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Full Length Article

Isolation, Characterization and Identification of Organophosphate Pesticide Degrading Bacterial Isolates and Optimization of their Potential to Degrade Chlorpyrifos

Samina Ambreen and Azra Yasmin*
Microbiology & Biotechnology Research Lab., Department of Biotechnology. Fatima Jinnah Women University, The Mall, Rawalpindi. Pakistan
*For correspondence: azrayasmin@fjwu.edu.pk
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Abstract

Current study was aimed to isolate indigenous organophosphate (OP) degrading soil bacteria from agricultural soils of Mochh, district Mianwali, Pakistan. Among a large number of bacterial isolates obtained, four best bacterial isolates (MB490, MB497, MB498 and MB504) were selected based on their tolerance against multiple OP pesticides (Chlorpyrifos, Triazophos and Dimethoate), for further analyses to optimize their potential to degrade Chlorpyrifos (CPF). These isolates could tolerate 0.8-8 g L^{-1} of Chlorpyrifos, 2-4 g L^{-1} of Triazophos and 0.22–4 g L^{-1} of Dimethoate. These bacteria showed multiple heavy metal resistance and possessed enzymes like Nitrate reductase, Oxidase and Catalase needed for biodegradation. When analyzed for CPF degradation by HPLC, these isolates exhibited 67.22 to 99.48% degradation of CPF (200 mg L^{-1}) in M-9 broth under wide range of pH (6, 7 and 8) and temperatures (25, 30 and 37◦C). Strain MB490 displayed maximum CPF degradation at pH 6, while other three isolates were best at alkaline pH 8. Isolates MB490 and MB498 were best degraders of CPF at 37◦C in comparison to MB497 and MB504, which showed highest degradation at 30◦C. These four strains were identified as *Pseudomonas kilonensis* MB490, *Bacillus thuringiensis* MB497, *Pseudomonas kilonensis* MB498 and *Pseudomonas* sp. MB504 based on 16S rRNA analysis. The above findings suggest that these novel isolates can be used efficiently under a wide range of temperature and pH for remediation of Chlorpyrifos polluted agricultural soils and water resources under local conditions. © 2020 Friends Science Publishers

Keywords: Biodegradation; Organophosphate; Pesticide
Abbreviations: OP = Organophosphate, CPF = Chlorpyrifos, TCP = 3, 5, 6-trichloropyridinol, RT = Retention time

Introduction

Organophosphate pesticides (OP) have been used in the agriculture all over the world to increase food productivity as well as to reduce vector-borne diseases in plants and animals**.** Their widespread use has resulted in prevalent environmental pollution, causing great damage to non-target species including humans through food chain (Kim *et al*. 2017). Chlorpyrifos (CPF), Triazophos (TAP) and Dimethoate (DM) are important members of organophosphate pesticides and have been extensively used in Pakistan for controlling agricultural, domestic and veterinary pests. These OP pesticides have a potential to inactivate acetyl cholinesterase enzyme in insects, human beings and many other animals, which causes nerve toxicity due to accumulation of acetylcholine (Liu *et al*. 2009). Chlorpyrifos has been applied on crops like cotton, wheat, fruit and vegetables against domestic and agricultural pests including corn rootworms, cockroaches, fleas, termites, flies, and lice (Das and Adhya 2015). The CPF pollutants are toxic and recalcitrant and so must be controlled. Both biotic and abiotic pathways contribute for the degradation of pesticides in soil and water. However, microbial degradation is considered as the chief route for pesticide breakdown and detoxification (Pankaj *et al*. 2016; Moorman 2018). The breakdown of the pesticide residues in the soil by the indigenous microflora is very slow and may be improved by the addition of more efficient biodegrading inoculum of microbes to the soil. This process is called bioaugmentation and is used to accelerate the bioremediation of polluted soils (Singh and Walker 2006). In recent times, many research studies have been focused on isolating indigenous bacteria from different sources that are capable to remove OP pesticides thus offering an environmentally friendly, effective and inexpensive method for *in situ* cleansing of pesticides (Tang *et al*. 2017). Biodegradation of CPF is possible only by the microbes having suitable mineralizing enzymes and

depends on some ecological, physiological, molecular and biochemical characteristics of these microbes. The most significant OP pesticide degrading enzymes includes Phosphotriesterases (PTEs) and Organophosphorus hydrolases (OPH) (Ghanem and Raushel 2005; Naphade *et al.* 2013; Schenk *et al.* 2016). There are many previous reports of CPF degradation by variety of bacterial isolates that are capable of using CPF as a sole source of carbon for their growth (Chishti and Arshad 2013; Supreeth and Raju 2016; Rayu *et al*. 2017). Chlorpyrifos breakdown products like CPF oxon and 3, 5, 6-trichloropyridinol have been considered as less toxic for nervous system than the parent compound (Wu *et al*. 2017). Soil pH and temperature is very crucial factor for microbial survival in soil, hence it profoundly affects microbial degradation of Chlorpyrifos (Singh *et al.* 2003). Therefore, temperature and pH optimization of CPF degrading bacterial strains is very critical in order to evaluate their potential to tolerate local temperature and pH conditions.

Present study was focused on the isolation and characterization of organophosphate pesticide degrading bacteria from agricultural soils having a history of OP pesticide treatment and screening of these local bacteria, optimization of conditions for their growth and CPF degradation in minimal broth.

Materials and Methods

The commercial-grade Organophosphate pesticides (Chlorpyrifos, Triazophos and Dimethoate) with 40% EC (400 g L^{-1}) were used throughout this study, which were procured from "Four Brothers Agri services, Pakistan". All other chemicals used in the study were purchased from Sigma-Aldrich or Merck.

Soil sampling and isolation of bacterial isolates

Soil samples in triplicate were taken from agricultural fields frequently treated with organophosphate pesticides, at Dera Saleemabad, Mochh, District Mianwali (Punjab), Pakistan. The soil samples were dried, sieved and mixed thoroughly to make the composite sample.

To isolate bacterial colonies, soil suspension was prepared, serially diluted (1/10, 1/100, 1/1000) and plated on nutrient agar medium. Total CFU g^{-1} of soil was calculated and among diverse colonies (10^5 CFU g^{-1} of soil), four bacterial colonies were selected on the basis of morphological variations and purified by single colony streaking for further studies.

pH and temperature optimization of bacterial growth

In order to study the effect of pH on bacterial growth, 25 μL inoculum for each isolate (MB490, MB497, MB498 and MB504) was given separately in 5 mL Nutrient broth having different pHs (5, 6, 7, 8, 9, 10 & 11) and incubated along with controls (without inoculum) under shaking conditions (150 rpm) of incubation at 37°C for 24 h. Similarly, for temperature optimization, bacterial isolates were grown in the nutrient broth at various temperatures (25°C, 30°C, 37°C and 42°C) at pH 7 and kept under static conditions along with controls for 24 h. After the completion of incubation period, bacterial growth was measured in terms of optical density (OD) using UV/Vis Spectrophotometer (BMS UV-160) at 600 nm.

Screening of bacterial isolates for CPF, TAP and DM resistance

Chlorpyrifos resistance assay was performed using M-9 minimal medium composed of following ingredients (g L^{-1}): Na_2HPO_4 (6.0), KH_2PO_4 (3.0), NaCl (0.5), NH_4Cl (1.0), Agar (15). Moreover, 1 mL of 0.1 *M* $CaCl_2$ solution, 1 mL of 1 *M* $MgSO_4$ solution and 5 g L^{-1} of Casein hydrolysate were also added after autoclaving (Sambrook *et al.* 1989) with and without 20% glucose (2 g L^{-1}) supplemented with varying concentrations (0.04, 0.22, 0.4, 0.6, 0.8, 2, 4, 6, and 8 g L^{-1}) of pure commercial grade (EC 400 g L^{-1}) CPF, TAP and DM separately. The four most promising bacterial isolates, designated as MB490, MB497, MB498 and MB504 having the highest tolerance for three OP pesticides, were selected for further CPF degradation studies.

Biochemical characterization

Four isolates were characterized using various biochemical tests (Oxidation fermentation test, Oxidase test, Nitrate Reductase test, Catalase tests, growth on MacConkey's agar medium and Eosin Methylene Blue (EMB) agar medium using standard methods.

Molecular analysis

Total genomic DNA was extracted from the bacterial isolates according to method described by Wilson (1990). The colony PCR was performed for the amplification of 16S rRNA gene. For this purpose, 0.4 μL (0.5 μM) each of forward (27F) and reverse primers (1492R), 12.5 μL of commercial master mix (Go Green Mastermix, Promega) and 8.7 μL PCR water were mixed together in a sterilized PCR tube. Then, 22 μL of this PCR mix was taken in another sterile PCR tube for each isolate and 3 μL of genomic DNA was mixed with it to obtain final volume of 25 μL of PCR reaction mixture. PCR reaction was established in the Thermocycler (96 universal Gradient Peq Star, Peq Lab., U.K.). During agarose gel electrophoresis of PCR products, loading dye was also integrated. PCR protocol was initiated involving denaturation at 95°C (for 4 min) then 29 cycles of denaturation at 94°C for up to 30 s, followed by annealing at 55°C for 1 min time period, next step was extension at 72°C for 1 min plus an additional final extension at 72°C for 10 min (Rayu *et al*. 2017). Blank

reaction without DNA was used as control. Purified PCR products were further submitted to Macrogen for 16S rRNA sequence-based identification of bacterial isolates. The evolutionary history of bacterial strains was inferred using the maximum likelihood method (Tamura *et al.* 2013) on the basis of maximum similarity and evolutionary analyses were conducted in MEGA6.

Heavy metal resistance profile

Bacterial isolates were tested for their heavy metal resistance by growing them on M-9 medium supplemented with 50–3000 μg mL^{-1} of various metals like Ni^{+2} ($NiCl_2$), Cr^{+6} ($K_2Cr_2O_7$), Mn^{+2} ($MnCl_2$), Cd^{+2} ($CdCl_2$), Cu^{+2} ($CuSO_4$), Zn^{+2} ($ZnSO_4$), Pb^{+2} [$Pb(NO_3)_2$], Co^{+2} ($CoCl_2$) and Fe^{+3} ($FeCl_3$) for 24 h at 37°C.

Organic pollutants profile

In the current scenario of increasing industrialization, organic pollutants are constantly contaminating our soil and water resources Therefore, bacterial isolates were also checked for their resistance towards various concentrations (0.1–6%) of different organic pollutants (Benzene, Toluene, Xylene, Aniline, Biphenyl and Naphthalene).

Optimization for CPF biodegradation

In order to study the effect of different pH and temperatures on the biodegradation of CPF, 500 μL of 24 h old bacterial inoculum (OD_{600} of 0.6 and CFU mL^{-1} g^{-1} of 10^6) was used for each isolate in 30 mL M-9 broth supplemented with 200 mg L^{-1} of CPF at different pHs (6, 7 and 8) along with un-inoculated controls and were incubated in shaker incubator (150 rpm) at 37°C for 24 h. While for temperature optimization CPF degradation was studied at different temperatures (25, 30 and 37°C) at pH 7 under static conditions along with controls. After incubation period of 24 h, bacterial growth was monitored in terms of OD 600 using UV-Vis Spectrophotometer (BMS UV-160). The extraction was done according to the method of Rokade and Mali (2013). For this purpose, 4 mL of the sample was centrifuged at 3500 rpm for 20 min and then ethyl acetate was added to supernatant in 1:1 ratio, and dried with 5g of anhydrous Na_2SO_4 to absorb moisture. After 30 min of shaking 1.5 mL of upper organic layer was collected in an eppendorf and used for HPLC analyses after being microfiltered by using Sartorius Ministart sterile syringe filters (0.45 μm) to remove any particles. The CPF degradation under shaking versus static condition was also studied using the same method as described above at pH 7 and 37°C.

HPLC conditions used for the analyses of chlorpyrifos degradation

The Chlorpyrifos degradation was analyzed using High-performance liquid chromatography (HPLC) LC-20AT equipped with a UV-Visible detector (SPD-20A) and a C18 column (0.46 x 15 cm). HPLC conditions used were adopted from Alvarenga *et al.* (2015). The retention times of 5.4 and 1.77 min for standard solutions of Chlorpyrifos and 3, 5, 6-trichloropyridinol respectively were determined by HPLC.

The percentage degradation of CPF was calculated by using the method given by Eissa *et al*. (2014). Whereas, concentration of residual CPF (mg L^{-1}) was calculated by comparing peak areas in the chromatogram of sample with that of peak area of the chromatogram of standard CPF as given below:

Concentration of CPF residue in sample (mg L^{-1}) = Peak area of chromatogram of sample ÷ Peak area of chromatogram of standard CPF× concentration of standard CPF (Bishnoi *et al*. 2009).

Results

Morphological and biochemical studies

Three isolates (MB490, MB498 and MB504) were found as Gram negative, while MB497 was observed Gram positive. All were rods, non-spore forming, non-capsulated, facultative anaerobes and non-lactose fermenters. All isolates were positive for Catalase, Oxidase, and Nitrate reductase activity.

pH and temperature optimization of bacterial growth

Strain MB490 was neutrophilic as it showed its optimum growth at pH 7, while isolate MB504 found slight acidophilic showing optimum growth at pH 6. Strains MB497 and MB498 were alkaliphilic with optimum growth at pH 8 and pH 9 respectively. However, there was less growth by all isolates at higher alkaline pHs of 10 and 11. All isolates were mesophilic with best growth at 30°C (MB497), 37°C (MB490, MB504) and 42°C (MB498). Moreover, all isolates showed considerable growth at all given pH (6–11) and temperature (25–42°C) ranges, thus indicating their ability to survive under extreme pH and temperatures.

Screening for OP pesticides tolerance

All bacterial isolates showed equally good growth on OP supplemented M-9 medium with and without glucose, thus proving that they could utilize OP pesticides as a sole source of carbon. For CPF, strain MB497 was noticed most tolerant (up to 8 g L^{-1}), whereas isolates MB490 and MB498 showed tolerance up to 6 g L^{-1} followed by MB504 (0.8 g L^{-1} of CPF). In case of TAP, isolates MB490, MB497 and MB498 proved equally good to tolerate up to 4 g L^{-1} of the compound as compared to MB504 which could resist up to 2 g L^{-1}. Likewise, MB490, MB497 and MB498 showed growth up to 0.4 g L^{-1} of DM, while MB504 was least tolerant (up to 0.22 g L^{-1}) of DM (Table 1).

Table 1: Comparison of maximum tolerance and growth of bacterial isolates against three OP pesticides (CPF, TAP and DM) in M-9 medium without glucose

Bacterial Isolates	Maximum OP pesticide tolerance (g L^{-1})		
	CPF	TAP	DM
MB490	(++) 6	(++) 4	(++) 0.4
MB497	(++) 8	(++) 4	(++) 0.4
MB498	(++) 6	(++) 4	(++) 0.4
MB504	(++) 0.8	(++) 2	(++) 0.22

++ = Good growth

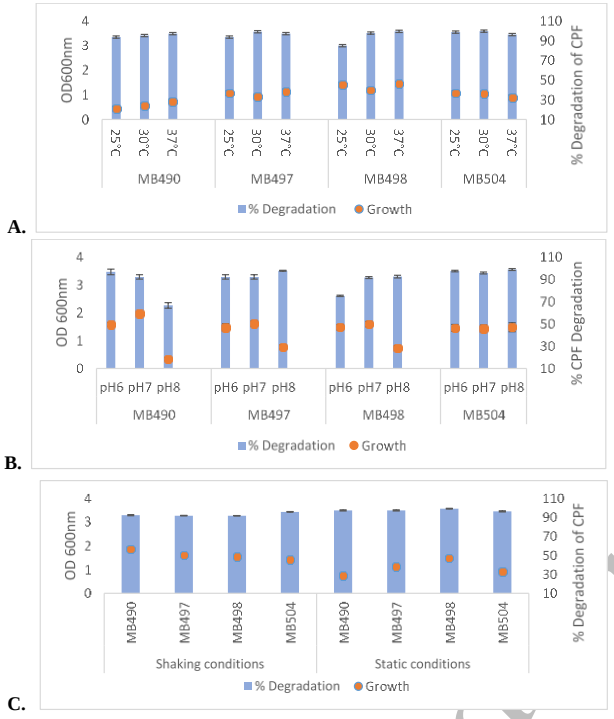

Fig. 1: Effect of (**A**) Temperature, (**B**) pH, (**C**) Shaking versus static conditions on growth and degradation of CPF by four isolates (MB490, MB497, MB498 and MB504) after 24 h. Error bars represent standard errors for values of three sample replicates

Heavy metal tolerance profile

In current study, all isolates could tolerate lead as [Pb $(NO_3)_2$] up to 2000 μg L^{-1}. Strain MB497 was most tolerant to 3000 μg L^{-1} of Manganese ($MnCl_2$), followed by isolates MB504, MB498 and MB490 which could grow up to 1000, 500 and 300 μg $L^{-1}$of Mn respectively. On the contrary, all isolates were very sensitive to Cadmium ($CdSO_4$). Isolates exhibited growth in the presence of different concentrations (50–300 μg L^{-1}) of Ni, Fe, Cu and Zn (Table 2).

Screening experiments against other organic pollutants

All isolates were capable of tolerating Benzene, Toluene, Xylene, Biphenyl and Naphthalene up to 5% (both with and without glucose). These showed sensitivities to Aniline having no growth, at all given concentrations (Table 3).

Effect of temperature on CPF degradation by bacterial isolates

All bacterial isolates exhibited significant CPF degradation (83.74–99.48%) at different temperatures (25, 30, 37°C) with different growth tendencies. Strains MB490 and MB498 exhibited highest CPF degradation (97 and 99.36% respectively) at 37°C, while strains MB497 and MB504 were performing best at 30°C with 98.88 and 99.48% CPF degradation respectively (Fig. 1A). There was only 3. 6 and 9% CPF degradation in the control was at 25, 30 and 37°C respectively, thus endorsing the key role of isolates in pesticide degradation. During HPLC analysis, all four isolates (MB490, MB497, MB498 and MB504) showed maximum peak reduction of CPF (RT = 5.4 min) at their respective optimum temperatures (Fig. 2, 3, 4 and 5). For example, the height of the CPF peak in the HPLC chromatogram for standard CPF was recorded at about 250 mAU (absorbance), while it was reduced to 15, 12 and 7.5 mAU after CPF degradation by MB490 at 25, 30 and 37°C, respectively (Fig. 2). Peak for 3, 5, 6-trichloropyridinol (RT = 1.77 min) was also prominent in many chromatograms which was determined by comparing with peak of standard TCP (Fig. 5E). Many different peaks for unknown metabolites also appeared at all given temperatures.

Effect of pH on CPF degradation by bacterial isolates

Strain MB490 showed best CPF degradation (97%) at acidic pH (pH 6), whereas isolates MB497, MB498 and MB504 showed maximum CPF degradation (99.39, 92.91 and 98.87% respectively) at alkaline pH 8 indicating involvement of alkaline phosphatases or some other similar degrading enzymes (Fig. 1B). HPLC chromatograms for effect of pH on CPF degradation by bacterial isolates are given in supplementary Fig. S1, S2, S3 and S4.

CPF degradation and growth by bacterial isolates under shaking versus static conditions of incubation

There was no substantial variation in CPF biodegradation and bacterial growth under shaking (aerobic) and static culture conditions after 24 h of incubation. In general, there was little more CPF degradation by the bacterial isolates (96 to 99.36%) under static than shaking conditions. Nevertheless, more than 90% CPF degradation was recorded under both conditions (Fig. 1C).

Molecular characterization

Four isolates MB490, MB497, MB498 and MB504 were characterized on molecular basis using 16S rRNA and identified as *Pseudomonas kilonensis* MB490 (accession no. MG685888), *Bacillus thuringiensis* MB497 (accession no. Kp886829), *Pseudomonas kilonensis* MB498 (accession no. MG685889) and *Pseudomonas* spp. MB504 (accession no. KP886830) respectively (Fig. 6A, B, C and D).

Table 2: Maximum tolerance of bacterial isolates against heavy metals

Bacterial isolates	Maximum Tolerance against heavy metals concentration (μg mL^{-1})								
	$NiCl_2$	$FeCl_3$	$CoCl_2$	$K_2Cr_2O_7$	$CuSO_4$	$ZnSO_4$	$PbNO_3$	$CdSO_4$	$MnCl_2$
MB 490	50	100	-	-	100	-	2000	-	300
MB497	300	50	50	50	100	200	2000	-	3000
MB498	50	100	-	50	100	200	2000	-	500
MB504	100	50	-	-	100	200	2000	-	1000

- = No growth

Table 3: Maximum tolerance of bacterial isolates against organic pollutants/chemicals

Bacterial isolates	Maximum Tolerance against organic chemicals concentration (%)					
	Benzene	Toluene	Xylene	Aniline	Biphenyl	Naphthalene
MB490	5	5	5	-	5	5
MB497	5	5	5	-	5	5
MB498	5	5	5	-	5	5
MB504	5	5	5	-	5	5

- = No growth

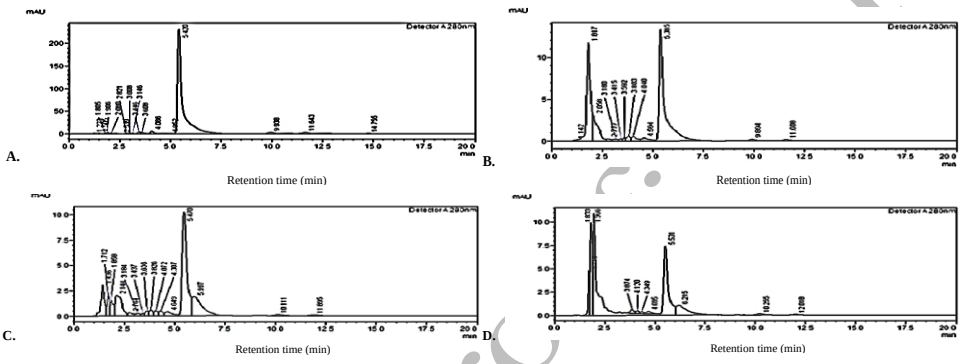

Fig. 2: Effect of temperature on degradation of CPF (RT= 5.4 min) by MB490. (**a**). Control, (**b**). MB490 at 25°C (**c**) 30°C (**d**) 37°C

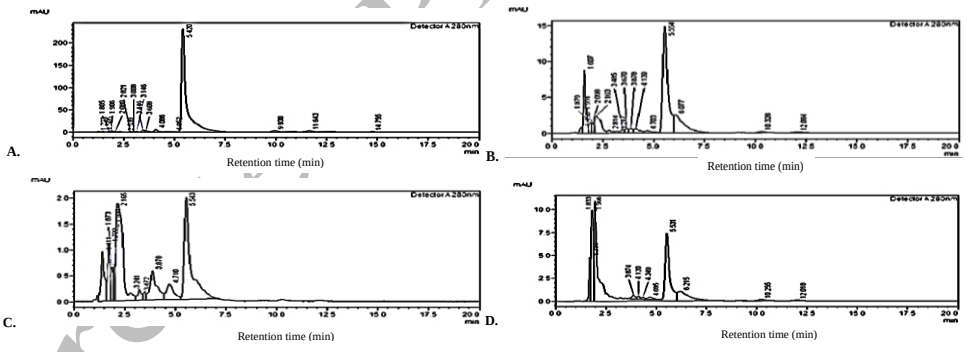

Fig. 3: Effect of temperature on degradation of CPF (RT= 5.4 min) by MB497. (**a**) Control, (**b**) MB497, at 25°C (**c**) 30°C (**d**) 37°C

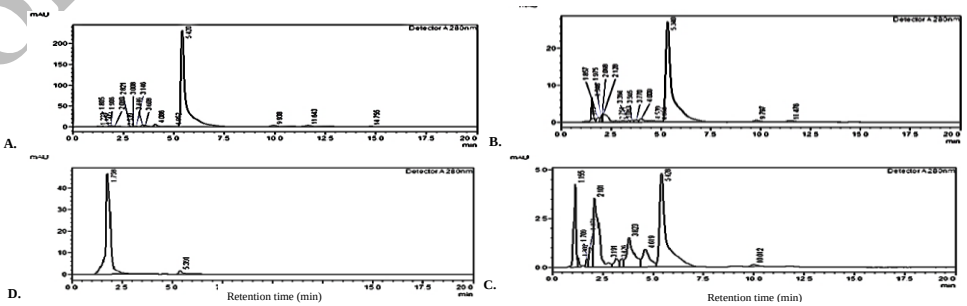

Fig. 4: Effect of temperature on degradation of CPF (RT= 5.4 min) by MB498. (**A**) Control, (**B**) MB498, at 25°C (**C**) 30°C (**D**) 37°C. A single and prominent peak of TCP can be noted at 1.77 min at 37°C

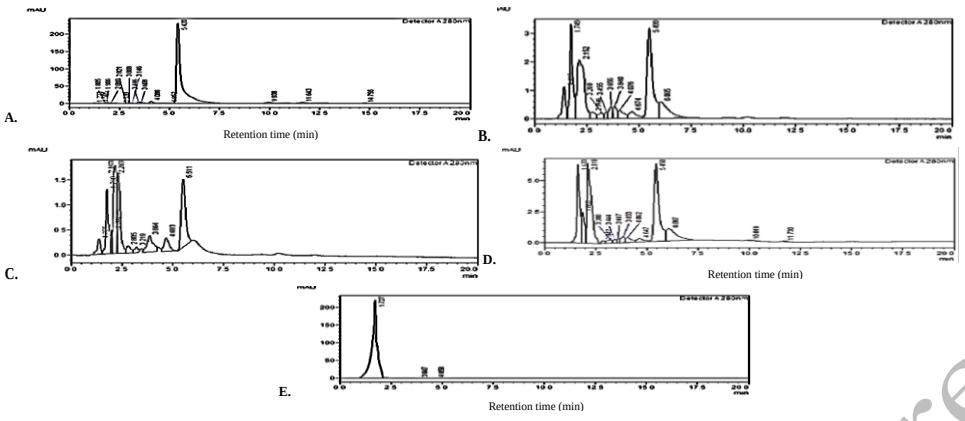

Fig. 5: Effect of temperature on degradation of CPF (RT = 5.4 min) by MB504. (**A**) Control, (**B**) MB504, at 25°C (**C**) 30°C (**D**) 37°C. (**E**) HPLC chromatogram of standard TCP for comparison to confirm formation of TCP during CPF degradation.

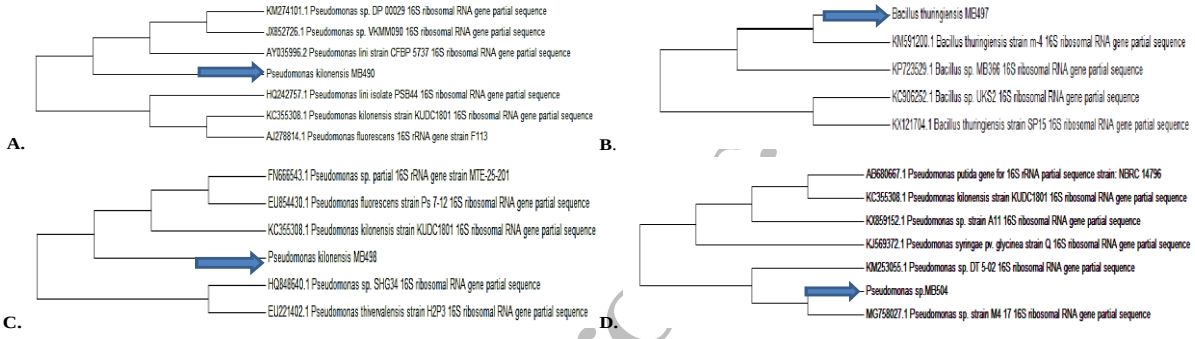

Fig. 6: Evolutionary relationships of **A**). *Pseudomonas kilonensis* MB490. **B**). *Bacillus thuringiensis* MB497. **C**). *Pseudomonas kilonensis* MB498. **D**). *Pseudomonas* spp. MB504 after 16S rRNA sequencing based on Neighbor-joining method

Discussion

There have been reports of biodegradation of many OP pesticides by isolated bacteria from different sources like agricultural soil, wastewater sludge, marine water, and even from fish (Ghanem and Raushel 2005; Mandal *et al*. 2005; Farhan *et al*. 2013). Earlier, Ghanem *et al*. (2007) revealed that 17.3 g L^{-1} of Chlorpyrifos was tolerated by isolate *Klebsiella* spp. Similarly, *Aeromonas*, *Pseudomonas* and *Klebsiella* could tolerate 4, 2 and 8 g L^{-1} of CPF respectively as demonstrated by Ajaz *et al.* (2005), which is comparable with present study outcomes. It was revealed that *Bacillus* TAP1 could tolerate TAP up to 100 mg L^{-1} (Tang and You 2012). Likewise, *Proteus vulgaris* and *Bacillus licheniformis* were capable of growing in presence of Dimethoate up to 0.005 mg mL^{-1} and 3.5 mg mL^{-1} respectively (Mandal *et al*. 2002, 2005). Earlier, Begum and Aundhati (2016) revealed that *Pseudomonas* spp. had potential of both heavy metal tolerance and organophosphate degradation. Wang *et al*. (2008) reported that certain marine bacterial species including *Pseudomonas* and *Bacillus* could tolerate benzene, toluene and xylene up to 5–20%.

Similarly, strain *Pseudomonas putida* was able to achieve maximum (76%) degradation of given 2% CPF at pH 7 and at 35°C (Vijayalakshmi and Usha 2012). Singh *et al*. (2004) revealed rapid degradation of Chlorpyrifos by *Enterobacter* spp. at 35°C. Likewise, CPF was degraded most efficiently by isolated strains of *Agrobacterium, Enterobacter* spp., *Pseudomonas* spp. and *Bacillus cereus* at 30°C and pH 7 (Awad *et al.* 2011; Liu *et al.* 2012; Chishti and Arshad 2013).

The bacterial growth (OD600 nm) mostly indicated an abrupt correlation with % CPF degradation at the given pH and temperature range, as there was less bacterial growth exhibiting maximum CPF degradation and vice versa (Fig. 1 and 2), probably indicating involvement of all bacterial machinery and energy for the production of OP degrading enzymes rather than cell division (Hett and Rubin 2008). Singh *et al*. (2003), reported maximum CPF degradation exhibited by an *Enterobactor* spp. at alkaline pH as compared to less degradation at acidic pH. Similarly, Anwar *et al*. (2009) reported highest degradation of CPF (40 mg L^{-1}) by *Bacillus pumilus* at higher pH (8.5). Present study results, where three of the four isolates (MB497, MB498 and MB504) exhibited maximum CPF degradation at pH 8, are in agreement with previous studies mentioned above, thus indicating that some key enzyme(s) involved in

Chlorpyrifos degradation may have their optimum enzymatic activity at alkaline pH. Current study results are partially in agreement with those reported by Chishti and Arshad (2013), who revealed higher CPF degradation under static conditions, by one strain SWLC2, while in contrast their other four isolates exhibited higher degradation under shaking condition. Likewise, *Pseudomonas putida* could have lesser CPF degradation at higher speed of shaking which was probably due to less contact between the pesticide and the culture (Vijayalakshmi and Usha 2012).

There are some earlier reports of isolation of *Bacillus* spp. capable of degrading CPF like *Bacillus pumilus* C2A1 and *Bacillus subtilis* (Anwar *et al*. 2009; El-Helow *et al*. 2013). Similarly, *Pseudomonas* spp. have been reported as capable of degrading OP-compounds such as *Pseudomonas putida* POXN01, *Pseudomonas* spp., *Pseudomonas kilonensis* SRK1 (Choi *et al*. 2009; Iyer *et al*. 2013; Khalid *et al*. 2016). Strain *Pseudomonas kilonensis* SRK1 was demonstrated by Khalid *et al*. (2016) to degrade 50% CPF (initial 150 mg L^{-1}) at pH 8 using glucose as extra carbon source. In contrast, two strains of *Pseudomonas kilonensis* MB490 and *Pseudomonas kilonensis* MB498 in present investigation, were able to remove 97 and 99.36% of CPF (initial 200 mg L^{-1}) respectively at optimum conditions of 37°C and pH 7 under static conditions without any supplementary carbon source in M-9 broth after 24 h thus indicating their higher capability to degrade CPF without depending on any extra nutrients like glucose for the induction of OP degrading enzymes.

Conclusion

On the basis of current findings, it can be suggested that *Pseudomonas kilonensis* MB490, *Bacillus thuringiensis* MB497, *Pseudomonas kilonensis* MB498 and *Pseudomonas* spp. MB504 are capable of tolerating high concentrations of multiple OP pesticides (CPF, TAP and DM) as well as heavy metals and organic compounds. Isolated strains showed ability to grow and degrade Chlorpyrifos under a wide range of temperature and pH. They were able to grow on selective M-9 medium with and without glucose, thus showing their capability to degrade and use pesticide as a sole source of carbon. They were capable of degrading CPF under both shaking and static conditions with very good results. In the current study, four strains were found to be facultative anaerobes thus showing their potential to grow under both aerobic and anaerobic conditions like those in the upper soil layers and in the deeper soil profiles, respectively. The biochemical tests also confirmed that isolated bacteria had all the enzymes necessary for biodegradation like Nitrate Reductase, Oxidase, Catalase *etc*. Thus, it can be strongly recommended that these indigenous bacteria have great potential to be applied for bioremediation of agricultural soils and water bodies contaminated with CPF.

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