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Original article (Orijinal araştırma)

Chlorpyrifos and deltamethrin degradation potentials of two *Lactobacillus plantarum* (Orla-Jensen, 1919) (Lactobacillales: Lactobacillaceae) strains

İki *Lactobacillus plantarum* (Orla-Jensen, 1919) (Lactobacillales: Lactobacillaceae) suşunun chlorpyrifos ve deltamethrini parçalama potansiyelleri

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Abstract

Many soil bacteria can degrade the synthetic insecticides chlorpyrifos and deltamethrin by their esterase enzymes and/or by using them as carbon and energy sources. The hypothesis tested was that similar degradation potential could be found in *Lactobacillus plantarum* (Orla-Jensen, 1919) (Lactobacillales: Lactobacillaceae) which is used in food fermentations. This study was conducted in-vitro in Bursa Uludağ University laboratories during 2017-2018 to demonstrate the two degradation mechanisms of *L. plantarum* strains LB-1 and LB-2, 4 d after inoculation. Significant growth in LB-1 found in mineral salt (MS) medium containing chlorpyrifos and deltamethrin compared with MS medium without insecticide and any carbon source. This strain also exhibited significantly enhanced hydrolysis activity. These capacities were found lower in LB-2 than LB-1. Based on periodically GC-MS analysis, degradation of chlorpyrifos and deltamethrin in MS medium proceeded by strains LB-1 and LB-2 reached the values of 96 and 90% and 24 and 53% after 3 d, respectively. Significant degradation of deltamethrin with both strains (86-82%) determined after 10 d. The study demonstrated that some *L. plantarum* strains could degrade chlorpyrifos and deltamethrin. Further studies should be conducted to show their effectiveness in the fermentation process of some fruits and vegetables and different bacteria inoculation rates.

Keywords: Degradation, esterase, insecticides, lactic acid bacteria, organophosphates, synthetic pyrethroids

Öz

Birçok toprak bakterisi chlorpyrifos ve deltamethrin gibi sentetik insektisitleri esteraz enzimleriyle ve/veya bunları karbon ve enerji kaynağı olarak kullanarak parçalayabilmektedir. Bizim bu çalışmadaki hipotezimiz gıda fermantasyonu aşamalarında kullanılan *Lactobacillus plantarum* (Orla-Jensen, 1919) (Lactobacillales: Lactobacillaceae)'un benzer bir insektisit parçalama potansiyelinin gösterilmesidir. Bu çalışma, *L. plantarum*'un iki farklı suşunun (LB-1 ve LB-2) aşılamadan sonraki 4 gün içinde, iki farklı insektisit parçalama mekanizmasını göstermek amacıyla 2017-2018 yıllarında, Bursa Uludağ Üniversitesi laboratuvarlarında, in-vitro koşullarda gerçekleştirilmiştir. Herhangi bir karbon ve enerji kaynağı içermeyen mineral tuz (MS) ortamı ile karşılaştırıldığında, chlorpyrifos ve deltamethrin içeren MS ortamında önemli düzeyde LB-1 gelişimi saptanmıştır. Ayrıca, bu suş için önemli düzeyde artan hidroliz aktivitesi de gözlemlenmiştir. Bu özellikler LB-2'de bir miktar daha düşük bulunmuştur. GC-MS cihazı ile yapılan periyodik analizler sonucunda, LB-1 ve LB-2 inoküle edilmiş MS ortamı içinde chlorpyrifos ve deltamethrin'in parçalanma oranları, 3 gün sonra chlorpyrifos için sırasıyla %96 ve 90, deltamethrin için %24 ve 53 olarak belirlenmiştir. Deltamethrin için önemli düzeyde bir parçalanma (%86-82) inkübasyondan 10 gün sonra gerçekleşmiştir. Bu çalışma, denemede kullanılan *L. plantarum* suşlarının chlorpyrifos ve deltamethrin parçalanma potansiyellerinin olduğunu göstermiştir. İleride bazı meyve sebzelerin fermentasyon süreçlerinde kullanımı ve bu suşların farklı inokülasyon oranlarında etkinliğinin belirlenmesi amacıyla daha fazla çalışma yapılması gerekmektedir.

Anahtar sözcükler: Parçalanma, esteraz, insektisitler, laktik asit bakterileri, organik fosforlular, sentetik piretroitler

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Introduction

Although the use of environmentally-friendly and target-specific pesticides have become widespread around the developed and the developing world in recent years, the broad-spectrum synthetic insecticides, organophosphorus and synthetic pyrethroids, are the most commonly used compounds for the control of the critical pests of many cultivated plants with 13 and 4% usage rates, respectively, among all insecticides (FAO, 2019). Also, chlorpyrifos and deltamethrin are the most commonly-used insecticides around the world (Maya et al., 2011; Cycon et al., 2014). Although a number of organophosphorus insecticides including chlorpyrifos have been banned due to hazard on the human, environment and non-target organisms in European Union Countries and Turkey, the use of these compounds has continued in a large part of the world (Anonymous, 2019; EC, 2019). Chlorpyrifos has been commercially used since the 1960s for the control of many insect pests in agriculture areas, is a moderately toxic compound having an acute oral LD_{50} of 135-163 mg/kg for rats and have a relative risk for lung cancer in human (Cho et al., 2009). Although the synthetic pyrethroids are much less toxic to mammals than organophosphorus, they have adverse health effects to human such as lymph node and splenic damage, carcinogenesis, and hormonal activity. The half-life (DT_{50}) of chlorpyrifos is between 27 and 386 d, and 386 d under laboratory conditions at 20°C. In the presence of hydroxyl radicals, this can be as short as 6 h. Sunlight, water content and/or soil microorganisms effect chlorpyrifos degradation (Anonymous, 2020; Eaton et al., 2008). The key metabolites are 3,5,6-trichloro-2-pyridinol and 2,3,5-trichloro-6-methoxypyridine, other known metabolite is O-ethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioic acid.

Deltamethrin has been used widely since the 1980s on various crops including all cultivated plants and human-disease vectors. The acute toxicity of deltamethrin was calculated orally as LD_{50} of 114-168 mg/kg for rats (Wu et al., 2006). Depending on the combined effects of some factors such as pH, temperature, microbial activity, metabolism and photolysis, approximately half-life of chlorpyrifos and deltamethrin is 36-46 d and 11-19 d, respectively (Roberts et al., 1998; Simon, 2014). The residues of the two compounds would be found on various foods depending on the degradation duration of the insecticide. The DT_{50} of deltamethrin changes between 21-58 d, and 28 d under laboratory conditions at 20°C. The key metabolites are decamethrinic acid and 3-phenoxybenzoic acid, other known metabolites are 3-(4-hydroxyphenoxy) benzoic acid, 4-hydroxydeltamethrin, 3-phenoxybenzoic acid (Anonymous, 2020).

Previous studies have shown that many soil bacteria could metabolize broad-spectrum synthetic insecticides, i.e., organophosphorus and synthetic pyrethroids by their esterase enzymes and/or by using the insecticides as carbon and energy sources (Lu et al., 2006; Singh & Walker, 2006; Yang et al., 2006; Lakshmi et al., 2008; Chen et al., 2011a, b, c, d, 2012a, b; Fenner et al., 2013). Chlorpyrifos degradation in soil and water occurred with both chemical hydrolysis and microbial activity. In that case, some aerobic bacteria can transform the compound by hydrolysis to produce diethyl thiophosphoric acid and 3,5,6-trichloro-2-pyridinol (Lu et al., 2006; Yang et al., 2006). Similarly, deltamethrin is degraded via both hydrolysis and microbial activity in soil, but slower under anaerobic conditions (Cycon et al., 2014). Deltamethrin degraded firstly to a-hydroxy-3phenoxy-benzeneacetonitrile and 3-phenoxybenzaldehyde with various genera soil bacteria using carboxyl ester. The latter compound is oxidized to 2-hydroxy-4-methoxy benzophenone (Chen et al., 2011b).

Similarly, some lactic acid bacteria (LAB) in some genera such as *Lactobacillus* (Cho et al., 2009; Islam et al., 2010; Zhao & Wang, 2012; Dordevic et al., 2013) and *Leuconostoc* (Cho et al., 2009) can metabolize insecticides by their specific enzymes such as esterase and/or by using these compounds as carbon and energy sources (Choi et al., 2004; Cho et al., 2009; Islam et al., 2010; Kumral & Kumral, 2013). LAB have gained much interest for their health benefits and are widely used as probiotics and starter culture for fermented products because of their generally recognized as safe status (Maragkoudakis et al., 2006). Mainly, the bacteria in fermented product play various roles during fermentation and the primary benefit of

its use is the preservative effect, by suppressing harmful bacterial growth during fermentation or storage periods. Biodegradation of pesticides is a promising technology since its potential for the removal of residues from food and agricultural products (Maragkoudakis et al., 2006). Some previous studies showed that some LAB including *Lactobacillus* spp. are involved in the degradation of insecticides but there is limited information about their potential to be used for the decontamination of foodstuff.

This study aimed to monitor the insecticide degradation potential and mechanisms of two *Lactobacillus plantarum* (Orla-Jensen, 1919) (Lactobacillales: Lactobacillaceae) strains isolated from fermented table olives. To demonstrate the use of insecticides as an energy/carbon source, the growth of the *L. plantarum* strains was monitored by plate counts in mineral salt (MS) medium supplemented with nitrogen, and containing chlorpyrifos or deltamethrin as the sole carbon sources. Metabolic capacities of the strains were determined by spectrophotometric esterase enzyme activity tests in the presence and absence of the insecticides. Insecticide degradation potentials of the strains were detected by GC-MS analysis in MS medium periodically.

Materials and Methods

This study was conducted in-vitro in Bursa Uludağ University laboratories during 2017-2018.

Test Microorganisms

Lactobacillus plantarum strains LB-1 and LB-2 used in this research were previously isolated from the fermentation brines of naturally fermented black olives of Gemlik variety in Bursa (Turkey). Strains were identified by 16s rRNA technique (Kumral et al., 2012) and differentiated from other group members according to Torriani et al. (2001). These strains were selected according to their survival potential in MS medium containing insecticides as sole carbon sources (Kumral & Kumral, 2014, 2016).

Chemicals and Reagents

Chlorpyrifos (O,O-diethyl-O-3,5,6-trichloro-2-pyridyl phosphorothioate) and deltamethrin ([(S)-cyano-(3-phenoxyphenyl) methyl] (1R,3R)-3-(2,2-dibromoethenyl)-2,2-dimethyl cyclopropane-1-carboxylate) were purchased from Sigma-Aldrich Chemical Company. All other reagents were of analytical grade.

Monitoring the Growth of Test Strains

The growth media used during the tests were De Man, Rogosa and Sharpe (MRS) broth, the optimum growth medium for LAB, and mineral salt (MS) medium containing no carbon source (Table 1). The growth of both test strains was monitored in; (i) MRS broth, (ii) MS medium, (iii) MRS broth supplemented with insecticides (chlorpyrifos and deltamethrin), (iv) MS medium supplemented with insecticides (chlorpyrifos and deltamethrin). Sterilized MRS broth and MS media tubes supplemented with filter sterilized insecticides at 100 mg/L. All the trials were inoculated with 18-24 h old test strains at a concentration of 10^8-10^9 colony forming units (CFU)/mL and left for incubation at 30ºC for 4 d (Cho et al., 2009). Microbial changes were monitored periodically using the spiral plating method.

Esterase Activity

The esterase activity of the test strains was detected by using α-naphthyl acetate (α-NA) as the substrate using the method which adapted in part from Morichi et al. (1968). Esterase activity was detected in MS media in the presence and absence of insecticides. MS media without insecticides were used as controls for both test strains. After 16 h of incubation, bacteria cultures were harvested by centrifugation at 10 000 g for 10 min at 4ºC. Bacteria cells were washed twice with 0.1 M phosphate buffer (pH 7.0) and resuspended with the same buffer. The assay mixture contained 60 µl of 100 mM sodium phosphate buffer, pH 7.0, 20 µl of α-naphthyl acetate (10 mM in dimethyl sulphoxide), 100 µl of cell suspension and 20 µl of fast blue RR (1.5 g/L). The absorbance change was kinetically measured at 500 nm at 23ºC in a Bio-Tek

Kinetic Microplate Reader (Winooski, USA) for 60 min. To determine the enhanced esterase activity of the strains, the assays performed in the presence and absence of the insecticides as an activator. The amount of protein in the enzyme source was determined according to the original procedure of Bradford (1976). The formation of the 1-naphthol-Fast Blue RR dye complex was measured at 500 nm and converted to specific activity using a standard curve of 1-naphthol and Fast Blue RR. All of the analysis was done in triplicate and the results were given as µmol p-nitrofenol per minute per mg protein.

Table. 1. Chemical composition of media (de Man et al., 1960; Cho et al., 2009)

Ingredients	Concentration (g/L)	
	MS Medium	MRS broth
Potassium dihydrogen phosphate	2.27	2.0
Sodium dihydrogen phosphate dodecahydrate	5.97	-
Sodium chloride	1	-
Magnesium sulfate heptahydrate	0.5	0.2
Calcium chloride dihydrate	0.01	-
Manganese sulfate tetrahydrate	0.02	0.05
Ferrous sulfate heptahydrate	0.05	-
Pepton from casein	0.01	10
Meat extract	-	8.0
Yeast extract	-	4.0
Glucose	-	20.0
Sodium acetate	-	5.0
Di-potassium hydrogen phosphate		2.0
Di-ammonium hydrogen citrate		2.0
Tween 80		1.0

Insecticide Residue Analysis

Extraction and purification of chlorpyrifos and deltamethrin were carried out based on the method of Aksu (2007). Liquid MS medium samples (5 mL) added with anhydrous $MgSO_4$ (2 g) and NaCl (0.25 g) were extracted with 5 mL of acetonitrile-dichloromethane (1:1, v/v) by vortexing for 2 min, and centrifuged at 7000 g for 5 min. The supernatant (2 mL) and $MgSO_4$ (0.15 g) were transferred to a new tube, and vortexed for 2 min, and centrifuged at 7000 g for 5 min. The liquid phase was used for further GC-MS analysis. Concentrations of chlorpyrifos and deltamethrin were measured using Perkin Elmer Clarus 680 Gas Chromatography-Clarus SQ8T Mass Spectrometry (Ohio, USA). Analyses were performed with a capillary column (PerkinElmer Elite-5MS, 30 m, 0.25 mm ID, film thickness 0.25 µm) and using Helium (1 mL/min) as a carrier gas. The injection temperature was 220ºC, and the injection volume was 1 µL. The oven temperature was increased linearly from 70 to 150ºC by 25ºC/min, and 150 to 200ºC by 2.7ºC/min and 200 to 285ºC by 6ºC/min. Retention time for chlorpyrifos and deltamethrin under these chromatographic conditions was 20.8 and 39.5 min, respectively (Figure 1). Five concentration levels from 0.01 to 100 mg/L were analyzed in the GC to show the linear range of detection of the insecticides or to calculate the standard curve by linear regression analysis.

The instrument was operated in SIR mode, and three selected ions for each compound for identification and quantification were monitored (chlorpyrifos, 97, 197 and 199 m/z; deltamethrin 181, 253 and 77 m/z). These ions were selected following the criteria of highest relative abundance, characteristic fragment ions and no interferences with the nearby peaks. Typical GC-MS peaks and selected ions of chlorpyrifos and deltamethrin standards are given in Figure1.

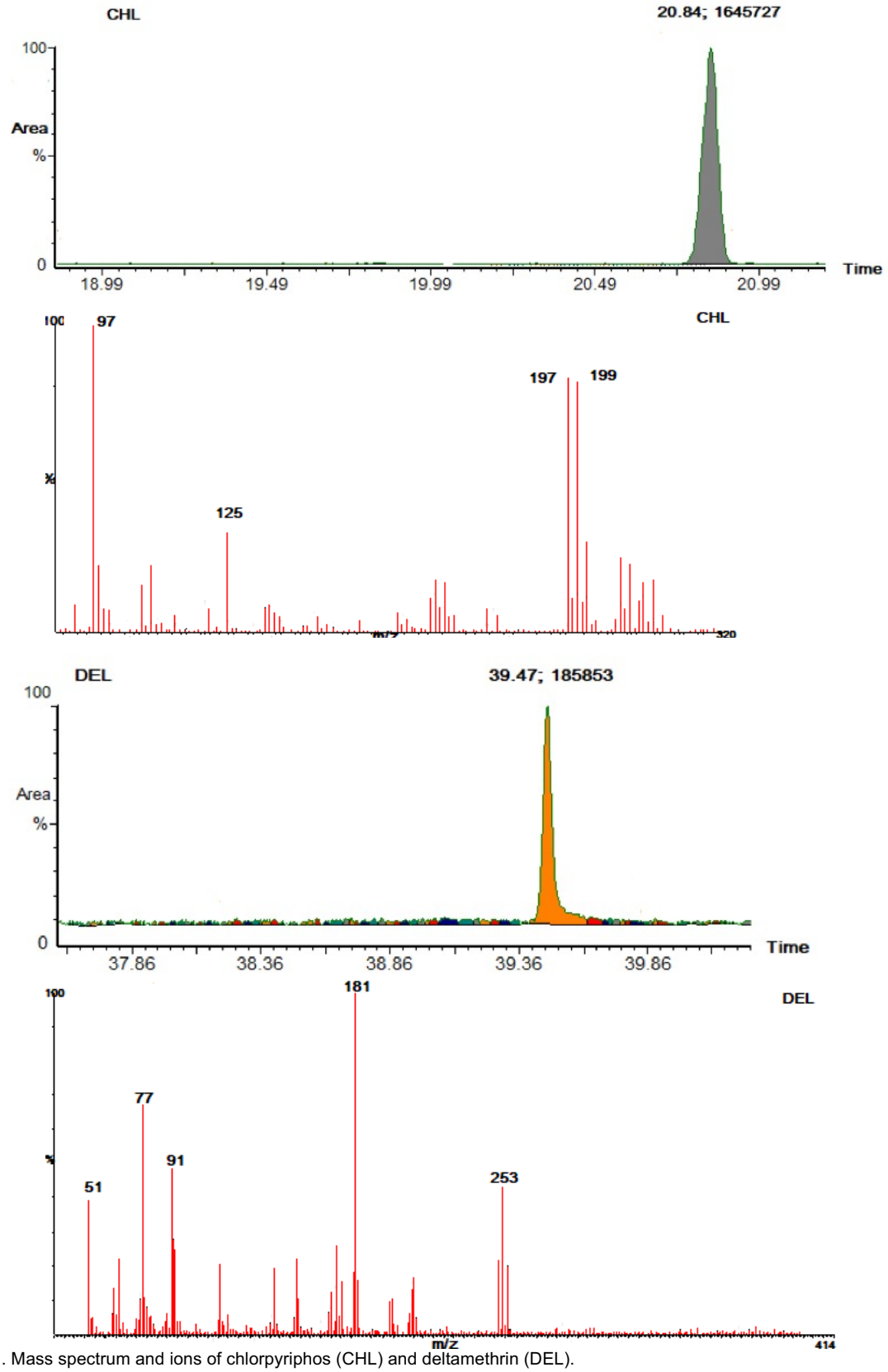

Figure 1. Mass spectrum and ions of chlorpyriphos (CHL) and deltamethrin (DEL).

Statistical Analysis

Two-way analysis of variance (ANOVA) (two-way) was performed on mean values for each detection time. The effects of time, LB strains, medium on bacteria growth were analysis fit model of SAS. Then post hoc testing ($p<0.05$) of multiple comparisons was performed by Tukey test (SAS 2007).

Results and Discussion

Growth of bacteria

LAB is used as starter cultures during industrial food processing to control the overall fermentation process, to reduce the risk of fermentation failure and fermentation period, to improve end product quality and to standardize the process (Kavitake et al., 2018). Especially they are of great industrial significance in that they play a vital role in the manufacturing, flavor, and texture development of fermented dairy foods. Furthermore, additional interest in starter bacteria has been generated because of the data accumulating on the potential health benefits of these organisms (Cogan et al., 2007). The effects of different groups of soil bacteria on pesticide degradation has reported previously (Lu et al., 2006; Singh & Walker, 2006; Yang et al., 2006; Lakshmi et al., 2008; Chen et al. 2011a, b, c, d, 2012a, b; Fenner et al., 2013), but there is scarce information about the potential of LAB. The results of this study showed that, the growth of bacterial strains (LB-1 and LB-2) in the presence and absence of chlorpyrifos and deltamethrin (at 100 mg/L) in MS and MRS medium are shown in Figure 2. In the MS medium containing chlorpyrifos and deltamethrin as the only carbon sources, after 2 d, an increase in the cell numbers of LB-1 was detected compared with that of control without insecticides (Figure 2) ($F_{5,11=}$40.3, P<0.01). In MRS medium containing alternative carbon and energy sources, in both of the insecticide containing treatments, a decline was observed in the cell growth of LB-1 in all treatments and control, probably as a result of decreasing energy sources. However, there is no significant difference between MS and MRS media containing insecticides with LB-1 after 4 d ($F_{5,11=}$13.6, P<0.01). This result may be evidence for the use of both chlorpyrifos and deltamethrin as a carbon and energy source by LB-1, in the absence of main nutrient sources (Figure 2).

For LB-2 strain, in MS media both with presence and absence of chlorpyrifos and deltamethrin, significant declines were observed in cell numbers after 2 and 4 d compared with those of MRS media ($F_{5,11=}$690, P<0.01). In MS medium containing deltamethrin, a slower declining trend was observed between 2 and 4 d (Figure 2). In MRS media containing both insecticides, a significant decline was observed in the cell number of LB-2 compared with that of control (Figure 2) ($F_{5,11=}$125, P<0.01). Bacteria growth models for LB-1 and LB-2 were significant ($F_{17,35=}$53.4, P<0.01; $F_{17,35=}$321, P<0.01). According to the fit model of LB-1 and LB-2, while MRS media significantly increased the growth of the bacteria ($F_{1,1=}$98.3, P<0.01; $F_{1,1=}$1720, P<0.01), addition of deltamethrin to both media was enhanced only the growth of LB-1 compared with no deltamethrin containing media ($F_{2,2=}$9.6, P<0.01).

In the present study, significant cell growth in one of the *L. plantarum* strains (LB-1) was detected in MS media containing chlorpyrifos and deltamethrin as the only carbon source compared to control (MS media without insecticide and any carbon source). Additionally, the growth capacity of LB-2 strain of *L. plantarum* was found weaker compared with LB-1. These results confirm that some strains of *L. plantarum* are capable of using chlorpyrifos and deltamethrin as carbon and energy source. Cho et al. (2009) reported that four LAB [*Lactobacillus brevis* (Orla-Jensen, 1919)*, L. plantarum, Lactobacillus sakei* Katagiri et al., 1934 and *Leuconostoc mesenteroides* (Tsenkovskii, 1878) van Tieghem, 1878] that they isolated from kimchi fermentation, grew well in the first day, but decreased slowly by day 2, and then increased gradually at day 6 in media containing 30 mg/L of chlorpyrifos. In accordance with our results, the authors showed that chlorpyrifos could be utilized by the strains as the sole carbon source. Additionally, it was reported that some soil bacteria [e.g., *Bacillus pumilus* Meyer & Gottheil, 1901*, Pseudomonas* spp., *Serratia liquefaciens* (Grimes & Hennerty, 1931) Bascomb et al., 1971*, Serratia marcescens* Bizio, 1823] metabolized chlorpyriphos

or other organophosphorus insecticides even in the presence of adequate nutrients in environment and its degrading ability was positively influenced by the presence of the supplementary nutrient sources (Nawab et al., 2003; Anwar et al., 2009; Cycon et al., 2009). Similarly, Cycon et al. (2014) showed that culturing bacteria in MS media containing two strains of soil originated *S. marcescens* were capable of using deltamethrin as sources of carbon and energy. Furthermore, pyrethroid-degrading soil bacteria were reported in various genera such as *Bacillus, Micrococcus, Ochrobactrum, Pseudomonas, Sphingobium, Stenotrophomonas* and *Streptomyces* (Madiha et al., 2013; Cycon et al., 2014). Our results are similar to those of other bacteria studies on deltamethrin, the findings obtained from this study are the first attempt of degradation of deltamethrin with *L. plantarum* strains.

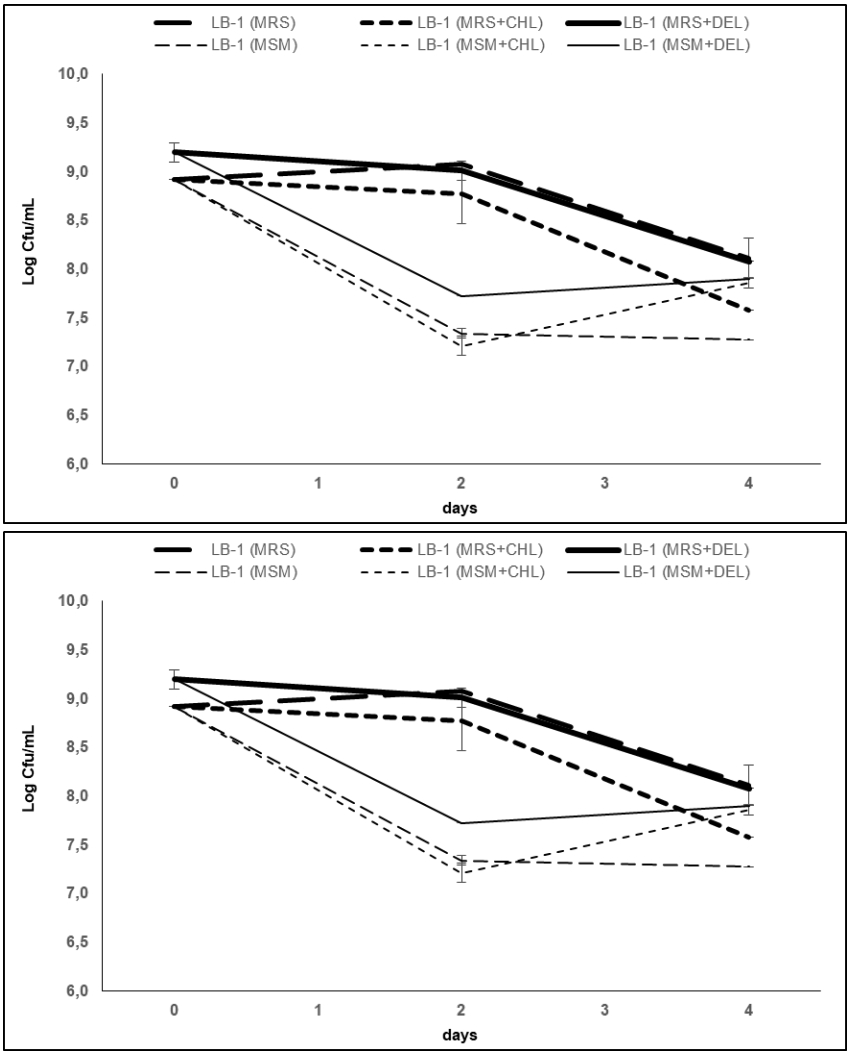

Figure 2. Mean bacterial growth of two *Lactobacillus plantarum* strains, LB-1 and LB-2, in the presence and absence of chlorpyrifos (CHL) and deltamethrin (DEL) in MS and MRS media (CFU, colony forming units).

Esterase Activity

The esterase activities in two *L. plantarum* strains at the presence and absence of chlorpyrifos and deltamethrin were given in Table 2. Based on the two-way ANOVA, the effects of strains and pesticide addition on the esterase activity were significant ($F_{5,17}$=17.6, P=0.01). There are no significant differences between esterase activities of both strains in the absence of the insecticides (Table 2). When chlorpyrifos or deltamethrin was added to the medium containing LB-1, the level of the esterase activity did not change

significantly ($F_{2,2}$=8.29, P=0.006). However, the esterase activity of LB-1 was inhibited 1.8% and 20% with deltamethrin and chlorpyrifos, respectively, and the effects were not significant. In LB-2, when deltamethrin added to the medium, the level of esterase activity decreased significantly. Similarly, the activity of LB-2 was affected by the addition of chlorpyrifos to the medium, but the effect (39.6%) was not found significant. The esterase activity of LB-1 was found significantly higher than that of LB-2 ($F_{1,1}$=53.4, P<0.01).

Table 2. The esterase activity in two *Lactobacillus plantarum* strains with and without chlorpyrifos (CHL) and deltamethrin (DEL) in MS medium

Treatments	Esterase activity (µmol p-nitrofenol/min/mg protein)*	Inhibition rate (%)**
LB-1	20532±2002 a	-
LB-1 + DEL	20165.4±3099 a	1.78
LB-1 + CHL	16389±110 ab	20.18
LB-2	15667.8±1054 ab	-
LB-2 + DEL	3002.7±481 c	80.84
LB-2 + CHL	9470±851 bc	39.56

* Means are not significantly different with same letters (Tukey, P<0.01);
** Inhibition rate was shown the inhibition of esterase activities of LB strains by insecticides as percentage.

In the present study, LB-1 grew faster and also had higher esterase activity. It is well known that some bacteria can metabolize insecticides by their specific enzymes such as esterase (Cycon et al., 2009). Enzymes initiate the significant mechanism for degradation, which depends upon the nature and type of substrates (Kumral & Kumral, 2013; Simon, 2014). Several studies have shown that many organophosphorus compounds, include esters of phosphoric acid, and can be hydrolyzed by carboxylesterase and phosphotriesterase (Simon, 2014). Cho et al. (2009) pointed out that some strains of *L. brevis, L. mesenteroides*, *L. plantarum* and *L. sakei* were capable of hydrolyzing five organophosphorus compounds, viz., chlorpyrifos, coumaphos, diazinon, parathion and methyl parathion. Similarly, Islam et al. (2010) demonstrated that one strain of *L. brevis* isolated from kimchi was capable of biodegrading chlorpyrifos. The researchers also pointed out that the *L. brevis* strain can hydrolyze chlorpyrifos with some enzymes including esterase and use the hydrolyzed products as their sole source of carbon. Accordance with our results, some investigators demonstrated that the purified recombinant esterase enzyme obtained from some bacteria species, viz., *Bacillus licheniformis* (Weigmann, 1898) Chester, 1901, *Bacillus stearothermophilus* Donk, 1920 and *Lactobacillus casei* (Orla-Jensen, 1916) Hansen & Lessel, 1971, can hydrolyze cypermethrin, permethrin, fenvalerate, deltamethrin, and malathion (Kim et al., 1998; Alvarez et al., 1999; Sogorb & Vilanova, 2002; Choi et al., 2004). Therefore, our results showed that some *L. plantarum* strains can utilize organophosphorus and synthetic pyrethroids through hydrolysis using bacterial enzyme-esterase.

Insecticide Residues

Changes in the chlorpyrifos levels of MS media inoculated with LB-1 and LB-2 strains detected with GC-MS analysis are given in Figure 3. During the first 3 d of incubation, the concentration of chlorpyrifos decreased quickly in media inoculated with LB-1 and LB-2 (96 and 90% reduction, respectively). Although there was no significant difference between the two test strains, the degradation rate in control was lower compared to test strains ($F_{2,9}$=13.3, P=0.002). After 7 d, a large portion of the chlorpyrifos (93-98%) was degraded in all media including control. Although the reduction rate (98%) in medium contains LB-2 strain was higher than the rates of LB-1 (97%) and control (93%), the differences were not significant ($F_{2,9}$=2.53, P=0.14). According to the fit model, LB strains have affected the degradation of chlorpyrifos ($F_{8,31}$=22.2; P<0.01). However, in these analysis time and time-treatment interactions were significant (time: $F_{2,2}$=88.7; P<0.01; time-treatment: $F_{4,4}$=0.55, P=0.69).

Changes in the deltamethrin levels of MS media inoculated with LB-1 and LB-2 strains detected with GC-MS analysis were given in Figure 3. Deltamethrin decomposed rapidly in medium containing LB-1 and LB-2 after 3 d (24 and 53% reduction, respectively). There is a significant difference among media inoculated with LB strains and control (no inoculation) ($F_{2,9}$=2.48, P=0.01). After 10 d, in media inoculated with the two strains, deltamethrin was degraded by a large proportion (86 and 82% for LB1 and LB2, respectively). The reduction (59%) in control was significantly lower compared to the media containing LB strains ($F_{2,9}$=11.7, P=0.003). Similarly, based on the fitted model, LB strains were affected to the degradation of deltamethrin ($F_{11,38}$=6.25, P<0.01). The effects of both time and treatment independently were found significant (time: $F_{2,2}$=16.2, P<0.01; treatment: $F_{2,2}$=5.59, P=0.007).

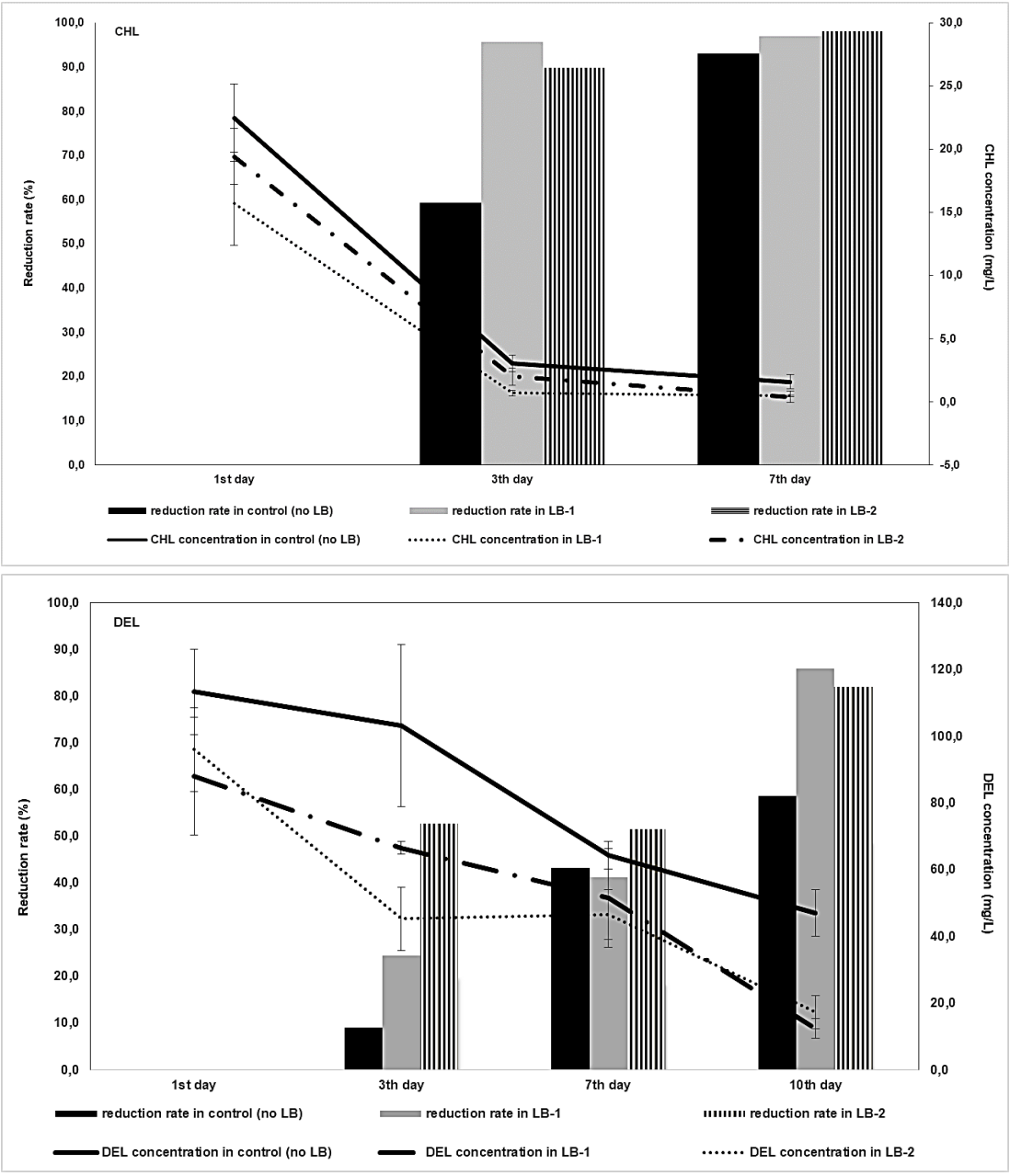

Figure 3. Effects of *Lactobacillus plantarum* strains LB-1 and LB-2 on degradation of chlorpyrifos (CHL) and deltamethrin (DEL) in MS media.

In the present study, degradation of chlorpyrifos and deltamethrin in MS media incubated with the LB-1 and LB-2 reached the values of 96 and 90% and 24 and 53% after 3 d, respectively, based on GC-MS analysis. Significant degradation of deltamethrin by LB-1 and LB-2 strains (86-82%, respectively) was determined after 10 d. This result was consistent with a previous study (Cho et al., 2009) reporting the effects of four LAB (*L. brevis, L. mesenteroides, L. plantarum* and *L. sakei*) on chlorpyrifos degradation. In that study, chlorpyrifos was degraded quickly within 3 d (83.3%), and then complete degradation occurred after 9 d during the fermentation of kimchi at 25ºC for 6 h. Additionally, Lakshmi et al. (2008) showed that some aerobic bacteria isolates obtained from soil samples [*Bacillius subtilis* (Ehrenberg, 1835) Cohn, 1872, *Brucella melitensis* (Hughes, 1893) Meyer & Shaw, 1920, *Pseudomonas aeroginosa* (Schroeter, 1872) Migula, 1900 and *Pseudomanas fluorescens* Migula, 1895] were capable of degrading chlorpyrifos (50 mg/mL) by an enrichment technique, significantly more (45, 50, 69 and 68%) compared with to control (5%) after 6 h of incubation and further increased to 69, 76, 100 and 93% after 12 h respectively. Dordevic et al., (2013), showed that the residue of bifenthrin, one of the synthetic pyrethroids, was reduced rate of 63% in wheat samples fermented with *L. plantarum* within 24 d at 30ºC. The findings obtained from this study are the first evidences about degradation rate of deltamethrin with *L. plantarum.*

Conclusion

In conclusion, it was shown that some strains of *L. plantarum* can metabolize insecticides by the esterase enzyme and use these compounds as carbon and energy sources. The results are consistent with other publications on the role of bacteria in insecticide degradation in different food commodities (Sogorb & Vilanova, 2002; Wu et al., 2006; Cho et al., 2009; Islam et al., 2010; Zhao & Wang, 2012; Dordevic et al., 2013). Also, the results demonstrated that *L. plantarum* strains have potential for the degradation of the residues. According to our results, there are some differences between strains in terms of growth rate, esterase activity and degradation potential. This could be due to the preadaptation of the bacteria to insecticides, pesticide concentration or bacteria population (Boethling, 1993). In the future, additional LAB species and strains having higher biodegradation capacity should be examined. Also, the potentials of the test strains on the degradation of other insecticides should be studied. Considering the adverse effects of insecticides on human health, to decrease insecticide residues in contaminated foods, experimental studies on the degradation potential of these in some food fermentation processes should be conducted.

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