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The Potentiality of *Lysinibacillus sphaericus* DM-3 and *Bacillus cereus* DM-5 in Degrading Dimethoate

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TWO BACTERIAL strains were isolated by soil enrichment technique and identified as *Lysinibacillus sphaericus* DM-3 and *Bacillus cereus* DM-5. Biodegradation experiments were performed in carbon free-mineral salt media supplemented with 100 mg/L of dimethoate. The ability of bacterial strains to degrade the dimethoate was evaluated under various conditions such as pH range, temperature and different concentrations of dimethoate. The dimethoate residue was determined quantitatively by HPLC method and the degradation byproducts were identified by GC/MS technique. The results revealed that both bacterial strains can utilize dimethoate as a sole carbon source up to a concentration of 500 mg/L. The optimum temperature for both strains to degrade dimethoate was 28°C. *L. sphaericus* DM-3 and *B. cereus* DM-5 showed maximum growth in the presence of dimethoate at pH 6.0 and 7.0, respectively. *L. sphaericus* DM-3 could degrade 24% of dimethoate within 72 h, whereas the degradation percentage using *B. cereus* DM-5 was 17% after the same incubation period. The main byproducts from the degradation of dimethoate by *L. sphaericus* DM-3 and *B. cereus* DM-5 was namely O,O,S-trimethyl phosphorothioate. This study reports for the first time the efficient ability of *L. sphaericus* DM-3 and *B. cereus* DM-5 to degrade dimethoate, up to 500 mg/L, over a wide range of pH.

Keywords: Dimethoate, Biodegradation, *Lysinibacillus sphaericus*, *Bacillus cereus*.

Introduction

O,O-dimethyl S-methyl carbamoyl methyl phosphorodithioate (dimethoate) is an anti-cholinesterase organophosphorus (OP) and carbamate pesticide characterized by the presence of carbamate group in its structure (Li et al., 2010 and Kumar & Gurupadayya, 2013). Dimethoate exerts its activity on insects by phosphorylation of the enzyme acetylcholinesterase at ending of nerves (Sogorb & Vilanova, 2002). The insect effector organs are overstimulated by the excess amount of acetylcholine, resulting in muscle contraction, i.e. symptoms of cholinergic poisoning (Vermeire et al., 2001 and Zhang et al. 2012). According to information published by the National Pesticide Information Center (2014), dimethoate is one of the most used OP pesticides worldwide. The continuous use of dimethoate has led to environmental pollution and disturbance in the biological ecosystem because of its toxicity (Chen et al., 2016). The relative high solubility of dimethoate in water indicates its low affinity for most types of soil and thus it has a relatively high potential for movement through farming soils (Van Scoy et al., 2016). Therefore, residues of dimethoate and its toxic analog omethoate were detected in soil and many crops (Al-Haifi et al., 2006).

The US environmental protection agency classified dimethoate as the third contaminant candidate list (CCL3), known to occur in public water systems that may impact human public health (U.S. EPA, 2009). The dimethoate adverse effect on the reproductive system and fertility of male rats was reported as the administration of dimethoate by oral route to adult male rats at the doses of 3.66, 5.50 and 11.00 mg/kg/day disrupted spermatogenesis process and reduced the rats fertility (Ngoula et al., 2014). Another study showed the carcinogenicity of dimethoate in male and female rats which developed monocytic leukemia (Reuber, 1984). Furthermore, the long-

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term effects of dimethoate exposure in mouse gastric tissue at low dose have been associated with potential carcinogenicity (Wang et al., 2013). Therefore, many western countries are banning the use of dimethoate such as France, Spain, and Italy (Mandal et al., 2005).

Microorganisms have a major role in environmental restoration through biodegradation of many pesticides as they have powerful mechanisms to clean up pesticide-contaminated soils (Essa et al., 2016 and Li et al., 2010). Microorganisms can aid in cleaning up pesticide-contaminated sites by oxidizing, binding, immobilizing, volatilizing or otherwise transforming contaminants through the extracellular enzymes production that act on a wide range of organic compounds (Lovley, 2003 and Ortiz-hernández et al., 2013).

Many attempts have been made for the degradation of dimathoate by microorganisms such as fungi; *Aspergillus niger* (Liu et al., 2001), Gram-negative bacteria; *Sphingomonas* sp. (Chen et al., 2016) and *Pseudomonas* sp. (Deshpande et al. , 2001) as well as Gram-positive bacteria; *Paracoccus* sp. (Li et al. 2010) and *Bacillus* species (Mandal et al., 2005 and Jayamadhuri, 2014). The efficiency of microorganisms to degrade pesticides has been reported to depend on several environmental parameters such as pH and temperature (Deshpande et al., 2001 and Ortiz-hernández et al. 2013). Despite many studies have been addressed the microbial degradation of dimethoate, the current understanding about the underlaying mechanisms and the byproducts produced is still fragmentary. Moreover, there is evidence that the microbial degradation of dimethoate is likely produces more toxic byproducts (Yao et al., 2011 and Van Scoy et al., 2016). Therefore, the aim of this study is to explore the soil microbial diversity, searching for new local bacterial isolate capable of degrading dimethoate effectively to less or nontoxic compounds.

Materials and Methods

Chemicals and cultivation media

High grade Dimethoate (99.40% purity) was purchased from Chem Service (West Chester, USA). Luria-Bertani (LB) medium and mineral salts medium (MS) were purchased from Bio basic (Ontario, Canada).

Isolation of dimethoate-degrading bacterial strains

In order to isolate dimethoate-degrading bacteria, soil samples were collected from the River Nile banks and agriculture fields treated with dimethoate (Giza, Egypt). The enrichment of the bacteria was done by inoculating ten grams of soil sample in 1000 ml of MS broth supplemented with dimethoate (100 mg/L) and incubated at 30°C for 48 h in a rotary shaker at 150 rpm. One ml of bacterial culture was serially diluted up to 10^{-6} dilution. The enrichment dilutions were plated onto solid MS media supplemented with dimethoate (100 mg/L) and incubated at 37°C for 48 h. Two bacterial isolates were selected, purified and maintained at 4°C on nutrient agar slants as well as glycerol stocks at -20°C for further investigation.

Identification of dimethoate-degrading bacterial isolates

The two potent bacterial isolates were selected, tested for their Gram stain, morphological shape, and spore formation ability under the microscope and identified based on both the morphological and biochemical properties with reference to Bergey's Manual of Determination Bacteriology (Sneath, 1986). The two bacterial isolates were designed as DM-3 and DM-5. Additional molecular confirmation, 16S rRNA gene sequence analysis was performed. Genomic DNA of the two isoltes was extracted using Genomic DNA Kit (GeneJET™,Thermo Scientific, USA) according to kit's instructions. Two PCR primers were used to amplify 16S rRNA gene; forward primer 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and reverse primer 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). PCR was carried out as the follow; 25 µl 2X DreamTaq master mix, 1 µl from each primer, 2 µl bacterial gDNA template (ca. 50 ng) and finally sterile water up to 50 µl. The PCR program was adjusted to initial denaturation at 95°C for 4 min, then denaturation at 95°C for 40 sec, annealing at 58°C for 40 sec, extension at 72°C for 1.5 min for 25 cycles, and a final extension step at 72°C for 10 min using GeneAmp 9700 thermal cycler (Applied Biosystems, USA). The amplicon was purified by PCR Purification kit (Biobasic, Canada). The purified amplicon was sequenced using the same primers by ABI3500 sequencer (Applied Biosystems, USA). The contig sequence was obtained from forward and reverse DNA sequence reads using DNA Baser Sequence Assembler software v.3.5.3. Identification of bacterial was performed by the BLAST server.

Phylogenetic analysis

The sequences of 16S rRNA gene of both isolates were compared to references of 16S rRNA

gene sequences of other bacterial isolates retrieved from NCBI database. Mega 7.0 software was used to construct the phylogenetic relationship with our isolated strain by neighbor-joining method.

The degradation ability of the two bacterial isolates at different incubation time

A single colony of two bacterial isolates inoculated into two test tubes containing 5 ml LB medium and incubated overnight at 37°C with moderate shaking (250 rpm). Fifty microliters from each culture (10^5 cfu/ml) were inoculated in different flasks containing 50 ml of MS broth supplemented with 100 mg/L of dimethoate as a sole carbon source. The growth was measured in terms of turbidity, by monitoring the optical density at 600 nm using spectrophotometer at different times of incubation lasting from 6 h to 72 h. The experimental units were performed with three replicates. Control sets without bacterial inoculums were incubated under the same conditions.

Effect of different dimethoate concentrations on the growth of the DM-3 and DM-5

In order to evaluate the capability of the two isolated bacteria to grow and utilize dimethoate, a single colony of DM-3 and DM-5 was inoculated into two test tubes containing 5 ml LB medium and incubated overnight at 37°C with moderate shaking (250 rpm). Fifty microliters from each culture (10^5 cfu/ml) were inoculated in different flasks containing 50 ml of MS broth supplemented with different concentration of dimethoate (100 to 500 mg/L) as a sole carbon source and incubated at 28°C for 72 h. The experiment was performed with three sets of replicates. Control sets without bacterial inoculums were incubated under the same conditions. The growth of bacteria in the medium was determined in terms of turbidity in the culture broth, by monitored the optical density at 600 nm using spectrophotometer.

Effect of different temperature and pH on the growth of dimethoate-degrading bacteria

Fifty microliters from each overnight grown culture (10^5 cfu/ml) were inoculated in different flasks containing 15 milliliters of MS media supplemented with 100 mg/L of dimethoate as sole carbon source. The pH was adjusted to different pH ranging from (4 – 12). The flasks were incubated on a rotary water bath shaker at room temperature and 200 rpm for 72 h. Depending on the optimal pH; the temperature values were adjusted to 15, 18, 37, 50 and 70°C with previously mentioned procedures and conditions. Control sets without bacterial inoculums were incubated under the same conditions. The growth of bacteria in the medium was determined in terms of turbidity in the culture broth, by monitoring the optical density at 600 nm:

Quantification of dimethoate residues using high performance liquid chromatography (HPLC)

Both bacterial isolates were grown overnight in LB medium, 10 µl of each bacterial culture was inoculated into 10 ml MS medium containing 100 mg/L of dimethoate and incubated in an orbital shaker at 28°C for 72 h as separate treatment. Samples were prepared referred to (Kumar & Gurupadayya, 2013). The concentration of utilized dimethoate was determined by high performance liquid chromatography (HPLC) (Binary LC Pump, USA). Standard (without inoculation of bacterial strain) was used to compare the inoculated samples. The separation was accomplished using Phenomenexluna C18 column. The solvent system was acetonitrile:water (60:40 v/v) at a flow rate 1 ml/min, and the volume of the sample injected was 20 µl.

Identification of the metabolites resulting from the degradation of dimethoate by gas chromatography/ mass spectrometric (GC/MS) analysis

The two bacterial strains DM-3 and DM-5 grown overnight in LB medium, 10 µl of both bacterial cultures was inoculated into 15 ml MS medium containing 100 mg/L of dimethoate and incubated in an orbital shaker at 28°C for 7 days under optimized conditions. Metabolites formed from dimethoate degradation by each bacterial isolates were determined separately using GC–MS equipped with an Aligent mass spectrometric detector, with a direct capillary interface and fused silica capillary column PAS-5 ms (30 m×0.32 mm× 0.25 µm film thicknesses). An equal volume dichloromethane was added to extract the residual dimethoate. After vigorous shaking for 5 min, the organic layer was separated and dehydrated by passing through anhydrous Na_2SO_4. Pulsed split in less-mode option was used to analyze samples at 280°C injection temperature. The carrier gas was helium at flow rate of ca. 1.0 ml/min. The solvent delay was 3 min and 1.0 µl injected. The electron multiplier voltage was maintained 1650 v. The GC/MS operation parameters were as follows: ionization potential 70 eV, interface temperature was 230 °C, and acquisition mass range scanning ranged from 50 m/z to 500 m/z. The instrument was tuned using perfluorotributylamine. GC column oven temperature was programmed for an initial hold of 2 min at 60 °C and the temperature was increased to 300°C, the rate was 5°C /min.

The identification of components was assigned by a comparison of their mass spectra and retention time with those of the authentic compounds and by matching with NIST, W9N11 and RTLPEST3 library.

Statistical analysis

Data were represented in figures as mean of replicates from three independent experiments and were analyzed for statistical difference using either Student's t-test or Duncan's test (SigmaStat version 3.5). Significant differences were based on the value of $p \leq 0.05$.

Results

Identification and characterization of the culture strain

Morphological identification

The isolation and enrichment techniques yielded two bacterial strains, designated as DM-3 and DM-5, demonstrated the ability to utilize and grow in MS media supplied with 100 mg/L of dimethoate as sole carbon source. Based on morphological and biochemical results, the two isolates were initially identified as *Lysinibacillus sphaericus* DM-3 (formerly known as *Bacillus sphaericus*) and *Bacillus cereus* DM-5. The morphological characteristics showed that the two strains have colonies that are different in shape and appearance. The results of microscopic examination revealed that both isolates are Gram-positive, rod-shaped and spore-forming bacteria. The biochemical tests revealed that both strains are motile, can grow at 5% NaCl, utilize citrate and glycerol, hydrolyzed casein, show hemolysis on blood agar media, did not produce pigments or indole and cannot hydrolyze the gelatin (Table 1). Unlike *B. cereus* DM-5, *L. sphaericus* DM-3 cannot grow at 45°C, failed to hydrolyze urea, ferment starch or utilize fructose. On the other hand, oxidase activity was only observed in *L. sphaericus* DM-3.

Molecular identification and phylogenetic analysis

The corresponding PCR amplicons of 16S rRNA of both strains were visualized on 1.5% agarose. Amplification of 16S rRNA and sequencing produced 1438 bp and 1432 bp fragments for DM-3 and DM-5, respectively. All the sequences were edited manually and trimmed to remove ambiguous region. The sequences were analyzed and submitted to GenBank under accession number MF967404 and MF967405 for DM -3 and DM- 5, respectively.

The sequences were aligned by multiple sequence alignment with other bacterial isolates and phylogenetic tree was constructed using Mega 7.0 software (Fig. 1 a, b), the phylogenetic analysis revealed that sequence of strain DM-3 showed 99% similarities with 16S rRNA sequence of *Lysinibacillus* sp. The biochemical tests results revealed that strain DM-3 was closest to *Lysinibacillus sphaericus* (Coorevits et al., 2012) Whereas the strain DM-5 showed 99% similarities with 16S rRNA sequence of *Bacillus cereus*.

The growth curve of both bacterial strains in presence of dimethoate

The growth of both strains showed a steady increase up to 36 h, which barely increased afterward (Fig. 2). During the exponential phase of growth the cell density of *L. sphaericus* DM-3 increased from 0.2 to 1.16 (OD 600 nm), while that for *B. cereus* DM-5 elevated from 0.1 to 1.10 (OD 600 nm). Moreover, the growth of *L. sphaericus* DM-3 was significantly higher than that for *B. cereus* DM-5 at most of time points.

Utilization of dimethoate by the degrading bacteria in different concentrations of dimethoate

The results indicate the ability of both strains to utilize different concentration range (100–500 mg/L) of dimethoate (Fig.3). The highest bacterial growth was recorded at the concentration of 100 mg/L. Furthermore, both strains were able to tolerate high dimethoate concentration up to 500 mg/L as indicated by the increase in cell density to 0.77 and 0.62 (OD 600 nm) by *L. sphaericus* DM-3 and *B. cereus* DM-5, respectively (Fig.3). There was a gradual significant reduction in *B. cereus* DM-5 growth proportionate with the increase in dimethoate concentration. However, there was no significant reduction in the growth of *L. sphaericus* DM-3 between both ranges 200-300 and 300-400 mg/ml of dimethoate.

Effect of different pH and temperature on bacterial growth in the presence of dimethoate

The results indicated that both bacterial strains could grow at pH range from 4 to 12 (Fig. 4 a). The optimal pH of *L. sphaericus* DM-3 and *B. cereus* DM-5 was 6 and 7, respectively. There was a significant reduction in the growth due to change in pH, acidic or alkaline, compared with optimum pH for each strain. *L. sphaericus* DM-3 and *B. cereus* DM-5 utilized dimethoate efficiently at a broad range of temperatures from 15 to 37°C (Fig.4 b). The optimal temperature for both strains was 28°C. *L. sphaericus* DM-3 showed significant reduction in growth after further increase in temperature higher than 37°C, whereas *B. cereus* DM-5 tolerated the increase in temperature showing no significant change in growth between the range 37-70°C.

TABLE 1. Morphological characteristics of colony and biochemical characterization of the two dimethoate-degrading bacteria

Test	*L. sphaericus* DM-3	*B. cereus* DM-5
Colony morphological		
• Shape	filamentous	round
• Color	white	yellow
• Elevation	flat	flat
Biochemical characterization		
Gram stain	+	+
Endospore formation	+	+
Shape	rod	rod
Motility	+	+
Pigment	-	-
Blood hemolysis	+	+
Growth at 45° C	-	+
Growth in 5% NaCl	+	+
Growth at pH 6	+	+
Hydrolysis of urea	-	+
Hydrolysis of gelatin	-	-
Hydrolysis of casein	+	+
Nitrate reduction	+	-
Oxidase	+	-
Utilization of citrate	+	+
Utilization of Fructose	-	+
Utilization of glycerol	+	+
Indole production	-	-
Fermentation of starch	-	+

+, positive reaction; -, negative reaction.

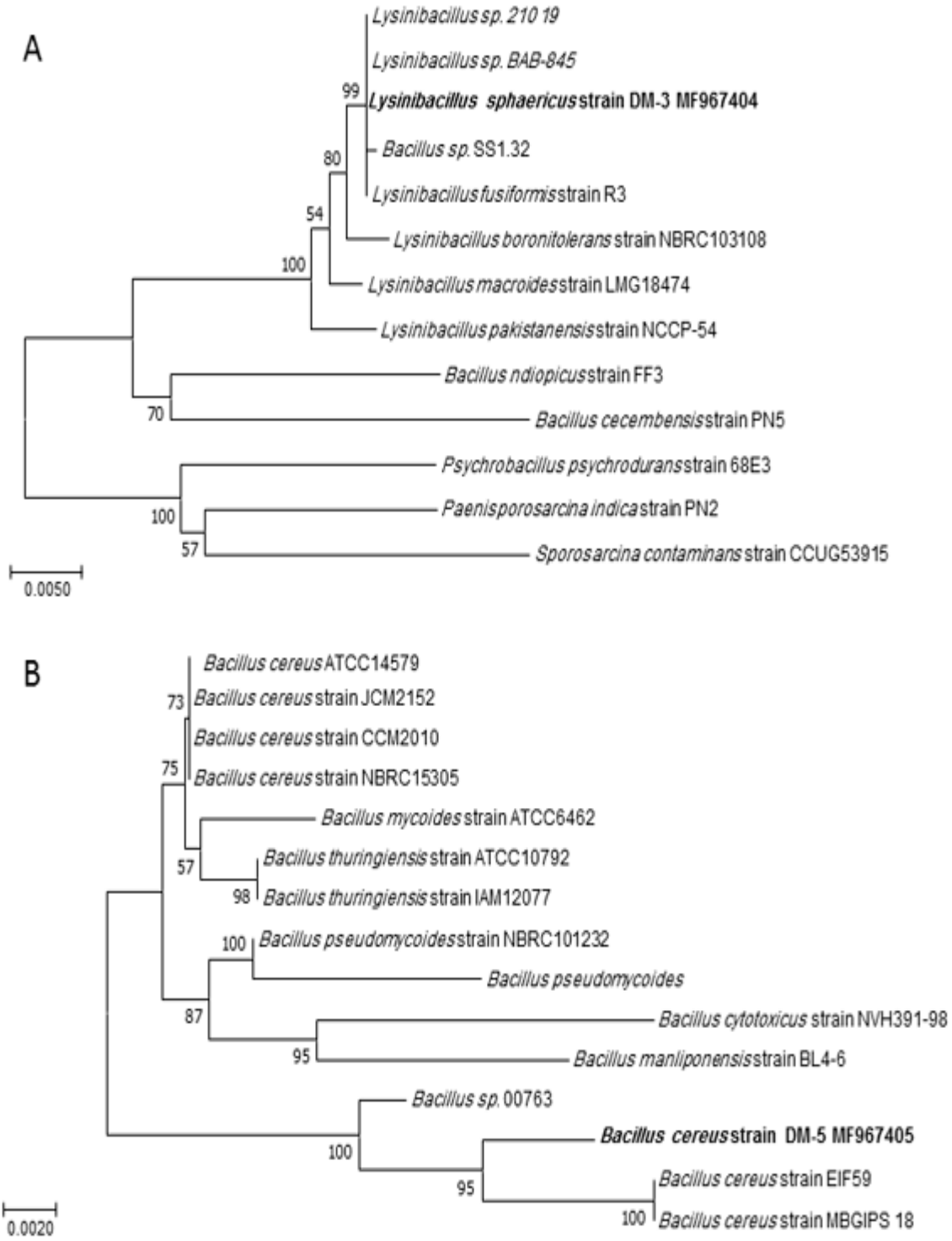

Fig. 1. 16S rRNA sequences based phylogenetic tree showing the phylogenetic relationship of both strains of *L. sphaericus* DM-3 (A) and *B. cereus* DM-5 (B) with different bacterial strains. Two phylogenetic trees were constructed by the neighbour-joining method using Mega 7.0 software. Numbers at nodes represents branch length. Bars represent nucleotide substitution per nucleotide position.

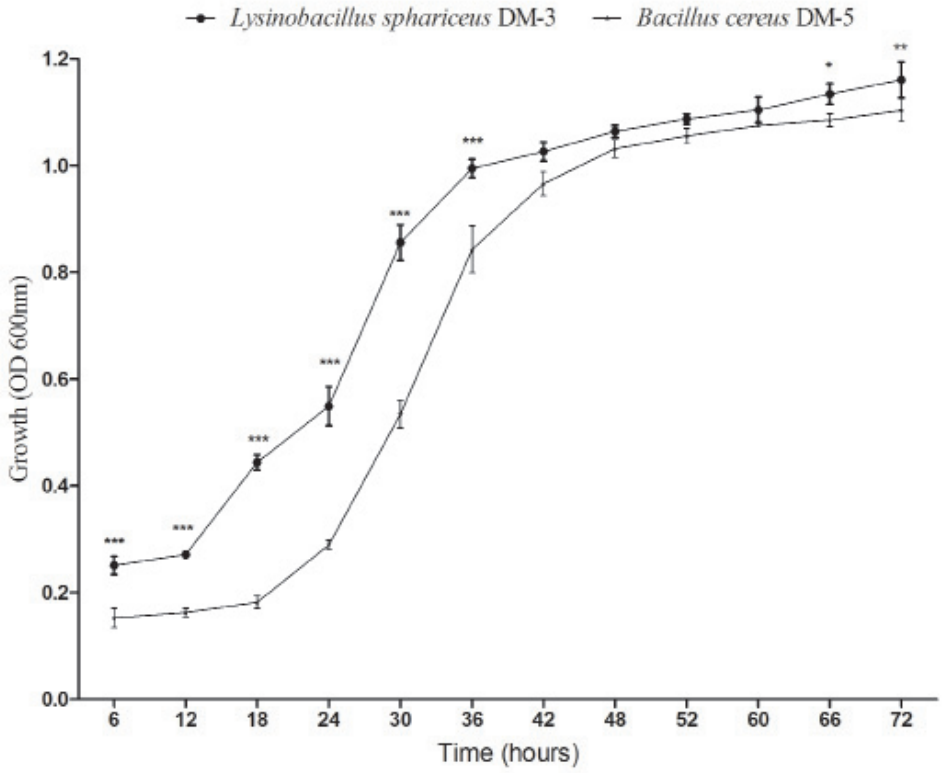

Fig. 2. The time course of the growth of *L. sphaericus* DM-3 and *B. cereus* DM-5 in MS containing dimethoate at the rate of 100 mg/L. Data were presented as mean of replicates and were analyzed statistically using Student's t-test for independent samples. *** p ≤0.001; ** p ≤0.01;* p ≤0.5.

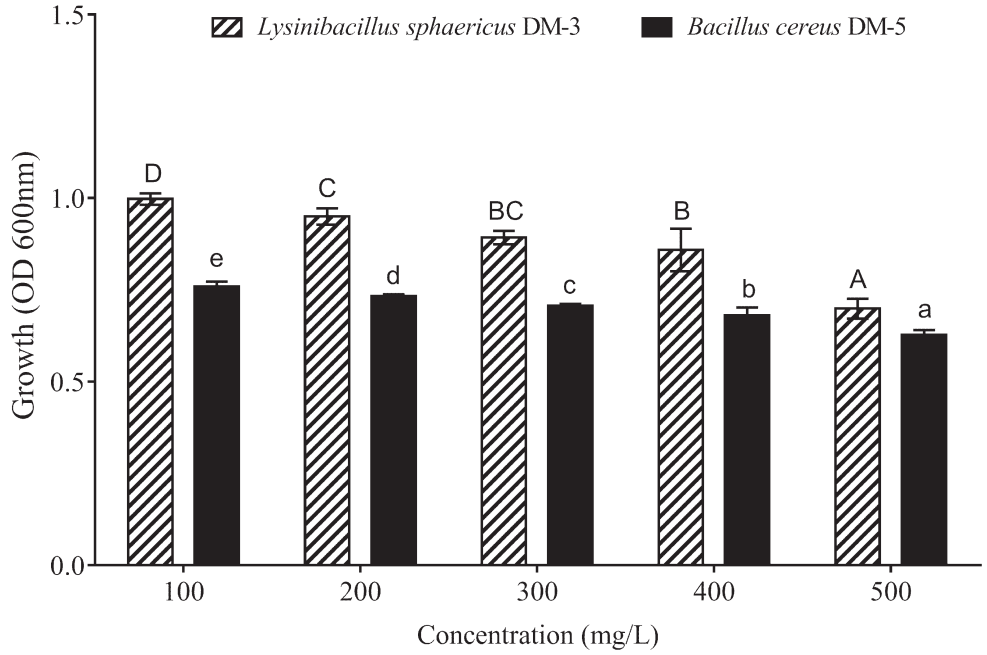

Fig. 3. Effect of different dimethoate concentrations (100-500 mg/L in MS media) on bacterial growth of *L. sphaericus* DM-3 and *B. cereus* DM-5. The data are represented as the mean ± standard deviation and analyzed statistically using Duncan's tests. Different letters on the bars indicate significant difference within the same strain, whereas bars with common letter are not significantly different. Upper case letters are for *L. sphaericus* DM-3 and lower case letters are for *B. cereus* DM-5.

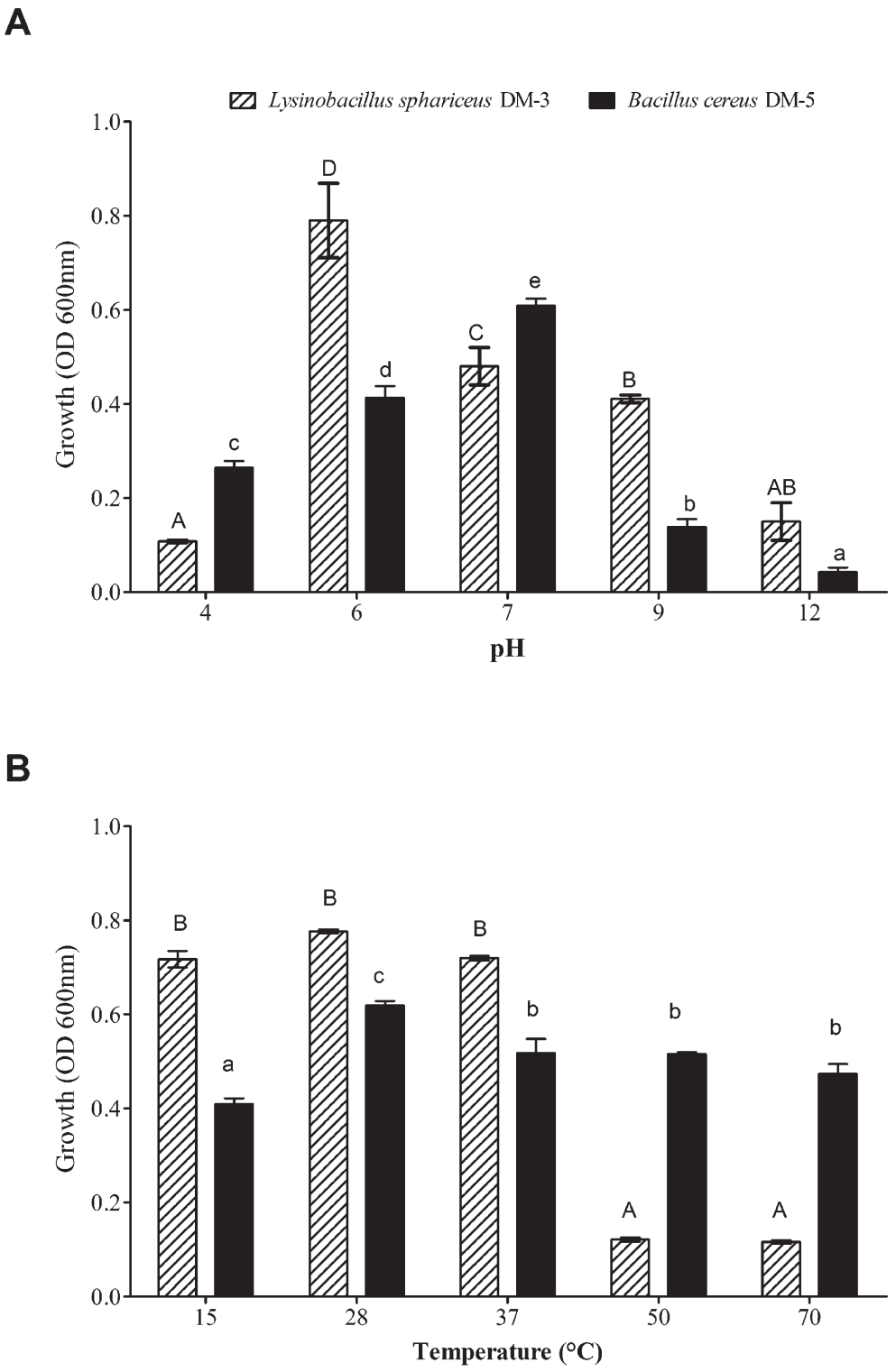

Fig. 4. Effect of changing pH (A) and different temperature (B) values on growth of *L. sphaericus* DM-3 and *B. cereus* DM-5 in the presence of dimethoate (100 mg/L) as a sole carbon source. The data are represented as the mean ± standard deviation as analyzed statistically using Duncan's tests. Different letters on the bars indicate significant difference within the same strain, whereas bars with common letter are not significantly different. Upper case letters are for *L. sphaericus* DM-3 and lower case letters are for *B. cereus* DM-5.

Quantification of dimethoate residues using High Performance Liquid Chromatography (HPLC)

The HPLC profile of MS media supplemented with 100 mg/L of dimethoate (positive control, Fig.5 a) showed 7 peaks with retention time (RT) of 1.46, 1.93, 2.43, 2.95, 3.80, 6.11 and 6.8 min (Fig.5 b). The HPLC profile obtained for the two bacteria treated samples significantly differed from the control in terms of number and height of peaks obtained and their RT. The HPLC profile of dimethoate treated with *L. sphaericus* DM-3 showed 4 peaks different from the control with RT 2.72, 3.52, 5.33 and 8.80 min (Fig.5 d). While the sample treated with *B. cereus* DM-5 showed two peaks different from the control with RT 1.91 and 2.71 min (Fig.5 c). These results suggested that there are degradation products are formed by action of both bacterial strains. The dimethoate concentration was reduced from the initial concentration 0.1 mg/ml to 0.076 (24% degradation) for *L. sphaericus* DM-3 and 0.083 mg/ml (17% degradation) for *B. cereus* DM-5, after 3 days of incubation.

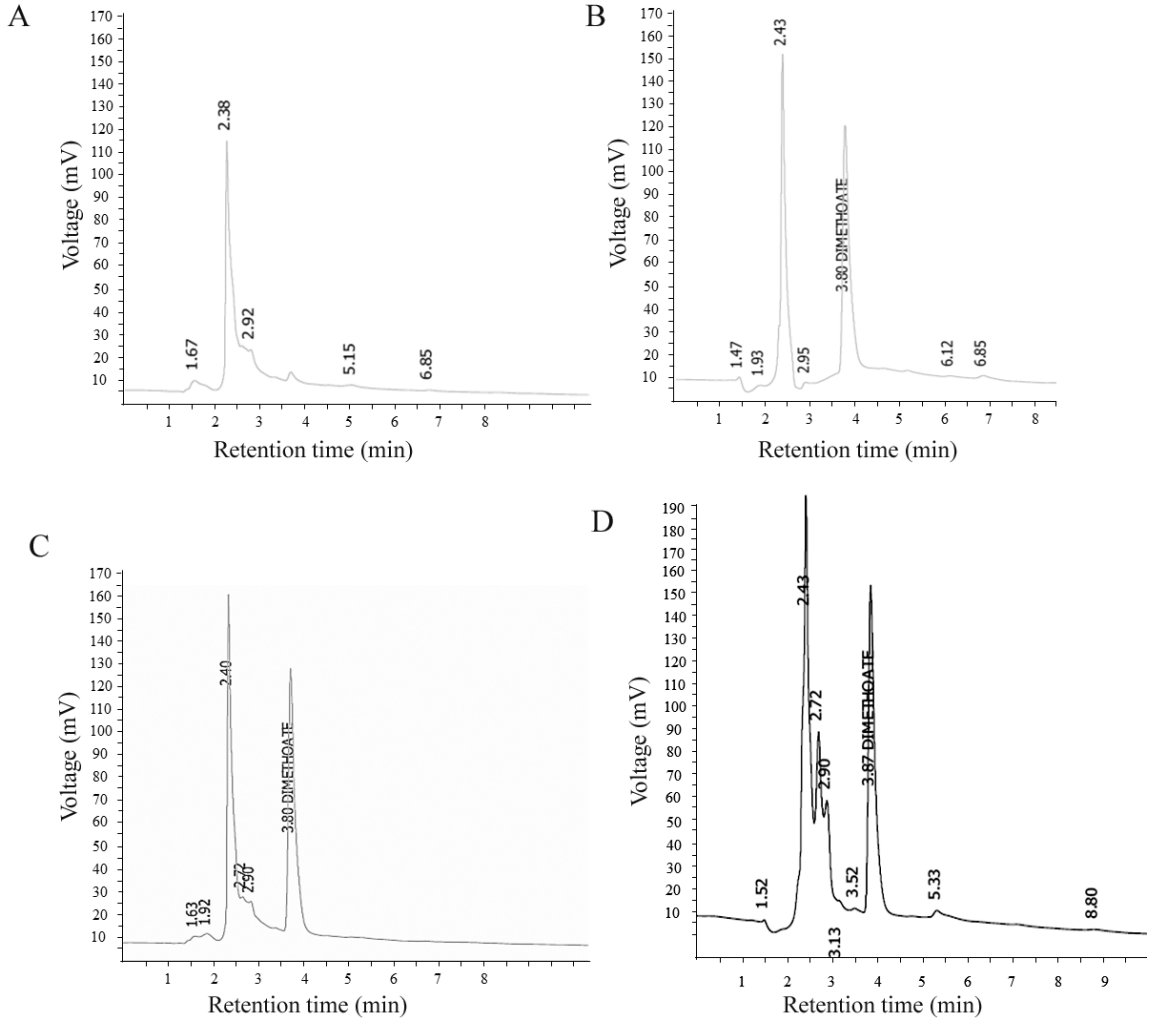

Fig. 5. Quantification of dimethoate degradation by *L. sphaericus* DM-3 and *B. cereus* DM-5 using HPLC analysis. Samples were prepared by inoculation of both strains in MS media supplemented with 100 mg/L dimethoate and 72 h of incubation. A) Control chromatogram of MS media without dimethoate B) Control chromatogram of MS media supplemented with 100 mg/L dimethoate C) HPLC chromatogram of MS media supplemented with 100 mg/L dimethoate and inoculated by *B. cereus* DM-5 D) HPLC chromatogram of MS media supplemented with 100 mg/L dimethoate and inoculated by *L. sphaericus* DM-3.

Identification of the metabolites resulting from the degradation of dimethoate using GC/MS

The metabolites produced during the degradation of dimethoate by *L. sphaericus* DM-3 and *B. cereus* DM-5 after 7 days of incubation, were detected using GC/MS. The spectrum patterns of dimethoate degrading residues by both strains were shown in Fig. 6. The GC/MS analysis of dimethoate degradation indicates that there were a considerable number of peaks. All the compounds were identified either according to NIST, W9N11 and RTLPEST3 library. Chemical names and retention times of the compounds produced by both strains are summarized in Tables 2 and 3. A peak with a retention time 10.54 was identified as O,O,S-trimethyl phophorothioic acid, was found to be the sole compound related to the biodegradation of dimethoate (Yao et al., 2011) in both strains with a 93% matching probability with W9N11 library. Many bacterial metabolic compounds, not related to dimethoate degradation, were detected in aqueous fractions of both strains such as oleic acid detected in *L. sphaericus* DM-3 (Table 2). The toxic metabolite, omethoate, was not detected in the aqueous extract of both strains.

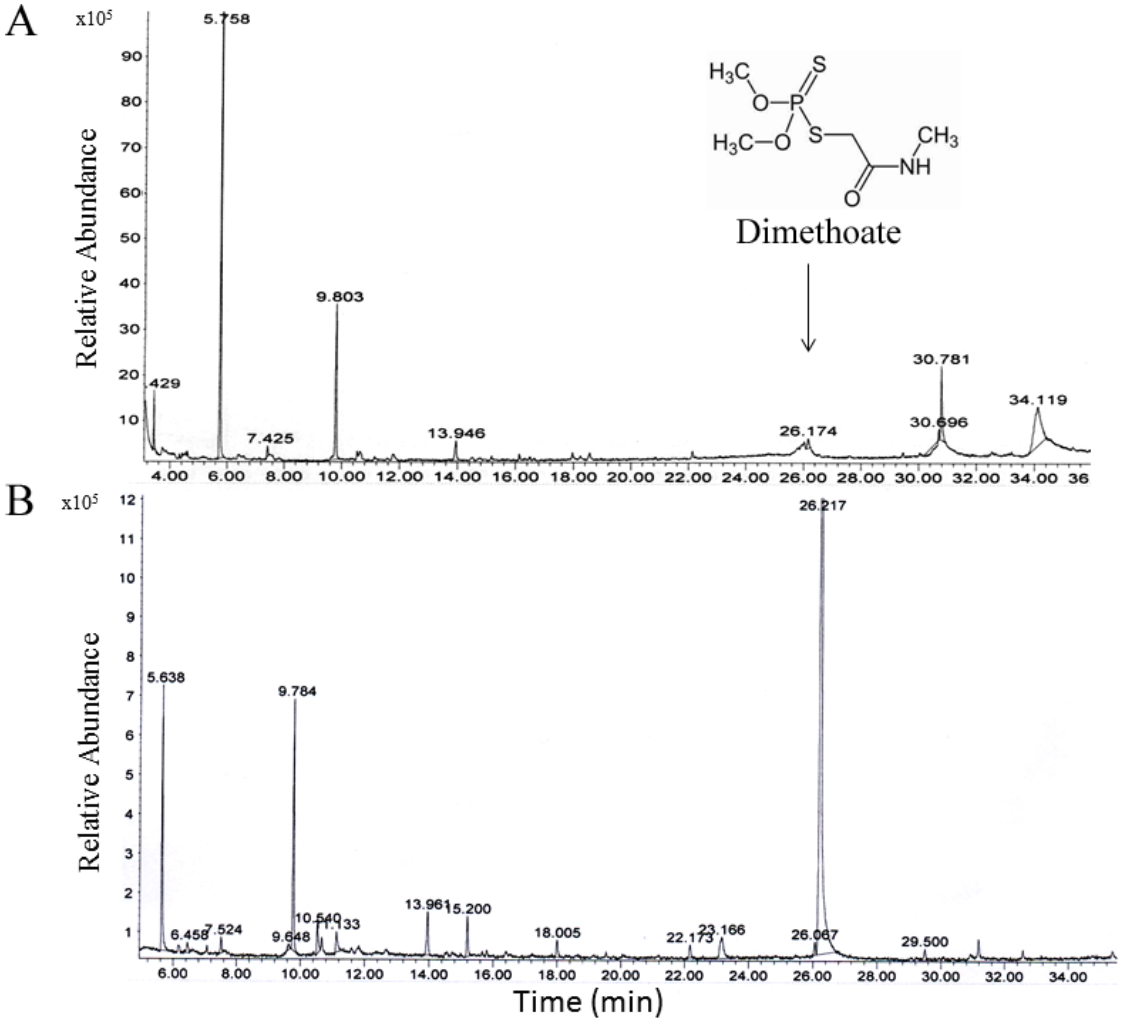

Fig. 6. Separation of metabolites resulting from dimethoate degrading strains *L. sphaericus* DM-3- and *B. cereus* DM-5 by GC/MS. Samples were prepared by inoculation of both strains in MS media supplemented with 100 mg/L dimethoate and incubated for 7 days. A) Metabolites produced due to degradation of dimethoate by *L. sphaericus* DM-3; B) Metabolites produced due to degradation of dimethoate by *B. cereus* DM-5. The metabolites were identified according to NIST, W9N11and RTLPEST3 library.

TABLE 2. The compounds detected by GC/MS in the aqueous fraction of culture filtrate of *L. sphaericus* DM-3 grown in MS broth supplemented with dimethoate.

Isolate	No.	Chemical Name	RT (min)	Area (%)	Quality
***Lysinibacillus sphaericus* DM-3**	1	Silanediol,dimethyl-dihyroxydimethylsilane, dimethylsilanediol	3.427	1.93	87
	2	2-Amino-6methylbenzoic acid, 6-amino-o-toluic acid	5.761	32.70	64
	3	Cyclotetrasiloxane, octamethyl	7.425	0.52	72
	4	Tetrasiloxane,decamethyl, decamethyltetrasiloxane	9.802	11.44	78
	5	Cyclotetrasiloxane, octamethyl,	13.945	2.36	87
	6	Dimethoate	26.177	1.06	96
	7	2-pentene,2-cyano-3-diethylboryl amino	30.693	2.18	43
	8	Meta-Methoxybenzenethiol m-methoxythiophenol	30.778	6.32	58
	8*	O,O,S-trimethyl phophorothioic acid	10.540	0.91	93
	9	Oleic Acid 9 octadecenoic acid (z)	34.123	17.10	99
	10	Hexanediooic acid, bis(2ethylhexyl) ester	38.529	1.04	64
	11	3,5,7-trimethyl-1-azaadamanatan-4,6,10-trione	39.004	1.44	25
	12	3,4,3a,4,7,7a-Hexahyro-5,6-bis(methoxymethoxy)-2-phenyl-4-[3-phenyl-1-(trimethy	39.318	19.13	37
	13	Bis (2-ethylhexyl) phthalate	40.905	1.87	83
	14	Cyclodecasiloxane, eicosamethyl-	41.236	1.16	58
	15	7,8,17,18-tetrahydro-35-methoxy-1,3,21,23-tetramethyl-16H,31H-5,9.15.19-dimethan	42.535	1.68	59
	16	1,2-Bis (trimethylesilyl) benzene	43.333	0.85	44
	17	23,24-Dicarbomethoxy-9,10-dimethoxyundecacyclo [16.10.1,1(4,15).1(21,26).0(2,17).	43.452	0,95	46
	18	2',4'-Dimethyloxaneilic acid N'-veratrylidenehydrazide	45.294	0.95	46

TABLE 3. The compounds detected by GC/MS in the aqueous fraction of culture filtrate of *B. cereus* DM-5 grown in MS broth supplemented with dimethoate.

Isolate	No.	Chemical Name	RT (min)	Area (%)	Quality
***Bacillus cereus* DM-5**	1	3,5-Dihyroxybenzamide	3.698	0.78	59
	2	Cyclotrisiloxane, hexamethyl 1,1,3,3,5,5-Hexamethyl-cyclohexasiloxane	3.885	2.55	72
	3	5,8-epoxy-15-nor-labdane	4.386	1.60	81
	4	Oxime, methoxy, phenyl-methyl N-hydroxybenzenecarboximidoate	5.642	8.17	83
	5	Trisiloxane,1,1,3,3,5,5-hexamethyl	6.457	0.70	49
	6	Cyclotetrasiloxane, octamethyl	7.527	0.78	74
	7	Cyclotrisiloxane, hexamethyl	9.649	0.04	59
	8	Cyclotisiloxane,hexamethyl-1,1,3,3,5,5-Hexamethyl-cycloHexasiloxane	9.785	9.48	83
	9	O,O,S-trimethyl phophorothioic acid	10.540	1.41	93
	10	4-ethylbenzamide	11.135	1.02	49
	11	Cyclotetrasiloxane,octamethyl-AS	13.961	2.23	74
	12	2-*p*-Nitrophenyl-oxadiazol-1,3,4-one-5	15.201	1.81	53
	13	Cyclopentasiloxane, decamethyl dimethysiloxanepentamer	18.002	1.14	90
	14	Cyclohexasiloxane,dodecamethyl	22.170	0.74	46
	15	1,2-benzenedicarboxylic acid, diethyl ester ethyl phthalate	23.163	2.83	93
	15’	Diethyl phthalate	23.163	2.83	96
	16	4-dimehylamino)phenyl-4’-(N-methyl)(N-ethyl) aminophenyl-imine	26.067	0.41	53
	17	Dimethoate	26.219	63.44	97
	18	Isoproterenol tri-TMS derivative	29.496	0.89	27

Discussion

Organophosphorus insecticides like dimethoate are considered potentially hazardous and have been known to cause several adverse effects on humans and other non-target organisms (Wang et al., 2013 and Qayoom et al., 2016). In this regard, microbial remediation of sites contaminated with chemical pesticides provides a robust and low cost removal process (Baez-Rogelio et al., 2017). Therefore, many studies were conducted to isolate bacterial strains capable of degrading OP compounds from contaminated soils (Wan et al., 2010; Chanika et al., 2011 and Geed et al., 2016).

In the present study, two dimethoate-degrading strains identified as *L. sphaericus* DM-3 and *B. cereus* DM-5 were isolated from dimethoate contaminated soil. The two degrading bacterial candidates were Gram-positive and endospore forming. This was a promising outcome as Gram-positive bacteria have a stronger cell envelope than Gram-negative bacteria. It was reported that bacterial endospore production ability is an adaptation mechanism for survival found in *Bacillus* species (Francis & Tebo, 2002). This may allow our two strains *L. sphaericus* DM-3 and *B. cereus* DM-5 to thrive in the highly variable environmental conditions.

The previously recorded bacteria degrading OP compounds are mainly *Bacillus* species, such as *B. licheniformis, B. pumilus, B. safensis, B. subtilis* and *B. cereus* **(**Mandal et al., 2005; Singh et al., 2006; Khan et al., 2016 and Begum et al., 2016). On contrary, few studies have pointed to the role of *Lysinibacillus* spp. in the degradation of pollutants (Wan et al., 2010; Manchola & Dussan, 2014 and Geed et al. 2016). To our knowledge, this is the first study to report the role of *Lysinibacillus* spp. in the degradation of dimethoate.

The degradation of dimethoate is affected by a large number of environmental factors (Deshpande et al., 2001). The pH value is an important factor that influences the microbiological metabolic activity and growth of microorganisms. Different microorganisms can grow over a wide pH range and every organism has its own tolerance level (Darsa et al., 2014). It was reported that dimethoate degrading-bacteria have optimum growth at pH ranges from 6 to 9 with maximum growth at pH 7 (Li et al., 2010 and Chen et al., 2016). Herein, *L. sphaericus* DM-3 and *B. cereus* DM-5 showed ability to degrade dimethoate within a broad range of pH 4-12. In general, neutral to slightly alkaline conditions are considered more favorable for bacterial metabolic activities and growth than acidic conditions (Padan et al., 2005).

Another factor that influences the growth and efficiency of pollutants-degrading bacteria is temperature. In this context, the rate of biodegradation mainly ceases at higher temperatures since the degradative enzymes are mostly plasmid-borne and bacterial cells are known to lose their plasmids at high temperatures (Deshpande et al., 2001). Furthermore, there are some evidences that bacterial incubation at 45°C may inhibit the synthesis of proteins that are required for survival of germinated spores at elevated temperature (Johnson & Busta, 1984). This is in line with our results which revealed that *L. sphaericus* DM-3 efficiently degrade dimethoate at temperatures ranging from 15 to 37°C and showed no significant growth at temperature higher than 37°C, suggesting that *L. sphaericus* DM-3 could have a plasmid encoding degradative enzymes. In contrast, *B. cereus* DM-5 showed the ability to degrade dimethoate at temperature above 37°C which indicate the high temperature tolerance of *B. cereus* DM-5 over *L. sphaericus* DM-3. In accordance with our results, Saleem et al. (2014) reported that *B. cereus* was effective in the decolonization of the industrial effluents at optimum pH 6.5 and temperature 45°C.

The utilization of dimethoate as the sole carbon source by bacteria has been reported in many studies (Deshpande et al., 2001 and DebMandal et al., 2008). The current study demonstrated that *L. sphaericus* DM-3 and *B. cereus* DM-5 could degrade dimethoate similar or even higher than previously reported bacterial strains. Both strains showed the ability to utilize 100 mg/L of dimethoate effectively and tolerate the presence of dimethoate up to concentration of 500 mg/L. Li et al. (2010) reported that *Paracoccus sp.* Lgjj-3 is able to utilize the same concentration of dimethoate (0.1 mg/ml). *Pseudomonas putida* and *Bacillus pumilus* reported maximum dimethoate degradation at concentration of 0.09 and 0.06 mg/ml of dimethoate, respectively (Jayamadhuri, 2014). In the present study, the growth in terms of optical density of *L. sphaericus* DM-3 was higher than the growth of *B. cereus* DM-5. Hence, the difference in degradation capability of various strains may be due to difference in enzyme system

required for degradation and/or difference in their growth rate (Bhattacharjee et al., 2014).

Four mechanisms have been proposed for dimethoate degradation: OH attack on the P=S bond, OH attack the P–S bond, the hydrolytic cleavage of thioester bond and pyrolytic decomposition of dimethoate (Yao et al., 2011). Moreover, various enzymes are involved in the degradation of dimethoate such as esterase, aldo–keto reductase, hydrolase and amidohydrolase (Liu et al 2001; Li et al. 2010 and Chen et al. 2016). The degradation of dimethoate can occur at the P–O–C, P–S or amide links of the molecule and then P=S bond may be oxidized by H_2O_2 to form P=O group (Wu et al. 2018).

In the current study, the main products of dimethoate biodegradation in aqueous fraction of culture filtrate of DM-3 and DM-5 were detected by GC/MS analysis. Among the various compounds detected, only one dimethoate degradation product, O,O,S-trimethyl phosphorothioate, was present in the degradation assay mixture for both strains. This result suggests that degradation of dimethoate by *L. sphaericus* DM-3 and *B. cereus* DM-5 is mainly preceded via enzymatic hydrolysis of S–CH_3 linkage**.** Supporting this explanation, Yao et al. (2011) proposed that O,O,S-trimethyl phosphorothioate could be produced by the cleavage of S–CH_3 linkage of dimethoate. Thereafter, O,O-dimethyl phosphorothioate could be further degraded into PO^{4+}, CO_2 and H_2O (Evgenidou et al. 2006).

Conclusion

the present study points out the efficiency of *L. sphaericus* DM-3 and *B. cereus* DM-5 to degrade dimethoate, up to 500 mg/L, over a wide range of pH (4-12). Such degradation was not accompanied by production of the toxic byproducts, omethoate, whereas O,O-dimethyl phosphorothioate was the major biodegradation product. Moreover, enzymatic hydrolysis of S–CH_3 linkage is the probable mechanism for degradation of dimethoate by the two bacterial strains.

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كفـــاءه كـــلا مـن لايسنوباسيللاس و باسيللاس سيريس على تكـســير الدايـمـيـثوات

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تـــم عزل سلالتين بكتريتين من تربـــة ملوثـــة بمبيد الدايميـثوات ثم تم تحديدهمـا و تعريفهمـا على أنهـما لايسنوباسيللاس و باسيللاس سيريس ثم أجريت تجارب لاختبار قدرتهما علي تكسيرمبيد الدايميثوات في وسط غذائي خالي من عنصر الكربون و يحتوي على تركيز 100 ملغم / لتر من الدايميثوات، وقد تم تقييم قدرة البكتريا على التكسير الحيـــــوي للدايميثوات من خلال اختبارات الظروف البيئية المختلفة (درجات الحموضة ودرجات الحرارة ومستويات التركيز المختلفة من الدايميثوات). كذلك تم تحديد بقايا الدايميثوات باستخدام تقنية الكروماتوجرافيا ذات الضغط العالى للسوائل HPLC وتم تحديد نواتج التكسير الحيوي باستخدام كروماتوجرافيا الغازGC/MS. كشفت النتائج المتحصل عليها قدرة كلا من السلالتين على النمو في تركيزات مختلفة من الدايميثوات 500- 100ملغم / لتر.

وكانت درجة الحرارة الملائمة لنمو السلالتين هي 28 درجة مئوية و كان أعلى نمو لكلا من السلالتين لايسنوباسيللاس و باسيللاس سيريس في وجود الدايميثوات في درجة حموضه 6 و 7 على التوالي. وأوضحت النتائج أيضا أن بكتريا لايسنوباسيللاس يمكن أن تحلل نسبة قدرها ٪24 من الدايميثوات خلال فترة حضانة 72 ساعة، في حين أن نسبة التحلل عن طريق بكتريا باسيللاس سيريس كانت ٪17 خلال 72 ساعة. بتحليل المركبات الناتجة عن عملية التكسير الحيوي بواسطة السلالتين تبين أن حمض الفوسفوثيويك، س، س، ث - ثلاثي ميثيل استر،هو المركب الوحيد المتعرف عليه كنتيجة للتكسير الحيوي لمبيد الدايميثوات في حين لا وجود لمركب أوميثوات الشديد السمية . تظهرنتائج هذه الدراسه كفاءة و قدرة عالية للسلالات المعزولة على التكسيرالحيوي لمبيد الدايميثوات في ظروف بيئية مختلفة و تركيزات عالية تصل إلى 500 ملغم/لتر.