 0.5                    | 0.01                     |                     |                          |                   |                          |      |                          |

and molecular weights of amplified DNA fragments (Table 1). Different polymorphic and monomorphic markers were obtained across RAPD profiles (Table 4 and 6). As regards genetic relationships, data presented in (Table 5) showed that, the highest genetic similarity was between CP8 and CP7& (66%), while the genetic similarity between CP8 and CP6 was the lowest (37%). All isolates gave the same pattern after digestion with two
restriction enzymes (EcoRI and AluI) separately except CP6 isolate (Figure 2). Two monomorphic bands were obtained (200 and 500 bp) among five bacterial isolates after digestion. Two specific bands for CP6 were identified (420 and 130 bp) with EcoRI and ALU1 respectively (Figure 2).

**DISCUSSION**

Obtained molecular genetic data documented that the studied isolates belongs to two different genera as showed in Figure 3. The dendrogram showed phylogenetic tree which divided into two main groups. The first main group consisted of two subgroups. The first subgroup contains CP7, CP8 and CP9 isolates. The closest genetic distance was found between CP7 and CP8 isolates, which were first clustered together and then with CP9 isolate. The second subgroup of the first main group includes CP5 isolate. On the other hand, the second group includes CP6 isolate. These two genera have focused on its wide range of diverse degrading capabilities and potential application in bioremediation. This is might be due to the great genetic diversity among these two genera which confirmed by different pattern of amplification and restriction enzyme digestion based on RAPD and PCR-RFLP. Chlorpyrifos has been reported to be degraded co-metabolically by bacteria, which needs extra carbon sources (Richins et al., 1997; Mallick et al., 1999). (Singh et al., 2004) reported isolation of...
Table 4. Specific markers for each chlorpyrifos degrading bacterial isolate across RAPD-PCR analysis.

| Primer | Bacterial strain | Primers | Total |
|--------|------------------|---------|-------|
| A2     | CP5, CP6, CP7, CP8, CP9 | 5(350,520,780,850,870bp) 1(580 bp) 1(320bp) | 7 |
| A3     | CP6, CP8          | 4(230,280,630,680bp) 1(430bp) 1(850bp) | 6 |
| B3     | CP5, CP7          | 2(680,550 bp) 4(500,530,630,700 bp) 2(580,620 bp) | 8 |
| B5     | CP6, CP8          | 1(600 bp) 3(150, 620, 670 bp) | 4 |
| C3     | CP5, CP7          | 2(280,680 bp) 4(480,520,620,740 bp) 2(270, 800bp) | 8 |
| C5     | CP5, CP7, CP8, CP9 | 6(250,280,320,430,550,740 bp) | 6 |
| Total  |                  | 5 26 0 4 4 | 39 |

Table 5. Similarity coefficient values among the five studied bacterial isolates.

| Isolates | CP5 | CP6 | CP7 | CP8 | CP9 |
|----------|-----|-----|-----|-----|-----|
| CP5      | 1.00|     |     |     |     |
| CP6      | 0.40| 1.00|     |     |     |
| CP7      | 0.44| 0.44| 1.00|     |     |
| CP8      | 0.53| 0.37| 0.66| 1.00|     |
| CP9      | 0.54| 0.40| 0.55| 0.62| 1.00|

Figure 3. Dendrogram demonstrating the relationships among the five studied bacterial isolates.

Enterobacter sp. B14, which can use CP for the supply of carbon and phosphorous sources, which it stopped degrading CP in the presence of other carbon sources. However, strain Pseudomonas stutzeri (B-CP5) shows a more rapid degradation in the presence of an additional fructose as carbon source. This indicates that CP could also be degraded co-metabolically by Pseudomonas stutzeri (B-CP5), which might signify the environmental adaptation of this bacterium. Pseudomonas stutzeri (B-CP5) was distinguished and characterized by three random primers (B3, C3 and B5) among six used primers. Primer B3 generate two polymorphic bands (550 and 680 bp), C3 obtain two polymorphic bands (280 and 680 bp) while B5 release one polymorphic band (600 bp). These polymorphic bands considered as specific markers for Pseudomonas stutzeri with the three mentioned primers Table 4. On the other hand CP6 belongs to other genera so it was generate 26 polymorphic bands.
Table 6. Specific markers for bacterial isolate across RAPD and 16SrRNA PCR-RFLP analysis.

| Primer | Isolate                        | Specific marker (M.W. bp) |
|--------|--------------------------------|--------------------------|
| C5     | CP6: Enterobacter aerogenes    | 250, 280, 320, 430, 550 and 740 |
|        |                                |                          |
| B3     | CP5: Pseudomonas stutzeri      | 550 and 680              |
|        | CP6: Enterobacter aerogenes    | 500, 530 and 680         |
|        | CP8: Pseudomonas maltophilia   | 520, 580 and 630         |
| C3     | CP5: Pseudomonas stutzeri      | 280 and 680              |
|        | CP6: Enterobacter aerogenes    | 480, 520, 620 and 740    |
|        | CP9: Pseudomonas vesicularis   | 270 and 800              |
| B5     | CP5: Pseudomonas stutzeri      | 600                      |
|        | CP6: Enterobacter aerogenes    | 150, 620 and 750         |
| A2     | CP6: Enterobacter aerogenes    | 350, 520, 780, 850 and 870 |
|        | CP8: Pseudomonas maltophilia   | 580                      |
|        | CP9: Pseudomonas vesicularis   | 320                      |
| A3     | CP6: Enterobacter aerogenes    | 230, 280, 630 and 680    |
|        | CP8: Pseudomonas maltophilia   | 430                      |
|        | CP9: Pseudomonas vesicularis   | 850                      |
| EcoRI  | CP6: Enterobacter aerogenes    | 420                      |
|        | Alu                             | 130                      |

among six used random primers and two polymorphic bands (420 and 130 bp) with EcoRI and AluI, respectively. These polymorphic bands considered as specific markers for Enterobacter aerogenes.

Chlorpyrifos has been reported to be degraded in liquid media by Flavobacterium sp. (Sethunathan et al., 1973), Pseudomonas diminuta (Serdar et al., 1982) and Arthrobacter sp. (Mallick et al., 1999), which were initially isolated to degrade other organophosphate compounds. However, these microorganisms do not utilize CP as a source of carbon but as a co-metabolite (Singh et al., 2003; Yang et al., 2006). Recently a Stenotrophomonas sp. has been isolated, which is capable of degrading both CP and TCP (Yang et al., 2006; Lakshmi et al., 2008). In conclusion, among the five studied isolates Pseudomonas stutzeri (B-CP5) was the most potent isolate to degrade the Chlorpyrifos. The results of this study demonstrate the usefulness of the RAPD-PCR analysis for detecting DNA polymorphism in bacterial isolates and establishing the relationships among different isolates. The majority of random primers used gave distinctly reproducible patterns in the entire isolates studied. However, primers are varied in the extent of information. Primers B5 and A2 revealed highly polymorphic patterns. Whereas, less polymorphic products were generated by C3 primer. Some DNA fragments were apparently similar in size among the studied isolates, whereas others were unique to a particular isolate. Therefore, different polymorphic and monomorphic bands were given and some of monomorphic considered as specific markers for identifying and tracking these bacterial isolates.

REFERENCES

Bayoumi RA, Awad NS, Ibrahim MMM (2010). Molecular genetic characterization of some Biosurfactant producing bacteria isolated from Egyptian red sea mangrove forests. Arab J. Biotech., 13(2): 209-222.

Bhagobaty RK, Joshi SR, Malik A (2007). Microbial degradation of organophosphorous pesticide: Chlorpyrifos. Internet J. Microbiol., 4: 1.

Conville PS, Fischer SH, Cartwright CP, Witebsky FG (2000). Identification of Nocardia species by restriction endonuclease analysis of an amplified portion of the 16S rRNA gene. J. Clin. Microbiol., 38: 158-164.

Holt JG, Krieg NR, Sneath PH, Staley JT, Williams ST (1994). Bergey's manual of determinative Bacteriology, 9th edn, Williams & Wilkins, Baltimore.

Krieg NR, Holt JG (1984). “Bergey's Manual of Systematic Bacteriology.” vol. 1, Williams & Wilkins Co., Baltimore, pp. 161–172.

Lakshmi CV, Kumar M, Khanna S (2008). Biotransformation of chlorpyrifos and bioremediation of contaminated soil. International Biodeterio. and Biodegrad. Under Publication, 33: 2195–2198.

Li X, He J, Li S (2007). Isolation of a chlorpyrifos –degrading bacterium, Shengomonas sp. strain Dsp-2 and cloning of the mpd gene. Res. Microbiol., 158: 143-149.
Mallick K, Bharati K, Banerji A, Shakil NA, Sethunathan N (1999). Bacterial degradation of chlorpyrifos in pure culture and in soil. Bull. Environ. Contam. Toxicol., 62: 48-54.

Marshall SM, Mello PI, Woodward DL, Johnson WM, Rodgers FG, Mulvey MR (1999). Rapid identification of Campylobacter, Arcobacter, and Helicobacter isolates by PCR-restriction fragment length polymorphism analysis of the 16S rRNA gene. J. Clin. Microbiol., 37: 4158-4160.

Nie NH, Hull CH, Jenkins JG, Steinbremmer, Bent DH (1975). Statistical package for the social sciences. 2nd edn. Megraw Hill, New York.

Nowrouzian F, Agnes E, Ingegerd A (2001). Computer-Based Analysis of RAPD (Random Amplified Polymorphic DNA) Fingerprints for Typing of Intestinal Escherichia coli. Mol. Biol. Today, 2(1): 5-10.

Racke KD, Laskowski DA, Schultz MR (1990). Resistance of chlorpyrifos to enhanced biodegradation in soil. J. Agric. Food Chem., 38: 1430-1436.

Richins RD, Kaneva I, Mulchanadani A, Chen W (1997). Biodegradation of organophosphorous pesticides by surface-expressed organophosphorous hydrolase. Nature Biotechnol., 15: 1984-987.

Serdar CM, Gibson DT, Munneck DM, Lancaster HM (1982). Plasmid involvement in parathion hydrolysis of Pseudomonas diminuta. Appl. Environ. Microbiol., 44: 246-249.

Sethunathan NN, Yoshida T (1973). A Flavobacterium that degrades diazinon and parathion. Canad. J. Microbiol., 19: 873-875.

Singh BK, Walker A, Morgan AJ, Wright DJ (2004). Microbial degradation of chlorpyrifos by Enterobacter strain B-14 and its use in bioremediation of contaminated soils. Appl. Env. Micro., 70: 4855-4863.

Singh BK, Walker A, Morgan AJ, Wright DJ (2003). Effects of soil pH on the biodegradation of chlorpyrifos--degrading bacterium. Appl. Environ. Microbiol., 9: 5198-5206.

Steinhauserova I, Ceskova J, Fojtikova K, Obrovska I (2001). Identification of thermophilic Campylobacter spp. by phenotypic and molecular meth. J. Appl. Microbiol., 90: 470-475.

Strong PJ, Burgess JE (2008). Treatment methods for wine-related and distillery wastewaters: a review. Bioremediation J., 12: 70-87.

Wilson MJ, Wade WG Weightman AJ (1995). Restriction fragment length polymorphism analysis of PCR amplified 16S ribosomal DNA of human Capnocytophaga. J. Appl. Bacteriol., 78: 394-401.

Woese CR (1987). Bacterial evolution. Micr. Rev., 51: 221-271.

Yang C, Liu N, Guo X, Qiao C (2006). Cloning of mpd gene from a chlorpyrifos-degrading bacterium. FEMS Microbiol. Lett., 251: 67-73.