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Research Article

IMPACT OF CHLORPYRIFOS ON *BACILLUS CEREUS* OP3 SOIL BACTERIA ISOLATED FROM RHIZOSPHERE

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ABSTRACT

Chlorpyrifos is a pesticide belongs to organophosphate group and widely used in Indian agriculture. The evaluation of chlorpyrifos removal from the soil using pesticide degrading bacteria will help in the resilience of soil fertility. Bacteria capable of degrading chlorpyrifos were isolated from chlorpyrifos-treated agricultural soil. The degradation of chlorpyrifos by a potential isolate was determined using FTIR and quantified using the molecular extinction coefficient in an ELISA reader. GC-MS was used to analyze the chlorpyrifos degradation metabolites. Additionally, the effect of chlorpyrifos exposure on genomic DNA and total protein content was determined. A total of 174 bacterial colonies were isolated, of which only 40 bacterial colonies were pesticide tolerant in 100ppm chlorpyrifos. Among them, only three isolates (OP3, OP5, and OP7) demonstrated tolerance above 100ppm. Within five days of incubation, OP3 demonstrated tolerance to chlorpyrifos concentrations up to 300ppm and 47 percent of chlorpyrifos was removed. By 16S rRNA sequencing, potential OP3 isolates were identified as *Bacillus cereus* OP3. FTIR spectra confirmed that *B. cereus* degraded chlorpyrifos, with 92.4 percent of chlorpyrifos degraded. In GC-MS analysis, metabolites such as M-Nitroaniline and 4-Methyl-2-{(4-Nitrophenyl)Thio}Pyrimidine were detected. Chlorpyrifos overexposure alters the total protein content of exposed cells and induces DNA breaks. As a result, it is concluded that *B. cereus* is able to degrade chlorpyrifos and the excessive use of pesticides make some changes in the soil bacteria. Overexposure of chlorpyrifos leads to an increase in total protein content and induce DNA strands breaks in pesticide degrading *Bacillus cereus*.

Keywords: *Bacillus cereus OP3*, Chlorpyrifos, Degradation, Metabolites.

1. INTRODUCTION

Chlorpyrifos (O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate} is an organophosphorus insecticide widely used throughout the world [1]. Chlorpyrifos (CP) has a half-life typically between 10 and 120 days in soil but can be as long as one year depending on abiotic factors such as temperature, moisture and pH [2].

The widespread use of chlorpyrifos pollutes the environment and causes significant harm to non-target species. Heavy soil application of chlorpyrifos increases the risk of pesticide residue accumulation and adverse health consequences for humans, including developmental toxicity, liver damage, reproductive defects, endocrine disruption, nervous system disorders, and immune system abnormalities [3, 4]. CP has also been shown to be acutely toxic to a variety of animals, including aquatic invertebrates and fish, arthropods, and soil microorganisms [5].

Continuous pesticide application over period of time can have a detrimental effect on soil microbial diversity. Although chlorpyrifos had null effect on soil microbial biomass [6], its effects on soil microbial properties such as enzymatic activity of microorganisms, microbial respiration, microbial biomass, and microbial diversity have been examined on a regular basis. Discoveries indicated that chlorpyrifos application and 3,5,6-trichloro-2-pyridinol accumulation resulted in statistically significant reductions in soil microbial biomass [7] and soil enzyme activities [8].

Chlorpyrifos pollutant removal is most critical when it becomes toxic and recalcitrant. The most potential cleaning mechanism for the bioremediation of chlorpyrifos-contaminated environments was done by the chlorpyrifos-degrading bacteria. The isolation of chlorpyrifos-degrading bacteria has been very complicated, considering the widespread use of chlorpyrifos for almost 50 years. However, researchers have attempted to isolate the bacteria that degrade chlorpyrifos [9, 10]. Hence, the current study focused

on isolating chlorpyrifos degrading bacterial strains from agricultural soil, determining their ability to degrade chlorpyrifos, and examining the effect of chlorpyrifos exposure on soil bacteria.

2. MATERIAL AND METHODS

2.1. Chemicals

Commercially available Chlorpyrifos (50%, Cheminova India Limited) was purchased and used throughout the study. Isolations of bacteria were carried out in a mineral salt medium (MSM) containing (g/L) K_2HPO_4, 1.5; KH_2PO_4, 0.5; NaCl, 0.5; $(NH_4)2SO_4$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2, and 10 mL of 100 × trace element solution. The 100× trace element solution was composed of (mg L^{-1}) $Na_2EDTA \cdot 2H_2O$, 500; $FeCl_2 \cdot 4H_2O$, 143; $ZnCl_2$, 4.7; $MnCl_2 \cdot 4H_2O$, 3.0; H_3BO_3, 30; $CoCl_2 \cdot 6H_2O$, 20; $CuCl_2 \cdot 2H_2O$, 1.0; $NiCl_2 \cdot 6H_2O$, 2.0; $Na_2MoO_4 \cdot 2H_2O$, 3.0; and $CaCl_2 \cdot 2H_2O$, 100.

2.2. Soil Collection

A total of 12 samples were collected from pesticide-applied agricultural soils exposed to various time points (2, 4, 6, and 10 years) from Karuppur area, Salem, Tamilnadu, India. Aseptically collected soil samples were placed in polythene bags and stored at 4°C until further processing.

2.3. Isolation of bacteria

One gram of soil was added to 100 mL of sterile water and aliquots of 1ml were diluted up to 10^{-8}. After serial dilutions, 0.1 ml of aliquots were spread on nutrient agar plates and incubated for 24 hours at 37°C. After the incubation, all isolated colonies were further purified. All isolates were screened for their ability to utilize pesticide primarily by streaking on MSM media containing 100ppm chlorpyrifos. Selected bacterial strains identified by using biochemical methods.

2.4. Chlorpyrifos Tolerance by the bacterial isolates

Selected bacterial strains were inoculated in Mineral salt medium with varying concentrations of chlorpyrifos (100, 200, 300, 400, and 500 ppm). All isolates were monitored for tolerance to chlorpyrifos for up to 48 hours using the tube dilution method. After 48 hours, isolates were streaked on MSM agar with various pesticide concentrations to determine their survival.

2.5. Efficacy of pesticide degrading bacteria

After 5 days of incubation, the removal of 200ppm chlorpyrifos by three selected bacterial isolates (OP3-*Bacillus sp*, OP5-*Pseudomaonas sp*, and OP7- *Micrococcus sp*) was determined using a UV spectrophotometer at 288 nm and the molar absorption coefficient (calculated value 6.07×10^3 $molL^{-1}$ cm^{-1}). Following that, the percentage of chlorpyrifos removed was determined using the following formula [11,12]:

$$A=\varepsilon_0.cl \quad (1)$$

2.6. Molecular identification of potential chlorpyrifos degrader

By using an alkaline lysis method, genomic DNA was isolated from a potential bacterial strain. The isolation genomic DNA was confirmed on the 0.4 % agarose gel. The genomic DNA was amplified and 16S rRNA sequencing was performed using universal primers (27F 5'-AGAGTTTGATCCTGGCTCAG-3'; 1492R 5'-GGTTACCTTGTTACGACTT-3').

2.7. Biodegradation of Chlorpyrifos

Chlorpyrifos was added at a concentration of 300ppm to 100mL of MSM medium, and the potential chlorpyrifos degrading bacterial strains were inoculated and incubated at room temperature for ten days. Every 24hours of incubation, 5ml of MSM cultured medium were collected and centrifuged at 10500×*g* for 10 min at 4°C. Thereafter, residual Chlorpyrifos was extracted twice with equal amount of ethyl acetate (1:1). After evaporating the solvent, the residue was resuspended in 3 mL of ethyl acetate. Residual amount of Chlorpyrifos was estimated by using Micro plate Absorbance reader (Bio rad Benchmark Micro plate Reader, India) at 288 nm by using molecular extinction coefficient (calculated value 6.07×10^3 $molL^{-1}$ cm^{-1}). Subsequently quantity of Chlorpyrifos degradation was monitored and calculated by using formula (1).

After 10days of degradation, 10 ml of samples were collected from flasks and centrifuged at 9,300×*g* for 10 min at 4°C. For confirmation of chlorpyrifos degradation, cell-free supernatant was used for liquid-liquid extraction with ethyl acetate (1:1) followed by FTIR in the frequency range 4000-500 cm^{-1} (Shimadzu, Japan). Mineral Salt Medium having 250ppm of Chlorpyrifos without inoculum was used as a control.

2.8. Detection of metabolites from pesticide degradation

A 5 ml of cell free supernatant was extracted with an equal volume of ethyl acetate following degradation. After evaporating the solvent, the residues were dissolved in methanol. The chlorpyrifos biodegraded metabolites were identified using gas chromatography

mass spectroscopy (Shimadzu, Japan) in conjunction with an analysis of a fused silica column packed with SH-RxiSil MS (30m× 0.25 mm ID ×250µm df). Helium was used as the carrier gas, flowing at a constant rate of 1ml/min. The injector temperature was set to 260°C during the chromatographic run.

2.9. Impact of chlorpyrifos on potential isolate

The potential bacterial isolates were cultivated in MSM for 48h at 37°C. Following inoculation, isolates were cultured in MS medium supplemented with pesticides at a concentration of 300ppm. The addition of 100ppm pesticides was continued for a total of 30 days at a 10-day interval. Bacterial cells were collected following exposure for further investigation.

2.9.1. Effect on total protein

Total protein of exposed and non-exposed of potential bacterial cells was estimated by using Lowry's method [13]. After incubation, bacteria were collected by centrifugation at 145×*g* for 5 minutes, and the pellet was resuspended in 1ml of TE buffer and centrifuged again at 14,534 ×*g* for 5 minutes. In a test tube, 1 ml of supernatant was collected and 4.5 ml of reagent 1 was added, followed by a ten-minute incubation. Then, 30 minutes later, 0.5ml of reagent 2 was added and incubated. After incubation, the optical density (OD) was determined at 660nm using a UV-Vis spectrophotometer.

2.9.2. Effect on DNA

After bacteria were exposed to chlorpyrifos, genomic DNA was isolated using the alkaline lysis method from both exposed and non-exposed bacteria. Effect of chlorpyrifos on genomic DNA of exposed and non-exposed bacterial cells was evaluated by alkaline gel electrophoresis assay [14].

2.10. Statistical analysis

All the experiments were performed with triplicates and the statistical analysis was performed using the GraphPad Prism version 5 software.

3. RESULTS AND DISCUSSION

3.1. Isolation of chlorpyrifos degrading bacteria

After incubation, different colonies were observed on the agar plates. Totally 174 bacterial colonies were chosen based on the morphology and appearance (Table 1). Among 174 bacterial colonies, 40 different bacterial strains were grown on MSM agar supplemented with 100ppm of chlorpyrifos (Fig. 1). For further processing, these 40 isolates were maintained in MSM medium containing chlorpyrifos. Based on biochemical tests the selected bacterial isolates were identified (table 2). Amongst 40 pesticide tolerant bacteria, *Bacillus sp* (25%) were predominant followed by *Pseudomonas Sp.* (17%), *Enterobacter Sp.* (17%), *Agrobacterium Sp.* (15%), *Micrococcus Sp.* (13%), *Azotobacter Sp.* (8%) and *Arthobacter Sp.* (5%) (Fig. 2).

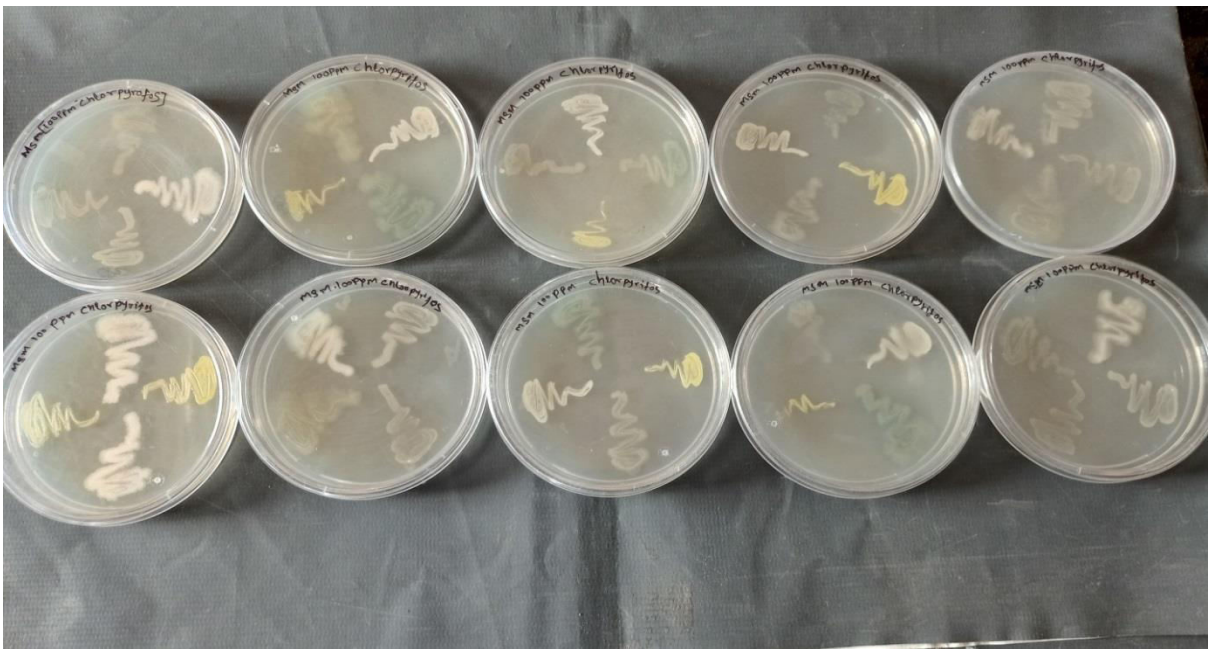

Fig. 1: Screening of chlorpyrifos tolerant bacteria

Table 1: Total Pesticide degrading bacterial strains isolated from different soil samples

Soil sample	No. of bacterial strains
Sample 1	18
Sample 2	12
Sample 3	16
Sample 4	11
Sample 5	13
Sample 6	10
Sample 7	17
Sample 8	19
Sample 9	17
Sample 10	12
Sample 11	13
Sample 12	16

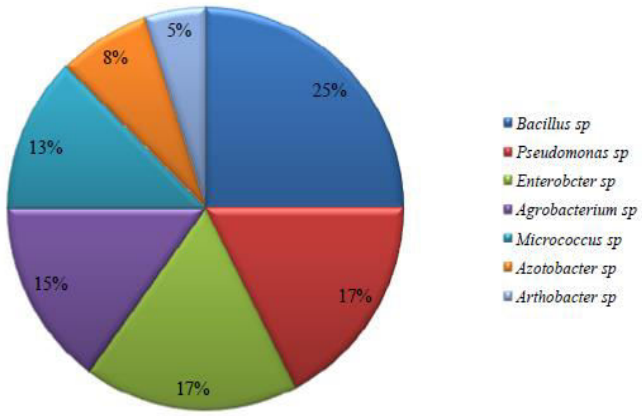

Fig. 2: Diversity of pesticide tolerant bacterial isolate

Table 2: Identification of chlorpyrifos degrading bacteria by biochemical characteristics

Isolates	Gram Staining	Spore staining	Motility	Indole	MR	VP	Catalase	Oxidase	Citrate	Nitrate	Urease	OF	Genus
OP1	Negative	-	+	-	-	+	+	-	+	+	+	-	*Enterobacter sp*
OP2	Positive	+	+	-	-	-	+	+	-	+	-	-	*Bacillus sp*
OP3	Positive	+	+	-	-	-	+	+	-	+	-	-	*Bacilus sp*
OP4	Negative	-	+	-	-	-	+	+	-	+	-	F	*Azotobacter* sp
OP5	Negative	-	+	-	-	-	+	+	+	+	+	F	*Pseudomonas* sp
OP6	Positive	+	+	-	-	+	+	+	+	-	-	O	*Arthobacter* sp.
OP7	Positive	-	-	-	-	+	+	+	-	+	+	F	*Micrococcus sp*
OP8	Positive	+	+	-	-	-	+	+	-	+	-	-	*Bacilus sp*
OP9	Negative	-	+	-	-	-	+	+	+	+	+	F	*Pseudomonas* sp
OP10	Negative	-	+	-	-	+	+	-	+	+	+	-	*Enterobacter sp*
OP11	Positive	+	+	-	-	-	+	+	-	+	-	-	*Bacillus sp*
OP12	Negative	-	+	-	-	-	+	+	+	+	+	F	*Pseudomonas* sp
OP13	Negative	-	+	-	-	-	+	+	-	+	-	F	*Azotobacter* sp
OP14	Negative	-	+	+	+	+	+	+	-	+	+	F	*Agrobacterium* sp
OP15	Positive	+	+	-	-	-	+	+	-	+	-	-	*Bacillus sp*
OP16	Positive	+	+	-	-	-	+	+	-	+	-	-	*Bacillus sp*
OP17	Negative	-	+	-	-	+	+	-	+	+	+	-	*Enterobacter sp*
OP18	Negative	-	+	-	-	+	+	-	+	+	+	-	*Enterobacter sp*
OP19	Positive	-	-	-	-	+	+	+	-	+	+	F	*Micrococcus sp*
OP20	Negative	-	+	-	-	-	+	+	+	+	+	F	*Pseudomonas* sp
OP21	Negative	-	+	-	-	-	+	+	+	+	+	F	*Pseudomonas* sp
OP22	Positive	-	-	-	-	+	+	+	-	+	+	F	*Micrococcus sp*
OP23	Negative	-	+	+	+	+	+	+	-	+	+	F	*Agrobacterium* sp
OP24	Negative	-	+	-	-	+	+	-	+		+	-	*Enterobacter sp*
OP25	Negative	-	+	+	+	+	+	+	-	+	+	F	*Agrobacterium* sp
OP26	Positive	+	+	-	-	+	+	+	+	-	-	O	Arthobacter sp.
OP27	Positive	+	+	-	-	-	+	+	-	+	-	-	*Bacillus sp*
OP28	Positive	+	+	-	-	-	+	+	-	+	-	-	*Bacillus sp*
OP29	Negative	-	+	-	-	+	+	-	+	+	+	-	*Enterobacter sp*
OP30	Positive	+	+	-	-	-	+	+	-	+	-	-	*Bacillus sp*
OP31	Negative	-	+	-	-	-	+	+	+	+	+	F	*Pseudomonas* sp

OP32	Positive	+	+	-	-	-	+	+	-	+	-	-	*Bacillus sp*
OP33	Negative	-	+	-	-	-	+	+	-	+	-	F	*Azotobacter* sp
OP34	Negative	-	+	+	+	+	+	+	-	+	+	F	*Agrobacterium* sp
OP35	Negative	-	+	-	-	-	+	+	+	+	+	F	*Pseudomonas* sp
OP36	Negative	-	+	+	+	+	+	+	-	+	+	F	*Agrobacterium* sp
OP37	Negative	-	+	+	+	+	+	+	-	+	+	F	*Agrobacterium* sp
OP38	Positive	-	-	-	-	+	+	+	-	+	+	F	*Micrococcus sp*
OP39	Negative	-	+	-	-	+	+	-	+	+	+	-	*Enterobacter sp*
OP40	Positive	-	-	-	-	+	+	+	-	+	+	F	*Micrococcus sp*

3.2. Chlorpyrifos Tolerance by the bacterial isolates

Isolates OP3 *Bacillus sp* showed more tolerance up to 300ppm, isolate OP5 *Pseudomaonas* sp and isolate OP7 M*icrococcus* showed tolerance up to 200ppm compared other bacterial isolates (Table 3). Isolate OP3 OP5and OP7 grown on agar plates containing chlorpyrifos which indicates these three isolates are viable after 48 hours incubation in the presence of chlorpyrifos (Fig. 3). So that chlorpyrifos tolerant isolate OP3- *Bacillus sp*, OP5- *Pseudomaonas sp* and OP7- M*icrococcus sp* were selected for the removal of pesticide.

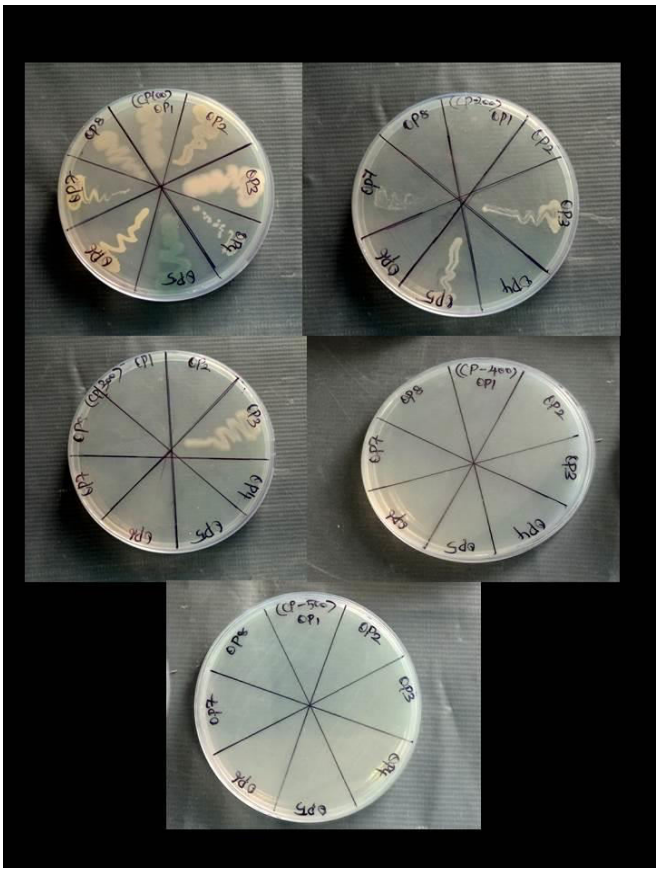

Fig. 3: tolerance of bacterial isolates against different concentration of chlorpyrifos

Table 3: Tolerance and survival of bacterial isolates at various concentrations of Chlorpyrifos

Isolate	Concentration of Chlorpyrifos (in ppm)				
	100	200	300	400	500
OP1	+	-	-	-	-
OP2	+	-	-	-	-
OP3	+	+	+	-	-
OP4	+	-	-	-	-
OP5	+	+	-	-	-
OP6	+	-	-	-	-
OP7	+	+	-	-	-
OP8	+	-	-	-	-
OP9	+	-	-	-	-
OP10	+	-	-	-	-
OP11	+	-	-	-	-
OP12	+	-	-	-	-
OP13	+	-	-	-	-
OP14	+	-	-	-	-
OP15	+	-	-	-	-
OP16	+	-	-	-	-
OP17	+	-	-	-	-
OP18	+	-	-	-	-
OP19	+	-	-	-	-
OP20	+	-	-	-	-
OP21	+	-	-	-	-
OP22	+	-	-	-	-
OP23	+	-	-	-	-
OP24	+	-	-	-	-
OP25	+	-	-	-	-
OP26	+	-	-	-	-
OP27	+	-	-	-	-
OP28	+	-	-	-	-
OP29	+	-	-	-	-
OP30	+	-	-	-	-
OP31	+	-	-	-	-
OP32	+	-	-	-	-
OP33	+	-	-	-	-
OP34	+	-	-	-	-
OP35	+	-	-	-	-
OP36	+	-	-	-	-
OP37	+	-	-	-	-
OP38	+	-	-	-	-
OP39	+	-	-	-	-
OP40	+	-	-	-	-

(+ : Viable, - : Nonviable)

3.3. Removal of chlorpyrifos

Chlorpyrifos degradation by bacterial isolates was determined using UV-VIS molecular extinction coefficient. After 5 days, isolate OP3 removed was able to remove 47% percentage of chlorpyrifos from medium supplemented 200ppm. The isolate OP3 was potential degrader of chlorpyrifos and selected for further work (Fig. 4).

3.4. Molecular identification of potential chlorpyrifos degrader

The molecular characterization of the potential bacterial isolate OP3 was performed using 16S rRNA sequencing and comparison to the NCBI database. According to NCBI BLASTn, the query sequence is 99.86 percent similar to *Bacillus cereus*, indicating that the bacterial isolate OP3 was *Bacillus cereus*. The phylogenetic tree analysis of *B. cereus* OP3 is depicted in Fig. 5. *B. cereus* OP3's 16S rRNA sequence was submitted to GenBank and assigned the accession number MH071172.

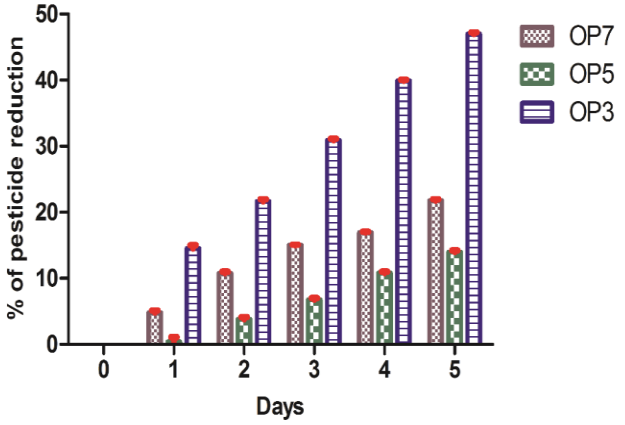

Fig. 4: Removal of chlorpyrifos by three chlorpyrifos tolerant bacterial isolates

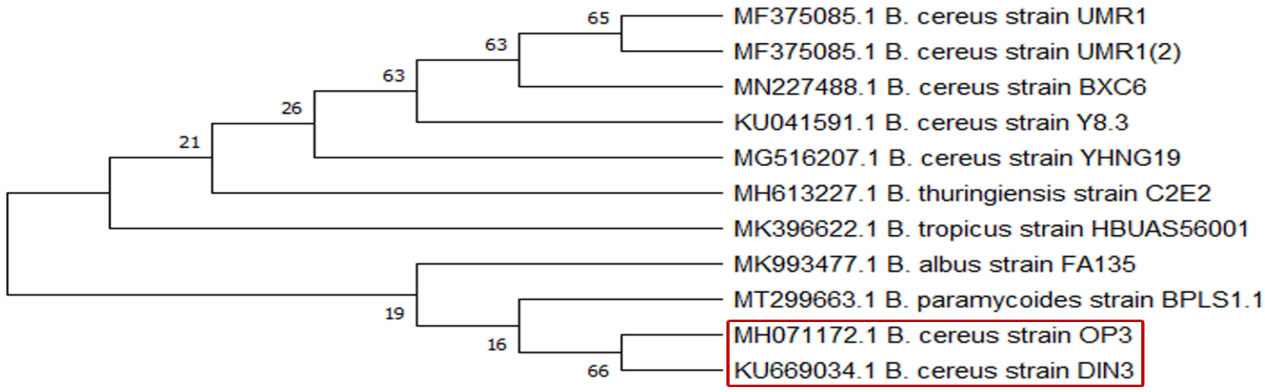

Fig. 5: Phylogenetic analysis of potential chlorpyrifos degrading bacteria *Bacillus* OP3

3.5. Biodegradation of Chlorpyrifos

Quantity of chlorpyrifos was monitored every 24 hours once up to 10 days of degradation. Amount of chlorpyrifos was gradually decreased day by day that indicates the potential bacterial isolate *Bacillus cereus* OP3 has been removed chlorpyrifos gradually. 92.4% of chlorpyrifos (300ppm) was degraded by *B. cereus* OP3 at 8th day of degradation and after 9th chlorpyrifos was reached at untraceable level (Fig. 6).

The FTIR spectrum provides more insight into the structural changes of chlorpyrifos. The IR spectra of the chlorpyrifos and degraded product by *B. cereus* OP3 are presented in Fig. 7. There is recognizable variation between the spectra, indicating degradation of chlorpyrifos. Chlorpyrifos (control) showed peaks at 3127.36cm^{-1}, 1401.19 cm^{-1}, 1120.56 cm^{-1} & 1094.53 cm^{-1} and 602.71 cm^{-1} which are characteristics of symmetric and asymmetric C-H stretching of CH_3 and CH_2 groups symmetric stretching vibrations of COO^-, C-Cl stretching and C-H deformation.

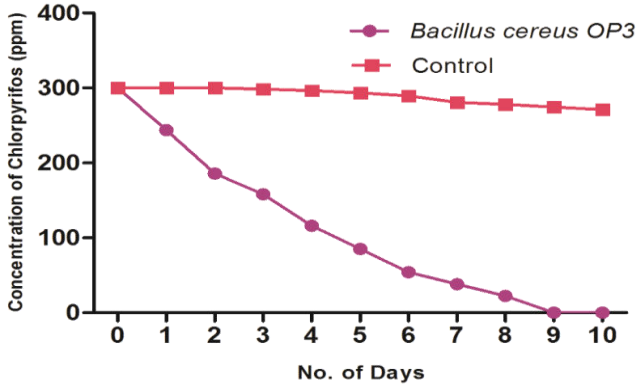

Fig. 6: Degradation of chlorpyrifos by potential isolate *Bacillus cereus* OP3

The spectrums of these peaks were entirely reduced after degradation of chlorpyrifos by *Bacillus cereus* OP3 whereas new peaks were observed at 1438.80 cm^{-1} and 1359.72 cm^{-1} which are characteristics of C-H bending vibrations of CH_3 and CH_2 groups and peaks at 1057.88 cm^{-1} characteristics of vibrations associated with phosphate groups (PO_4) which indicates hydrolytic degradation of chlorpyrifos by *B. cereus* OP3.

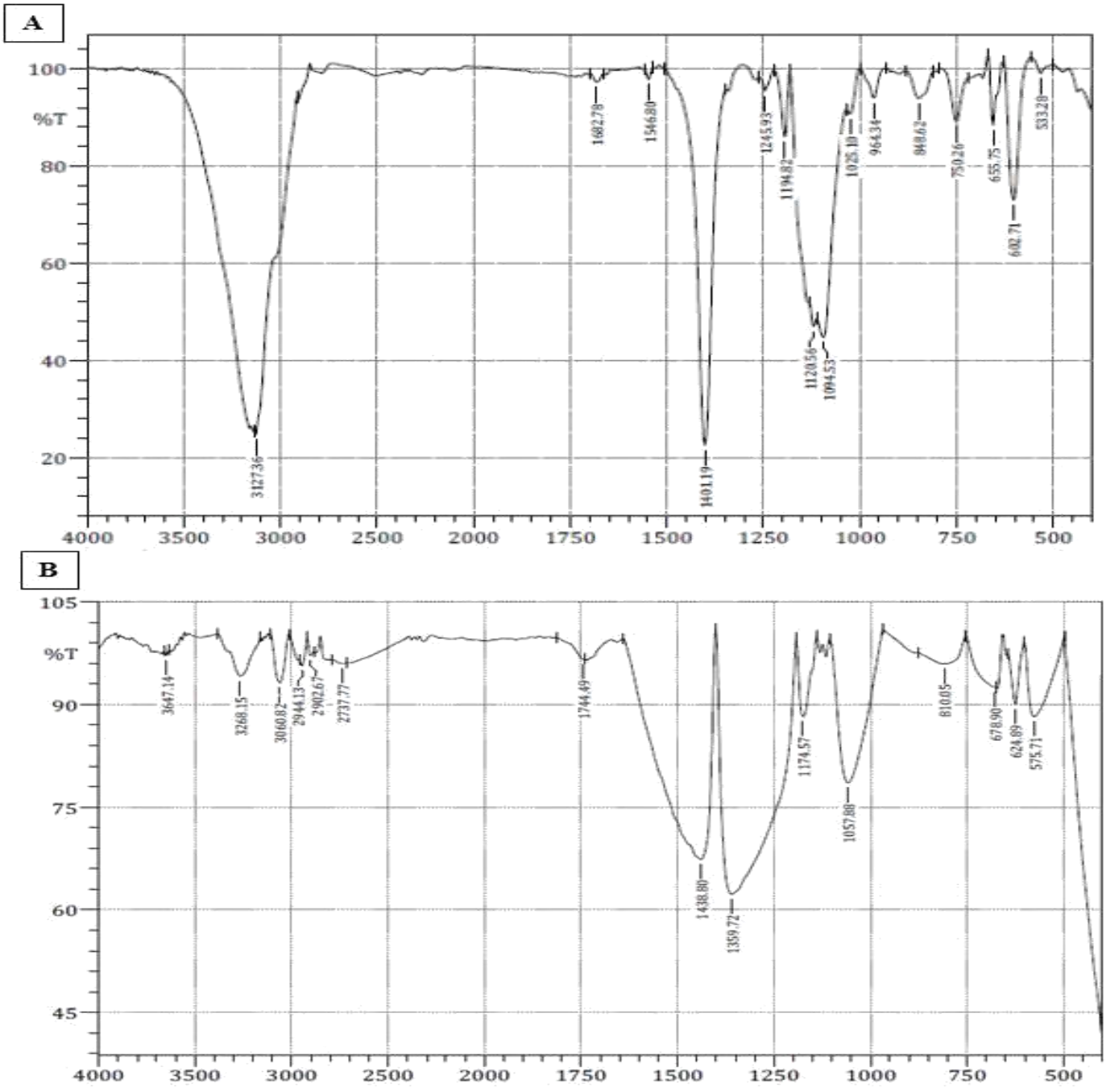

Fig. 7: FTIR Spectrum of chlorpyrifos degradation by *Bacillus cereus* OP3. Chlorpyrifos (A) and chlorpyrifos degradation by *Bacillus cereus* OP3(B).

3.6. Detection of metabolites from pesticide degradation

GC-MS analysis showed two peaks (Fig. 8) with retention time of 17.99 min and 26.33 min. compared with GC-MS NIST library, compound detected at 17.99 min waws determined as M-Nitroaniline and detected at2 6.33 min was determined as 4-Methyl-2-[[4-Nitrophenyl] Thio] Pyrimidine. M-Nitroaniline and 4-

Methyl-2-{(4-Nitrophenyl)Thio}Pyrimidine were metabolites relesed after degradation of chlorpyrifos by *Bacillus cereus* OP3.

3.7. Effect on total protein

Total protein content of potential *Bacillus cereus* OP3 bacterial cells was estimated by Lowry's method after the exposure to chlorpyrifos. Total protein of exposed and non-exposed *Bacillus cereus* OP3 was calculated (Fig. 9) by comparing with bovine serum albumin standard. It was observed that the total protein content of isolates was slightly increased throughout exposure.

3.8. Effect on DNA

Bacterial DNA damage by chlorpyrifos in potential isolate *B.cereus* OP3 was analyzed through alkaline gel electrophoresis (Fig. 10). The results indicated chlorpyrifos able to induce DNA strand breaks in exposed *B. cereus* cell DNA was damaging after 20 day exposure to chlorpyrifos compare to non-exposed cells.

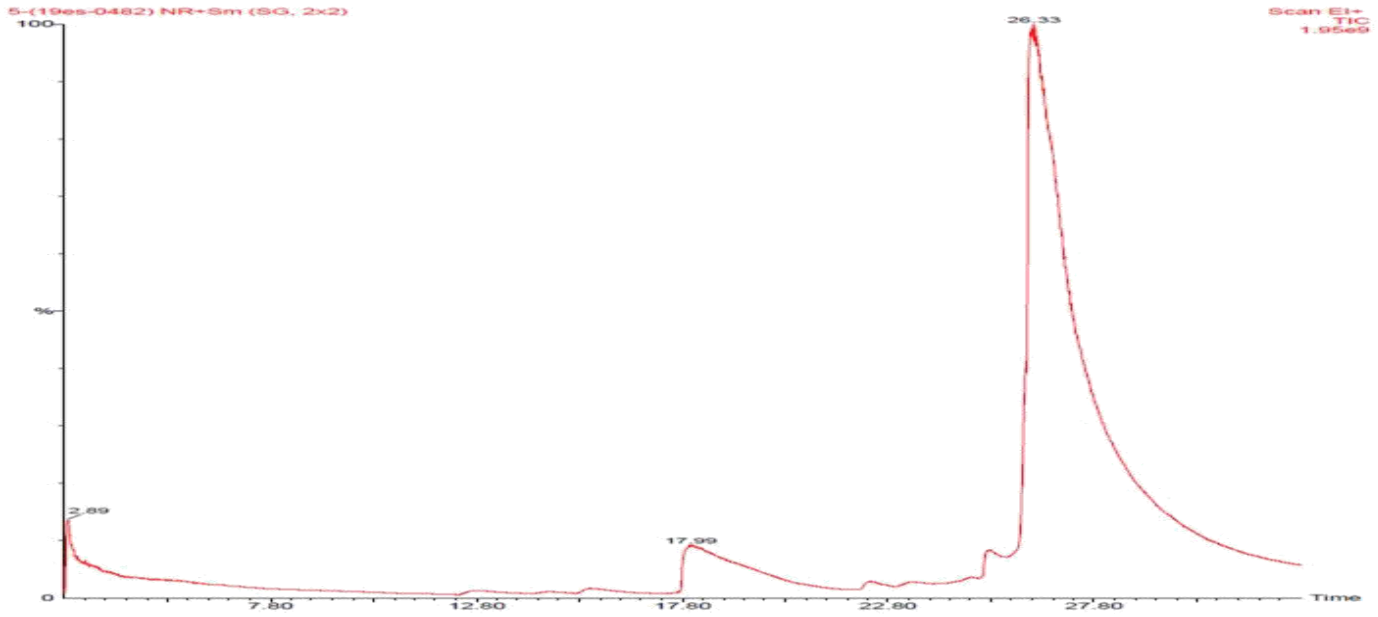

Fig. 8: GC-MS analysis of Chlorpyrifos degraded metabolites by *Bacillus cereus OP3*

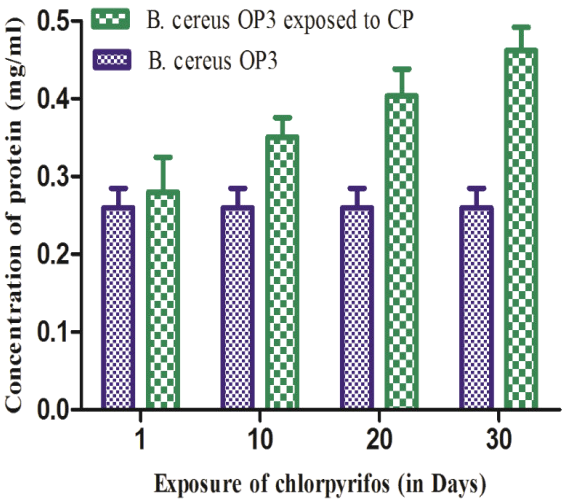

Fig. 9: Total Protein concentration of exposed and non-exposed *B. cereus*

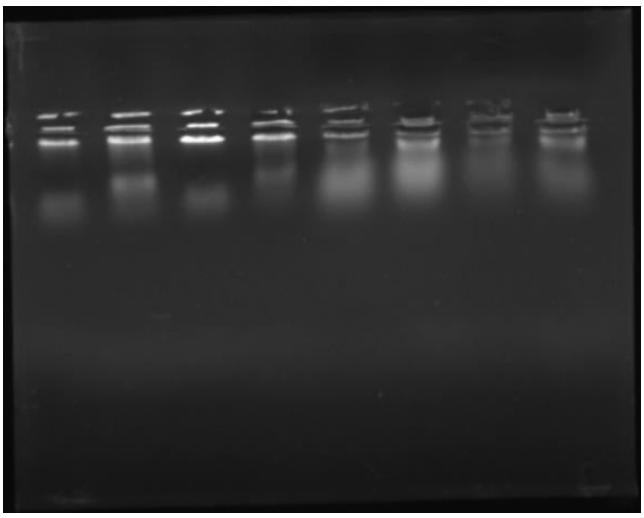

Fig. 10: Effect of chlorpyrifos on *B. cereus* DNA (From Left to right Lane 1-4; non-exposed *B. cereus* after 1, 10, 20, 30 days, lane 5-8: exposed *B. cereus* after1, 10, 20, 30 days)

4. DISCUSSION

Organophosphate pesticides are used to control the major insect pests in agriculture. Among organophosphate pesticides, Chlorpyrifos is most commonly used [15]. Chlorpyrifos is distinct from other organophosphate compounds and it has been reported to be recalcitrant. Because of its primary metabolite TCP stops propagation of microorganisms in soil [16]. However, many bacteria have been reported which degrade chlorpyrifos incompletely.

In this study, 40 morphologically different bacterial isolates were grown on MSM agar plates containing 100ppm of chlorpyrifos. All the forty chlorpyrifos tolerant isolates were identified based on biochemical characteristics. Amongst all, tolerant *Bacillus sp* (25%) are abundant compared to other bacterial isolates. Isolate OP3- *Bacillus sp*, OP5-*Pseudomaonas sp* and OP7-M*icrococcus sp* grown on agar plates containing over 100ppm of chlorpyrifos and also viable after 48 hours incubation in the presence of chlorpyrifos. In that, *Bacillus* sp OP3 showed more resistance to chlorpyrifos up to 300 ppm and survival after 48 hours compared to other three isolates. In screening, isolate *Bacillus* sp OP3 rapidly removed 47% of chlorpyrifos after 5 days compared to all other isolates. The potential isolate OP3 was identified as *Bacillus cereus* OP3 by 16S rRNA sequencing using universal primers.

Previously many researchers have demonstrated that biochemical tests can be used to isolate and identify chlorpyrifos degrading bacteria, as well as their resistance to chlorpyrifos. Four bacterial colonies with distinct morphologies on mineral salts agar enriched with chlorpyrifos. *Providencia stuartii, Serratia marcescens, Klebsiella oxytoca*, and *Bacillus subtilis* were identified as the four isolates. The growth experiments demonstrated that *P. stuartii* strain MS09 can grow in the presence of extremely high concentrations of chlorpyrifos and utilize it as a carbon source [17]. *Pseudomonas* sp. (Ch1D) isolated from agricultural soil using the enrichment culture technique in the presence of chlorpyrifos (0.01 g l^{-1}) [18]. Similarly, researchers discovered that three isolates, *Klebsiella* sp., *Pseudomonas putida* and *Aeromonas* sp., all exhibited resistance to chlorpyrifos at concentrations of up to 2mg ml^{-1}, 4mg ml^{-1}, and 8 mg ml^{-1}, respectively [19].

In the present study, about 92.4% of chlorpyrifos (300ppm) was degraded at 8th day by *Bacillus cereus* OP3 and potential isolate used chlorpyrifos for the growth. Degradation of chlorpyrifos was confirmed by FTIR Spectrum and it revealed hydrolytic cleavage of chlorpyrifos by *Bacillus cereus* OP3.

Similar result obtained from various researchers that *B. cereus* MCAS 02 reached a maximum chlorpyrifos degradation (89%) and chlorpyrifos was used as a sole nutrient source for the bacterial growth. *B. cereus* MCAS 02 can degrade chlorpyrifos and convert it into non-toxic metabolites [20]. 70% of chlorpyrifos degraded by *Lactobacillus fermentum*, 61% of chlorpyrifos degraded by *L. lactis* whereas *E. coli* degraded 16% a lesser concentration of chlorpyrifos [21]. Some bacteria such *as P. aeruginosa, Brucella melitensis*, *P. fluorescence*, *B. subtilis*, *B. cereus*, *S. marcescens* and *Klebsiella* sp. degraded 75-87% of chlorpyrifos [22].

FTIR Spectrum results similar to *A. terreus* JAS1 chlorpyrifos-degraded IR spectrum of chlorpyrifos showed bands at 3,444 and 1,627 cm^{-1} which are the physical characteristics of the N-H and C=O amide groups, peaks at 1,415, 1,050, 845, and 670 cm^{-1} those indicates C=C stretching, C-O stretching, C-H bending, and C-Cl stretching, respectively. *A. terreus* JAS1 chlorpyrifos-degraded samples' IR spectrum peaks were observed at 3,430, 3,433, and 3,401 cm^{-1}, respectively, which are corresponding to the N-H group. Peaks observed 1,415-, 1,050-, 845-, and 670-cm^{-1} were IR spectrum of chlorpyrifos; those peaks disappeared in the chlorpyrifos-degraded samples in soil and Mineral salt media [23].

Bacillus cereus OP3 metabolized chlorpyrifos and produce M-Nitroaniline and 4-Methyl-2-{(4-Nitrophenyl)Thio}Pyrimidine. These metabolites were identified using Gas Chromatography-Mass Spectrometry. *Bacillus pumillus* strain C2A1 was capable of degrading chlorpyrifos to 3, 5, 6-trichloro-2-pyridinol (TCP) as the primary hydrolysis product [24]. *Stenotrophomonas* YC-1 strain is capable of degrading chlorpyrifos to Diethyl thiophosphoric acid (DETP) and its intermediate TCP [25]. *Lactobacillus fermentum* degraded chlorpyrifos to 3,5,6-trichloro-2-pyridinol (TCP), *L. lactis* degraded chlorpyrifos to chlorpyrifos oxon, and *E. coli* degraded chlorpyrifos to chlorpyrifos oxon and diethylphosphate [21].

Continuous exposure of potential isolates *Bacillus cereus* OP3 to chlorpyrifos may make some changes and responsible to increase the total protein content after exposure. Significant variation was observed in total protein of some bacterial isolates exposed to chlorpyrifos during growth throughout 10 days [26]. In alkaline electrophoresis assay, streaking of DNA bands was observed after exposure to chlorpyrifos which clearly indicates that chlorpyrifos tempted or cause

damage in genetic material of potential *B.cereus* OP3. Gel densitometrical analysis revealed that AB1157 was the strain displaying the bacterial DNA damage, analyzed through alkaline gel electrophoresis, that $SnCl_2$ is able to induce DNA damage [14]. Higher concentrations and longer exposures to chlorpyrifos resulted in increased DNA fragmentation in the cells. These findings imply that chlorpyrifos causes the typical chromatin DNA damage or fragmentation associated with cell apoptosis [27]. Exposure to organophosphorus pesticides can result in DNA oxidative damage, inhibition of AChE function, and an increase in serum levels of inflammatory markers in humans [28]. As per United States Environmental Protection Agency (USEPA), chlorpyrifos is not mutagenic to bacteria but can induce minor genetic changes in yeast and DNA damage in bacteria [29].

5. CONCLUSION

Thus, this study concluded that *B. cereus* OP3 isolated via enrichment is capable of degrading up to 91.2% of chlorpyrifos on the eighth day of incubation. The FTIR spectrum of chlorpyrifos degradation indicated that hydrolysis took place and that the phosphate group was released during degradation. Excessive use of the organophosphate pesticide chlorpyrifos increased total protein in *B. cereus* OP3 and induced DNA damage. As a result, repeated chlorpyrifos application may lead to changes in the characteristics or viability of soil bacteria.

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Conflict of interest

None of the authors declare conflict of interest.

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