JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Biodegradation of Chlorpyrifos by Lactic Acid Bacteria during Kimchi Fermentation

KYE MAN CHO, REUKARADHYA K. MATH, SHAH MD. ASRAFUL ISLAM, WOO JIN LIM, SU YOUNG HONG, JONG MIN KIM, MYOUNG GEUN YUN, JI JOONG CHO, AND HAN DAE YUN*

Division of Applied Life Science (BK21 Program), Research Institute of Agriculture and Life Science, Gyeongsang National University, Chinju 660-701, Korea

We examined the role of microorganisms in the degradation of the organophosphorus (OP) insecticide chlorpyrifos (CP) during kimchi fermentation. During the fermentation of kimchi, 30 mg L^{-1} of CP was added and its stability assayed during fermentation. CP was degraded rapidly until day 3 (83.3%) and degraded completely by day 9. Four CP-degrading lactic acid bacteria (LAB) were isolated from kimchi fermentation in the presence of 200 mg L^{-1} CP and were identified as *Leuconostoc mesenteroides* WCP907, *Lactobacillus brevis* WCP902, *Lactobacillus plantarum* WCP931, and *Lactobacillus sakei* WCP904. CP could be utilized by these four strains as the sole source of carbon and phosphorus. Coumaphos (CM), diazinon (DZ), parathion (PT), and methylparathion (MPT) were also degraded by WCP907, WCP902, WCP931, and WCP904 when provided as sole sources of carbon and phosphorus.

KEYWORDS: Organophosphorus insecticides; chlorpyrifos; *kimchi*; lactic acid bacteria; multiplex PCR

INTRODUCTION

Organophosphorus (OP) compounds are the most widely used insecticides. They support increased world food production and account for an estimated 34% of worldwide insecticide sales. However, OP compound poisoning is a worldwide health problem with about three million poisonings and 200,000 deaths annually (*1, 2*). Among OP compounds, chlorpyrifos (CP, *O*,*O*-diethyl-*O*-3,5,6-trichloro-2-pyridyl phosphorothioate) has been commercially used since the 1960s for the control of foliar insects of cotton, paddy fields, pasture, and vegetable crops. CP degradation in soils and the aquatic environment involves both chemical hydrolysis and microbial activity. In most cases, aerobic bacteria tend to transform CP by hydrolysis to produce diethylthiphosphoric acid (DEPT) and 3,5,6-trichloro-2-pyridinol (TCP) (*3, 4*). Several effective CP-degrading strains have been previously described: Singh et al. (*5*) reported that two bands were found to be associated with enhanced degradation of chlorpyrifos by molecular fingerprinting of bacterial 16S rRNA genes by PCR-denaturing gradient gel electrophoresis (DGGE), and they isolated the potential six CP-degrading bacteria showing efficient degradation of CP from an Australian soil, and finally obtained the CP-degrading bacterium of *Enterobacter* sp. B-14 (*6*). Yang et al. (*7*) isolated an *Alcaligenes* sp. that could use not only CP but also parathion as a source of carbon. Also, Yang et al. (*3*) and Li et al. (*8*) reported that CP-degrading strain *Stenotrophomonas* sp. YC-1 and *Sphingomonas* sp. Dsp-2, respectively, were isolated from the sludge of a wastewater treating system of an OP manufacturer.

The widespread use of CP in agriculture has raised public concern about potential human health risks that may result from acute or chronic dietary exposure to CP residues in foods (*1, 9*). The Korean government sets the maximum residue limit (MRL) of CP in stored fruits and vegetables at 0.1 to 2.0 mg kg^{-1}. CP is defined as a moderately toxic compound having an acute oral LD_{50} of 135−163 mg kg^{-1} for rats and 500 mg kg^{-1} for guinea pigs. In humans exposed to CP, expression of CD26 cells and the frequency of autoantibodies both increased (*10*). Lee et al. (*11*) reported that individuals in the highest quartile of CP lifetime exposure-days (>56 days) had a relative risk of lung cancer 2.18 times that of individuals in the lowest quartile of exposure. Thus, there is a need to monitor and minimize the CP content in fermentation foods destined for human consumption.

Kimchi is a traditional Korean fermented vegetable food made of Chinese cabbage, radish, green onion, and red pepper, garlic, ginger, and fermented seafood (*jeotgal*) as the major raw materials, which traditionally has been served as side dishes at meals along with cooked rice and other dishes. Kimchi may differ in *baechukimchi* (Chinese cabbage kimchi), *chonggak kimchi* (Baby radish kimchi), *Dongchimi* (watery radish kimchi) and *yeulmu-mulkimchi* (watery young radish kimchi) etc. In the summer, *yeulmu-kimchi* tends to taste better and be more economical to prepare (*12*). *Yeulmu-mulkimchi* is another traditional Korean fermented food; typically, it is a soup

* To whom correspondence should be addressed. Phone: +82-55-751-5469. Fax: +82-55-757-0178. E-mail: hdyun@nongae.gsnu.ac.kr.

10.1021/jf803649z CCC: $40.75
Published on Web 02/06/2009

Table 1. List of Lactic Acid Bacteria and Oligonucleotide Primers Used in This Study

primer names	primer sequences (5′ → 3′)	species/genes (accession number)	type strain no.	PCR product (bp)	limited detection (log cfu mL^{-1})
LcaF	GAT TGT TGC TGC AGG TAT CGA GAA G	*Leuconostoc carnosum*/DNA-depend RNA polymerase (X95811)	KCTC[a] 3525	506	0.74
LcaR	TCC AAC GTA TCT GTG ACA GAC AAT AGC				
LmeF	GAG CCG TTA TTC AAG CAC CAA TC	*Leuconostoc mesenteroides*/alcohol-acetaldehyde dehydrogenase (AY804189)	KCTC 3530	358	0.70
LmeR	CCT GCG CCT TGA TAG TTT AAC AAG				
LbrF	AAA GCC AGG TAC TGT TAA GGG TGC	*Lactobacillus brevis*/surface layer protein	KACC[b] 10553	482	0.58
LbrR	AAC CAT CAG CCT TAG TGT CAG CG	(AY040846)			
LplF	AAG GCC GTA GTC AGT CGT CTA TGG	*Lactobacillus plantarum*/cadmium−manganese	KCTC 3104	313	0.52
LplR	TCA ACCA CAC GAA TAT CAG CCG G	transport ATPase (AF136521)			
LpeF	TCC GGT TTA CGC GGA ACA TTT	*Lactobacillus pentosus*/recombinase A	KCCM[c] 35472	398	0.60
LpeR	CCT TGA TTT GTT CAG CAC GAC G	(AJ621666)			
LsaF	TTA AAG GCA TTA GCT GAA GGC TGT	*Lactobacillus sakei*/putative lipase-esterase	KCTC 3603	274	0.56
LsaR	CGC CAT GGT GGC CAC GAT	(YP_395501)			
LlaF	TTG CAT GGA ATG AGC GGA AAC	*Lactococcus lactis*/tributyrin esterase	KCCM 40104	248	0.98
LlaR	TAT CCT CCC ATT GAT AAA CCA GCG	(AF157484)			
PpeF	CTT TGT GCC CGG TGG ATC CT	*Pediococcus pentosaceus*/esterase-lipase	KCTC 3507	330	1.24
PpeR	AAA GGC TGC AAT GTA GTT GAT GCT A	(ZP_00323987)			
WcoF	AAG CGT ATC TTG AAC CAA GCT GG	*Weissella confusa*/D-alanine: D-alanine ligase	KCTC 3499	208	0.54
WcoR	GCA AAG CGT CCT TAA CAC CAG C	(U08910)			
WkoF	TCT GCC GAA GCT TGA CCG G	*Weissella koreensis*/maltose phosphorylase	W602	154	0.62
WkoR	GCC GAA TTA AGT AGT GTA AAG TCA AAT G	(this study)	(this study)		

[a] KCTC, Korean type culture collection. [b] KACC, Korean agricultural culture collection. [c] KCCM, Korean culture center of microorganisms.

prepared from fermented kimchi and fresh red peppers as a minor ingredient. *Yeulmu-mulkimchi* can be acidified and softened more rapidly than other kimchi because it contains more lactic acid (produced by lactic acid bacteria, LAB) and bacterial enzymes that promote softening during storage and distribution (*13*). Furthermore, the *yeulmu-mulkimchi* experiment system has two main advantages: (1) ease of inoculation, pesticide addition, and sampling; and (2) shortening the batch time by accelerating fermentation. On the basis of acidity, kimchi was divided into five stages: initial stage, acidity >0.2; immature stage, 0.2 > acidity > 0.4; optimum-ripening stage, 0.4 > acidity >0.8, over-ripening stage, 0.8 > acidity > 1.2; rancid stage, 1.2 < acidity. Kimchi has the best flavor, taste, and texture when the pH and acidity are 0.6 to 0.8%, respectively (*14*). Previously, many researchers have reported that lactic acid bacteria (LAB) contribute to kimchi fermentation including *Le. mesenteroides*, *Le. citreum*, *Le. gasicomitatum*, *Lb. brevis*, *Lb. curvatus*, *Lb. plantarum*, *Lb. sakei*, *Lc. lactis*, *Pe. pentosaceus*, *We. confusa*, and *We. koreensis* (*15*−*17*).

The daily intake of kimchi by the Korean population is about 120 g per person, which constitutes about 10% of the total food intake (*18*). The assurance of safety from contaminants in such staple food items is very important, and the residue level of pesticides widely used for vegetable production should be carefully monitored and managed. In previous studies, Yun (*19*) and Park et al. (*20*) reported that CP and other OP were degraded during the fermentation of kimchi. However, LAB is the primary fermenting organisms in kimchi fermentation, and no OP-degrading LAB had been reported. The objectives of this work were to measure the degradation of CP and other OP during kimchi fermentation, and to isolate OP-degrading strains from a fermentation of kimchi.

MATERIALS AND METHODS

Chemicals. Cadusafos (CS; *S*,*S*-disec-butyl *O*-ethyl phosphorodithioate), chlorpyrifos (CP; *O*,*O*-diethyl-*O*-3,5,6-trichloro-2-pyridyl phosphorothioate), coumaphos (CM; *O*-3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl *O*,*O*-diethyl phosphorothioate), diazinon (DZ; *O*-3-chloro-4-methyl-2-oxo-2*H*-1-chromen-7-yl *O*,*O*-diethyl phosphorothioate), dyfonate (DF; *O*-ethyl *S*-phenyl ethylphosphonodithioate), ethoprophos (EP; *O*-ethyl *S*,*S*-dipropyl phosphorodithioate), fenamiphos (FA, *R*,*S*-ethyl 4-methylthio-*m*-tolyl isopropylphosphoramidate), methylparathion (MPT; *O*,*O*-dimethyl *O*-4-nitrophenyl phosphorothioate), parathion (PT; *O*,*O*-diethyl *O*-4-nitrophenyl phosphorothioate), 3,5,6-trichloro-2-pyridinol (TCP), and diethylthiophosphoric acid (DETP) were obtained from ChemService (West Chester, PA, USA) and Sigma Chemical Co. (St. Louis, MO, USA). HPLC-grade water, methanol, acetonitrile, and glacial acetic acid were purchased from Fisher Scientific (Fairlawn, NJ, USA). All reagents and samples used in the present study were of analytical grade and were used without further purification.

Bacterial Strains and Growth Medium. Ten lactic acid bacterial strains tested in this study are shown in **Table 1**. Reference strains were collected from the Korean type Culture Collection (KTCC), Korean Culture Center of Microorganisms (KCCM), and Korean Agricultural Culture Collection (KACC). The reference strains and isolates of kimchi were grown overnight at 28 °C in Lactobacilli MRS broth/agar (MRSB/MRSA, Difco, Becton Dickinson Co., Sparks, MD, USA).

Preparation and Fermentation of *Yeulmu-mulkimchi*. Six raw pesticide free vegetables such as young radish, onion, garlic, red pepper, scallion, and ginger were obtained from Gyeongnam Agricultural Research and Extension Services (GARES) in Jinju, Korea, and pear, sugar, salt, and wheat flour were purchased at a commercial supermarket. The water used in all processes of *yeulmu-mulkimchi* preparation was tap water. All of the raw materials were washed with tap water and sliced and used to prepare *yeulmu-mulkimchi* as previously described by Park (*12*). Prepared *yeulmu-mulkimchi* samples were placed in a 1,000 mL glass jar (1,800 mL) with a cap and fermented at 25 °C for 6 h and after at 8 °C for day 12. Each samples of *yeulmu-mulkimchi* were collected, 100 mL at 0, 1, 2, 3, 6, 9, and 12 days.

pH and Acidity. Each blended *yeulmu-mulkimchi* sample was filtered to collect the fluid portion, and the pH was measured with a pH meter (MP220, pH meter, UK). Twenty milliliters of *yeulmu-mulkimchi* filtrate (*yeulmu-mulkimchi* juice) was titrated with 0.1 N NaOH at pH 8.2 ± 0.2 for acidity estimation (AOAC, 2000). The acidity was calculated on the basis of lactic acid as stated below:

$$\text{Acidity (\%, as lactic acid)} = 0.009 \times \text{mL of 0.1 N NaOH} \times F \times 100/\text{Sample (mL)}$$

where *F* is a factor of 0.1 N NaOH (*F* value, 1.010).

Total LAB Viable Cells and Isolation of Lactic Acid Bacteria. One milliliter of each blended *yeulmu-mulkimchi* sample was diluted in 9 mL of sterile 0.85% NaCl water, and serial dilutions (10^{-1} to 10^{-12}) were made. First, total LAB cell numbers were determined by spread-plating 100 μL on MRSA, and all plates were incubated at at 28 °C for 48 h, after which colony counts were carried out. Total LAB viable cells were performed in triplicate. Second, 96 colonies were randomly

selected from the total LAB viable cells (approximately 30 to 100 cells) on MRSA plates. Finally, our designed multiplex PCR was carried out on the isolated 96 LAB colonies for the determination of specific LAB strains.

DNA Extraction. Genomic DNAs were extracted using the G-spin Genomic DNA Extraction Kit (iNtRON Biotechnology, Suwon, Korea) or by the method of boiling and vortexing bacterial pellets for 10 min at 80 °C. The extracted DNA was used as a template for the uniplex and multiplex PCR.

Primer Design and Multiplex PCR Reaction. The designed 10 new species-specific primers and oligonucleotide sequences are shown in **Table 1**. Individual multiplex PCR (using a mixture of 10 primer sets) was carried out on a single LAB colony and was performed with a thermalcycler (PC802, Astec, Tokyo, Japan). On the basis of the manufacturer's instructions, the PCR reaction mixture (50 μL) contained 1 μL of Super-Therm DNA polymerase (5.0 unit, JMR, Side Cup, Kent, UK), 2 μL each of primers (20 pmol), and 5 μL of reaction buffer with 2.5 mM $MgCl_2$, 5 μL of 2.5 mM deoxynucleosied triphophate (dNTP), 5 μL of template DNA, and 30 μL of sterile water. Multiplex PCR was carried out through 35 cycles following a preheat step at 95 °C for 5 min. Each cycle consisted of denaturation at 94 °C for 30 s, annealing at 60 °C for 1 min, and extension at 72 °C for 2 min. The last cycle was followed by a final extension at 72 °C for 10 min. The same PCR condition was used for single PCR analysis for reference strains.

Agarose Gel Electrophoresis. The PCR amplicons were analyzed by agarose gel electrophoresis. The 30 μL mixer samples (individual PCR products) were loaded onto a 2.5% agarose gel and subjected to electrophoresis for 6 h at 80 V in 1 × TAE buffer. The gel was stained with 1 μg mL^{-1} ethidium bromide (EtBr) solution. Gels were observed and documented on a CCD-camera based gel documentation system (Model GelDoc 1000, Bio-Rad, USA). A 100 bp DNA ladder (Bioneer Inc., Daejeon, Korea) was included in each gel to determine the molecular size of the PCR products.

Inoculation and Degradation in *Yeulmu-mulkimchi*. The samples of the *yeulmu-mulkimchi* were treated under aseptic conditions with 30 mg L^{-1} (*19*, *20*) for the degradation of CP during the fermentation of *yeulmu-mulkimchi*. For screening of CP-degrading LAB, the samples of the *yeulmu-mulkimchi* were treated under aseptic conditions with 200 mg L^{-1} CP. One set of CP treatment *yeulmu-mulkimchi* in triplicate was inoculated, and another set without CP was kept as controls. CP was extracted and determined by TLC and HPLC.

16S rDNA Analysis of Four Chlorpyrifos-Degrading Lactic Acid Bacteria. Four CP-degrading LAB were selected (strains WCP907, WCP902, WCP931, and WCP904) from the fermentation of *yeulmu mulkimchi* with CP at day 9. The 16S rDNA sequence analysis of four CP-degrading LAB (strains WCP907, WCP902, WCP931, and WCP904) was previously described by Cho et al. (*21*). Nucleotide sequence data reported for the 16S rDNA of the strains of WCP907, WCP902, WCP931, and WCP904 are available in the GenBank database under the accession numbers FJ480207, FJ480208, FJ480209, and FJ480210, respectively.

Growth and Degradation of Chlorpyrifos. Two different media, a diluted water 1/25 MRS medium and a mineral slat medium supplemented with nitrogen (MSMN, 2.27 g L^{-1} KH_2PO_4; 5.97 g L^{-1} $Na_2HPO_4 \cdot 12H_2O$; 1.0 g L^{-1} NaCl; 0.5 g L^{-1} $MgSO_4 \cdot 7H_2O$; 0.01 g L^{-1} $CaCl_2 \cdot 2H_2O$; 0.02 g L^{-1} $MnSO_4 \cdot 4H_2O$; 0.025 g L^{-1} $FeSO_4$; 0.01 g L^{-1} peptone; pH 6.9), were modified and used in a liquid culture of isolated bacteria. The total of 500 μL of bacterial culture suspension (8.0 log cfu mL^{-1}) was inoculated into 50 mL of 1/25 MRS with CP (100 mg L^{-1}), and finally bacterial growth was confirmed in MSMN medium with CP (100 mg L^{-1}). At periodic intervals, the 15 flasks were sacrificed, and its contents were used to determine the growth and degradation of CP. Each strain culture, such as WCP907, WCP902, WCP931, and WCP904, and *Escherichia coli* DH5α, was run in triplicate to ensure accuracy. Growth was monitored by spectrophotometer at 600 nm (Spectronic 2D, USA). Inoculated culture of *E. coli* DH5α with CP (100 mg L^{-1}) was used as a control. The degradation ability of the isolate was assayed with TLC and HPLC. In order to investigate if the CP-degradation isolates could use CP as the sole source of carbon and phosphorus, the MSMN medium was modified as described by Cullington and Walker (*22*).

Substrate Range. Degradation of other organophosphate insecticides was also carried out using identical conditions. The prepared *yeulmu-mulkimchi* and the liquid medium were supplemented with CS, CM, DZ, DF, EP, FA, MPT, and PT at 100 mg L^{-1}. The insecticide residues were measured by HPLC. Growth of strain WCP907, WCP902, WCP931, and WCP904 cells on methanol, ethanol, propanol, isopropanol, and acetone were tested by using MSMN medium with one of these compounds as the sole source of carbon.

Thin Layer Chromatography and High Performance Liquid Chromatography Analysis. Thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) were performed in order to determine the residual concentration of nine OP during the fermentation of *yeulmu-mulkimchi* and the degradation by the strains of WCP907, WCP902, WCP931, and WCP904.

Five milliliters of the culture was collected and centrifuged. Four milliliters of supernatant was extracted with ethylacetate (8 mL × 3), and the organic layer was dried with Na_2SO_4 and concentrated under vacuum. The extracted sample was spotted on a precoated silica gel aluminum plate (0.25 mm, Merck, Germany). The TLC plate was developed with a chloroform and hexane (4:1, v/v) solvent system for the detection of CP. An ethylacetate, isopropanol, and NH_4OH (5:3:2, v/v) system was used for the detection of TCP. Finally, the target compounds were detected with UV wavelength (254 nm). In TLC, TCP was confirmed as the spot with approximately 0.66 of *Rf* value, and the more polar CP was determined as the spot with about 0.57 of *Rf* value.

One milliliter of supernatant was mixed with 1 mL of methanol for the preparation of an HPLC sample. The above mixed solution was filtered through a 0.45 μm Minipore PVDF filter (Schleicher & Schuell, GmbH, Dassel, Germany) for HPLC analysis. Injection volume was the 10 μL of filtered sample. The analysis of OP and TCP was carried out on HPLC (HPLC, Perkin-Elmer 200 series, Perkin-Elmer Co., Norwalk, CT, USA) using a C18 column (250 × 4.6 mm, 5 μm, Phenomenx, CA, USA). The mixture of 0.5% acetic acid and methanol (1:4 v/v) was eluted with a flow rate of 1 mL min^{-1} at 30 °C. Target compounds, OPs and TCP, were measured at 214 nm of a UV detector (Perkin-Elmer UV 200 series, Perkin-Elmer Corp., Norwalk, CT, USA). In HPLC analysis, TCP was detected approximately at 5.5 min, and OPs were detected between 4.6 to 14.0 min depending on the feature of the each functionality.

The calibration curves for nine OPs and TCP were made from the serial dilutions of the samples dissolved in 100% methanol. The linear range and the equation of linear regression were obtained sequentially at 0, 10, 25, 50, 75, and 100 μg mL^{-1}. The above serial standard solutions were filtered through a 0.45 μm Minipore PVDF filter (Schleicher & Schuell, GmbH, Dassel, Germany) for HPLC analysis. Injection volume was 10 μL of serial standard solutions. Mean areas ($n = 3$) generated from the standard solution were plotted against concentration to establish the calibration equation. The concentrations of the nine OPs and TCP were determined on the basis of the peak areas in the chromatograms.

Statistical Analysis. Data were analyzed statistically by ANOVA and Duncan's multiple range tests using the SPSS software program (SPSS Inc., Chicago, IL, USA). A p value <0.05 was considered significant.

RESULTS

Degradation of Chlorpyrifos during *Yeulmu-mulkimchi* Fermentation. CP was added to *yeulmu-mulkimchi* fermentation at 30 mg L^{-1}, and the CP concentrations were monitored by HPLC analysis. The CP was degraded rapidly through day 3 (to about 5 mg L^{-1}), and there after, complete degradation occurred within 9 days during the fermentation of *yeulmu-mulkimchi* at 25 °C for 6 h and at 8 °C for day 12 (**Figure 1A**).

When 200 mg L^{-1} of CP was added to the fermentation, it was degraded to about 179 mg L^{-1} at day 1 and to about 49

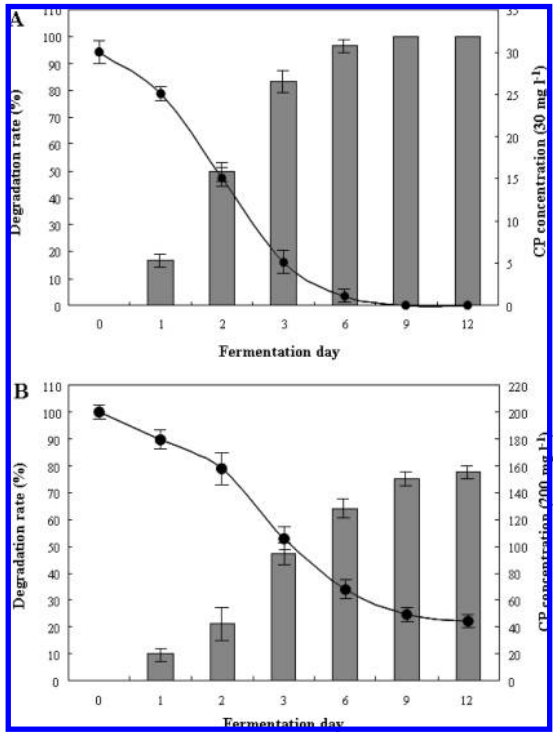

Figure 1. Degradation degree and concentration during *yeulum-mulkimchi* fermentation with 30 mg L^{-1} (**A**) and 200 mg L^{-1} (**B**) of chlorpyrifos (CP) at 25 °C for 6 h and 8 °C for day 12. The black circle indicates the CP concentration, and the gray box indicates the CP degradation rate. Values indicate the means of three replications ($n = 3$). A p value <0.05 was considered significant.

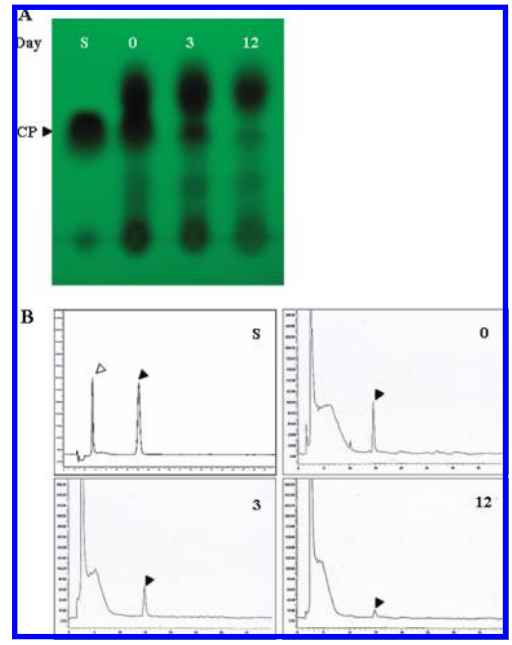

Figure 2. TLC (**A**) and HPLC (**B**) profiles of chlorpyrifos (CP) during *yeulmu-mulkimchi* fermentation with 200 mg L^{-1} of CP at 25 °C for 6 h and 8 °C for day 12. S, standard; 0, fermentation of *yeulmu-mulkimchi* at day 0; 3, fermentation of *yeulmu-mulkimchi* at day 3; 12, fermentation of *yeulmu-mulkimchi* at day 12. The white arrowhead indicates the 3,5,6-trichloro-2-pyridinol (TCP, retention time: approximately 5.5 min) peak, and the black arrowheads indicates the CP (*Rf* value, approximately 0.57; retention time, approximately 14 min) band and peak.

mg L^{-1} at day 9 (**Figure 1B**). Thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) analysis were used to follow CP degradation during *yeulmu-mulkimchi* fermentation. Degradation of CP was observed by comparing samples after 6 and 12 days (**Figure 2**).

Other OP compounds were also degraded during *yeulmu-mulkimchi* fermentation. Concentrations of CS, CP, CM, DZ, DF, EP, FA, MPT, and PT decreased by 3, 41, 5, 13,0, 7, 14, 38, and 42%, respectively, by day 3, and by 37, 80, 48, 74, 22, 45, 51, 79, and 82%, respectively, by the final day of fermentation (day 12). pH values of the fermentation mixture decreased from ca. 6.18 (at day 0) to 3.69 to 3.65 (on the final day) of *yeulmu-mulkimchi* fermentation. At the same time, the acidity of the mixture increased from 0.21% to 1.25% to 1.23% (data not shown).

Detection of Lactic Acid Bacteria during *Yeulmu-mulkimchi* Fermentation. Specificities of the 10 species-specific primer pairs were evaluated by PCR with genomic DNAs. All species-specific primer pairs produced a single PCR product with an expected product size, representing the species specificity of the designed primers. Therefore, we used primers specific for the 10 LAB species to determine which LAB were present during *yeulmu-mulkimchi* fermentation. The PCR products detected for the different types of LAB strains ranged from 154 to 506 bp (**Table 1** and **Figure 3**) Detection of the 10 LABs was possible after 4 h (0.52 to 1.24 log cfu mL^{-1}, respectively) on MRS liquid culture (**Figure 3A**).

At the start of *yeulmu-mulkimchi* fermentation, before the addition of CP (day 0), seven species of lactic acid bacteria were detected including *Le. carnosum*, *Le. mesenteroides*, *Lb. plantarum*, *Lb. sakei*, *Lc. lactis*, *Pe. pentosaceus*, and *We. confusa*. During *yeulmu-mulkimchi* fermentation without CP, lactic acid bacteria of eight species were detected at the optimum-ripening stage (day 3) including *Le. carnosum*, *Le. mesenteroides*, *Lb. brevis*, *Lb. pentosus*, *Lb. plantarum*, *Lb. sakei*, *We. confuse*, and *We. koreensis*. Four LAB species, *Lb. plantarum*, *Lb. sakei*, *We. confuse*, and *We. Koreensis*, were detected at the rancid stage (day 12). During *yeulmu-mulkimchi* fermentation with CP, the lactic acid bacteria detected included *Le. mesenteroides*, *Lb. brevis*, *Lb. plantarum*, *Lb. sakei*, *We. confuse*, and *We. koreensis* at the optimum-ripening stage (day 3), and *Lb. brevis*, *Lb. plantarum*, *Lb. sakei*, and *We. koreensis* at the rancid stage (day 12) (**Figure 3B**).

Comparison of Major LAB during Fermentation with and without Chlorpyrifos. The pH of the fermentation mixture decreased from 6.20 at day 0 to a final pH (day 12) of, respectively, 3.69 and 3.65 for the without CP and with CP fermentations (**Figure 4A**). However, the acidity increased from 0.20% to about 1.24% in both cases (**Figure 4B**). In the *yeulmu-mulkimchi* fermented without CP, the highest level of total LAB was observed on day 2 (13.4 log cfu mL^{-1}). The level of total LAB in *yeulmu-mulkimchi* fermentation with CP increased greatly from 4.4 log cfu mL^{-1} at day 0 to 13.2 log cfu mL^{-1} at day 3, and then decreased gradually (**Figure 4C**).

Table 2 shows the distribution of LAB species among the without CP and with CP samples during *yeulmu-mulkimchi* fermentation. *We. confusa* was dominant in fermented *yeulmu-mulkimchi* in both without CP and with CP samples at the initial and immature stages. The predominant lactic acid bacteria varied at the different stages of fermentation during the *yeulmu-mulkimchi* fermented without CP: *We. confusa* at the initial (55.2%) and immature (39.5%) stages, and *We. koreensis* at the optimum-ripening (22.9%), over-ripening (28.1%), and rancid (42.8%) stages. The predominant lactic acid bacteria also varied during fermentation with CP at the different stages of fermentation: *We. confusa* at the initial stages (55.2%) and *Lb. sakei* at the immature (32.6%), optimum-ripening (61.0%), over-

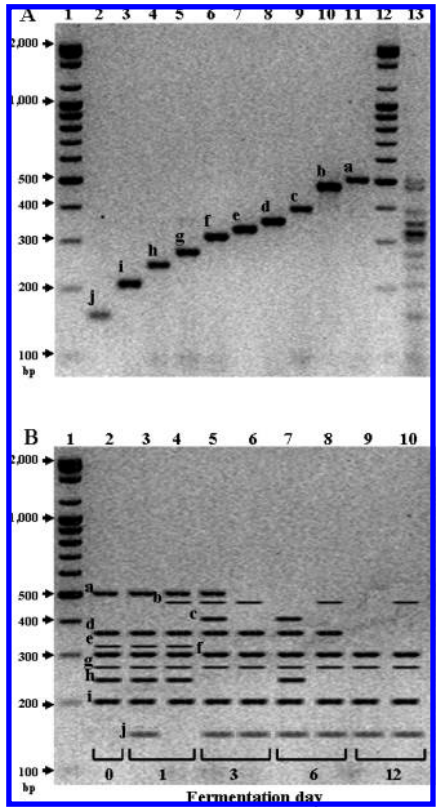

Figure 3. Agarose gel electrophoresis of the multiplex PCR products obtained from the 10 references of lactic acid bacteria (**A**) and the lactic acid bacteria during *yeulmu-mulkimchi* fermentation without and with 200 mg L^{-1} of chlorpyrifos (CP) (**B**). Lanes A1, A12, and B1, size marker (100 bp-ladder); 1ane A2, *We. korenesis* (j, 154 bp); lane A3, *We. confusa* (i, 208 bp); lane A4, *Lc. lactics* (h, 248 bp); lane A5, *Lb. sakei* (g, 269 bp); lane A6, *Lb. plantarum* (f, 313 bp); lane A7, *Pe. pentosaceus* (e, 330 bp); lane A8, *Le. mesenteroides* (d, 358 bp); lane A9, *Lb. pentosus* (c, 398 bp); lane A10, *Lb. brevis* (b, 482 bp); lane A11, *Le. carnosum* (a, 506 bp); lane A13, mixer of the 10 PCR products; lane B2, *yeulmu-mulkimchi* fermentation at initial stage (0 day); lane B3, *yeulmu-mulkimchi* fermentation without CP at immature stage (day 1); lane B4, *yeulmu-mulkimchi* fermentation with 200 mg L^{-1} of CP at immature stage (day 1); lane B5, *yeulmu-mulkimchi* fermentation without CP at optimum-ripening stage (day 3); lane B6, *yeulmu-mulkimchi* fermentation with 200 mg L^{-1} of CP at optimum-ripening stage (day 3); lane B7, *yeulmu-mulkimchi* fermentation without CP at over-ripening stage (day 6); lane B8, *yeulmu-mulkimchi* fermentation with 200 mg L^{-1} of CP at over-ripening stage (day 6); lane B9, *yeulmu-mulkimchi* fermentation without CP at rancid stage (day 12); lane B10, *yeulmu-mulkimchi* fermentation with 200 mg L^{-1} of CP at rancid stage (day 12).

ripening (70.7%), and rancid (83.4%) stages. *Le. mesenteroides* was present at higher levels in *yeulmu-mulkimchi* with CP than without CP at the immature stage. *Lb. brevis* and *Lb. plantarum* were present at higher levels in the fermentation with CP than in that without CP at the optimum-ripening and over-ripening stages. These results suggest that *Le. mesenteroides*, *Lb. brevis*, *Lb. plantarum*, and *Lb. sakei* may be able to hydrolyze CP and utilize DEPT to support their growth.

Identification of Four Chlorpyrifos-Degrading Lactic Acid Bacteria. PCR amplification of the total DNA from each lactic acid bacteria with bacteria-specific primers produced a single amplification product of about 1.5 kb. The 16S rDNA sequence of strains WCP907, WCP902, WCP931, and WCP904 were compared with those of all validly described LAB, representatives of some related taxa. The highest 16S rDNA sequence

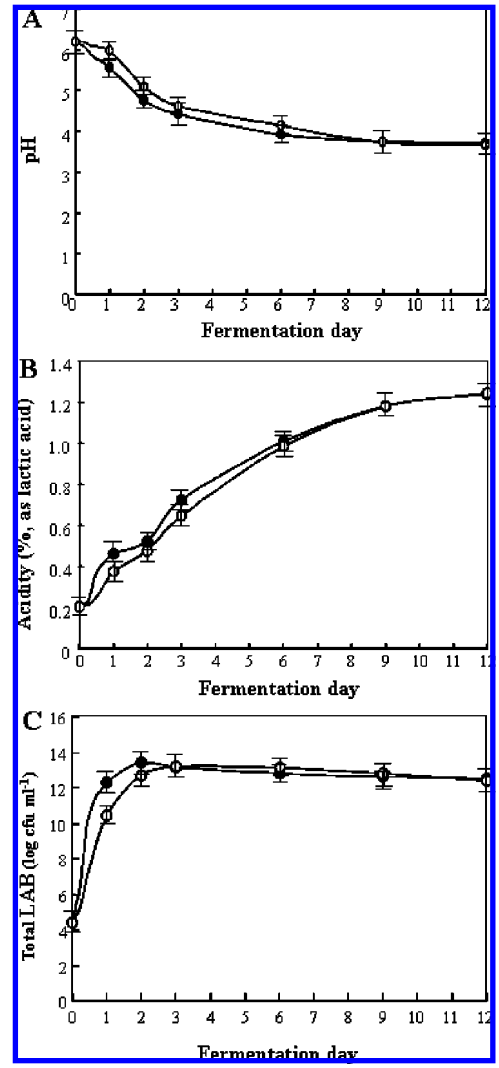

Figure 4. Change of pH (**A**), acidity (**B**), and total lactic acid bacteria (LAB) cell numbers (**C**) during *yeulmu-mulkimchi* fermentation with 200 mg L^{-1} of chlorpyrifos (CP) at 25 °C for 6 h and 8 °C for day 12. Twenty milliliters of kimchi filtrate (kimchi juice) was titrated with 0.1 N NaOH at pH 8.2 ± 0.2 for acidity. One milliliter of each blended kimchi was serially diluted, and the dilutions were plated onto MRSA and incubated at 28 °C for 48 h. The black circle indicates the *yeulmu-mulkimchi* fermentation without CP, and the white circle indicates the *yeulmu-mulkimchi* fermentation with 200 mg L^{-1} of CP. Values indicate the means of three replications ($n = 3$). A p value <0.05 was considered significant.

similarity is observed between strains WCP907, WCP902, WCP931, and WCP904 and *Le. mesenteroides* NCIMB 8023 (99.6%), *Lb. brevis* ATCC 14869 (99.4%), *Lb. plantarum* JCM 1149 (99.2%), and *Lb. sakei* DSM 20017 (99.7%), respectively (data not shown).

Cell Growth and Degradation of Chlorpyrifos in Liquid Culture. We examined the growth of the potential CP-degrading LAB (WCP907, WCP902, WCP931, and WCP904) in media with CP and also their ability to degrade CP in culture. As shown in **Table 3**, the four strains grew well until day 1, decreased slowly by day 2, and then increased gradually at day 6. CP was detected only at very low concentrations in samples taken after 6 day. During the first three days, these four LAB strains exhibited an initial rapid degradation of CP from a 100 mg L^{-1} starting concentration to approximately 32 to 39 mg L^{-1} at day 3. After day 3, the CP concentration decreased more slowly over time. These results support the hypothesis that *Le. mesenteroides*, *Lb. brevis*, *Lb. plantarum*, and *Lb. sakei* may

Table 2. Lactic Acid Bacteria (LAB) Population during Different Stages of *Yeulmu-kimchi* Fermentation[a] in the Absence[c] or Presence[d] of Chlorpyrifos (200 mg L^{-1})

	fermentation stage[b]/day				
LAB species	initial/0 [%, n/96]	immature/1 [%, n/96]	optimum-ripening/3 [%, n/96]	over-ripening/6 [%, n/96]	rancid/12 [%, n/96]
Le. carnosum	2.1	2.1[c]	2.1	1.0[d]	0
		1.0	0	0	0
Le. mesenteroides	4.2	12.6	9.4	8.3	0
		18.7	6.3	2.1	0
Lb. brevis	0	0	1.0	0	0
		2.1	2.1	4.2	7.3
Lb. pentosus	0	0	2.1	3.1	0
		0	0	0	0
Lb. plantarum	3.1	5.2	8.3	5.2	12.6
		7.3	17.8	19.6	10.4
Lb. sakei	6.3	10.4	16.7	19.8	29.0
		32.6	61.0	70.7	83.4
Lc. lactic	9.4	6.3	0	2.1	0
		5.2	0	0	0
Pe. pentosaceus	2.1	1.0	0	0	0
		0	0	0	0
We. confusa	55.2	39.5	11.4	8.3	10.4
		24.7	11.8	6.3	0
We. koreensis	0	4.2	22.9	28.1	42.8
		0	1.0	2.1	2.1

[a] *Yeulmu-mulkimchi* fermentation at 25 °C for 6 h and 8 °C for day 12. [b] Kimchi fermentation was divided into five stages on the basis of acidity as follows: initial stage, acidity >0.2; immature stage, 0.2 > acidity >0.4; optimum-ripening stage, 0.4 > acidity >0.8, over-ripening stage, 0.8 > acidity >1.2; rancid stage, 1.2 < acidity. [c] *Yeulmu-mulkimchi* fermented without CP. [d] *Yeulmu-mulkimchi* fermented with CP.

be able to utilize CP to support their growth. The four strains were also tested for their ability to degrade other OP compounds: CP, CM, DZ, MPT, and PT, which all have DEPT as a side chain, and CS, DF, EP, and FA, which do not have DEPT as a side chain. All OP compounds with the exception of DF (that is, CS, CP, CM, DZ, EP, FA, MPT, and PT) were hydrolyzed at a phosphoester bond when added to cultures of strains WCP907, WCP902, WCP931, and WCP904 (**Table 4**).

DISCUSSION

In the present study, we demonstrate the degradation of CP by lactic acid bacteria during the fermentation of *yeulmu-mulkimchi*. When CP was added at 30 mg L^{-1}, it was degraded by 83.3% at optimum-ripening stage (3 day, pH 4.51, acidity 0.72) and completely degraded by day 9 (over-ripening stage, pH 4.02, 0.99). Yun (*19*) reported that the removal rate of CP was 36% after one week, 57.8% after two weeks, 79.7% after three weeks, and 88.4% after four weeks at 4 °C during the fermentation of *baechukimchi*. During the fermentation of kimchi at 4 °C for 24 day, the pH became low (pH 4.5 to 5.8), and the residual amount of pesticides was decreased by 51.4% to 69.4% for three pesticides (pirimiphos-methyl, chlorpyrifos, and rothiofos) after washing and salting (*20*). When the *yeulmu-mulkimchi* was fermented with 100 mg L^{-1} of other OP, the degradation rates of CS, CP, CM, DZ, DF, EP, FA, MPT, and PT (100 mg L^{-1}) reached up to 37, 80, 48, 74, 22, 45, 51, 79, and 82%, respectively, by day 12 (with pH values ranging from 3.69 to 3.65). Generally, OP insecticides are stable in mild acidic to neutral pH and easily decomposed in alkaline pH, with the exception that the DZ is unstable in acidic pH (*18*). Previously, the environmental rate of CP has been studied extensively. Degradation in soil involves both chemical hydrolysis and microbial activity. Initially, the high rate of CP degradation in soils with alkaline pH was attributed to chemical hydrolysis. Later, Racke et al. (*23*) concluded that the relationship between high soil pH and chemical hydrolysis was weak and that other factors such as soil silt content might be important in determining environmental factors, such as temperature, moisture content, organic carbon content, and pesticide formulation. Recently, several researchers reported that the degradation of CP was very slow in acidic soils but that the rate of degradation increased considerably with an increase in soil pH. Additionally, Li et al. (*8*) showed that high moisture, high organic nitrogen content, and high inoculum density resulted in rapid degradation. However, the fumigation of soil samples completely inhibited the hydrolysis of CP, suggesting an involvement of soil microorganisms (*3*, *5*, *7*). Practically, Nam et al. (*24*) reported that the removal rates of pesticides were reduced 27 to 100% during fermentation, milking, canning, and processing of raw food materials.

Yun (*19*) and Park et al. (*20*) suggested that CP was degraded by LAB during kimchi fermentation. The highest levels of total LAB in *yeulmu-mulkimchi* fermentation appeared at 13.4 log cfu mL^{-1} (without CP) at day 2 and 13.2 log cfu mL^{-1} (with CP) at day 3. This result presumes that LAB growth may be affected by high CP concentration. We used multiplex PCR to detect the LAB species during *yeulmu-mulkimchi* fermentation with CP and without CP at 200 mg L^{-1} of CP. *We. koreensis* predominated after day 3 (optimum-ripening stage) in samples without CP, whereas *Lb. sakei* species were dominant in the case of WCP. *Le. mesenteroides* was higher with CP than in samples without CP at the immature stage. *Lb. brevis* and *Lb. plantarum* were higher in samples with CP than those without CP at the optimum-ripening and over-ripening stages. Four CP-degrading LAB strains were isolated on day 9 from the *yeulmu-mulkimchi* fermented with 200 mg L^{-1} of CP. To our knowledge, this is the first report that four CP-degrading *kimchi*-LAB species, such as *Le. mesenteroides* WCP907, *Lb. brevis* WCP902, *Lb. palntarum* WCP931, and *Lb. sakei* WCP904, were isolated and identified from kimchi fermentation. Also, these same strains are present in the absence of CP. These bacteria are unusual in that they can hydrolyze CP and utilize part of the compound (DEPT) as a sole source of carbon. Our TLC and HPLC analysis supports this proposed pathway, along with the finding that these four strains grew to higher population density when CP was present in the culture medium. Previously, many researchers reported that lactic acid bacteria (LAB) contribute to kimchi fermentation including *Le. mesenteroides*, *Le. citreum*, *Le. gasicomitatum*, *Lb. brevis*, *Lb. curvatus*, *Lb. plantarum*, *Lb. sakei*, *Lc. lactis*, *Pe. pentosaceus*, *We. confusa*, and *We. koreensis*. In particular, *Lb. brevis*, *Lb. plantarum*, *Lb. sakei*, and *We. koreensis* were reported as acidic tolerance bacteria. These strains generally proliferate in the late stage of kimchi fermentation, reportedly playing an important role in its over-fermentation (*15*−*17*). These results suggest that *Lb. brevis*, *Lb. plantarum*, and *Lb. sakei* may be tolerant against the high concentration of acid and CP.

In previous reports, the OP are degraded by microorganisms because they can be utilized by the metabolic pathway for carbon and phosphorus (*25*, *26*). Here, we demonstrate conclusively that *Le. mesenteroides* WCP907, *Lb. brevis* WCP902, *Lb. plantarum* WCP931, and *Lb. sakei* WCP904 can utilize OP insecticides as a source of carbon and phosphorus in a defined culture medium. In addition, these strains were capable of hydrolyzing five OP insecticides: CP, CM, DZ, MPT, and PT. The isolates exhibited versatility, utilizing dimethyl compounds such as MPT and diethyl compounds such as CP, CM, DZ, and

Table 3. pH, Optical Density (OD), and Degraded Concentration of the Strains of DH5α, WCP907, WCP902, WCP931, and WCP904 Grown on MSMN Medium Contained in Chlorpyrifos (100 mg L^{-1}) at the Incubation Time

	pH/OD_{600nm}/residual CP concentration (mg L^{-1})				
	strains				
incubation day	*Eco* DH5α[b]	*Lme* WCP907[c]	*Lbr* WCP902[d]	*Lpl* WCP931[e]	*Lsa* WCP904[f]
0	7.01ab/0.24 cd/98b[a]	7.03 h/0.23gh/101ab	7.00ab/0.24 cd/99b	7.02ab/0.23 cd/100ab	7.01ac/0.23d/102bd
1	7.00d/0.31gh/96 g	5.01d/0.88ab/86c	5.06gh/0.83a/87i	5.02d/0.85ij/87j	4.99ab/0.86ji/89ac
2	6.92ac/0.33b/96a	4.98 g/0.86hi/61h	5.01ef/0.81g/66gh	4.98ȉcd/0.84hi/65g	4.86abc/0.84bc/63 cd
3	6.91a/0.28 cd/95ab	4.72ab/0.89a/32b	4.73bcd/0.87gh/39 cd	4.70d/0.87/34gh	4.65a/0.87ij/31gh
6	6.93b/0.24ac/94d	4.53bc/0.96ac/26ef	4.55d/0.92e/31ef	4.51cde/0.94bc/22f	4.50 g/0.95d/24ab
9	6.92ab/0.22c/92d	4.51ac/0.84 cd/16g	4.53ab/0.84hi/21bc	4.47ac/0.86d/14ef	4.48a/0.85abc/12d

[a] Values indicate the means of three replications ($n = 3$). A p value <0.05 was considered significant. [b] *Escherichia coli* DH5α for negative control. [c] *Leuconostoc mesenteroides* WCP907. [d] *Lactobacillus brevis* WCP902. [e] *Lactobacillus plantarum* WCP931. [f] *Lactobacillus sakei* WCP904.

Table 4. Degradation Concentration of Different Organophosphate Insecticide Growth of the Strains of DH5α, WCP907, WCP902, WCP931, and WCP904 on MSMN Medium Contained in 100 mg L^{-1} of the Nine Organophosphorus (OP) Insecticides

	residual OP concentration (mg L^{-1})				
	strains				
organophosphorus insecticides[b]	*Eco* DH5α[c]	*Lme* WCP907[d]	*Lbr* WCP902[e]	*Lpl* WCP931[f]	*Lsa* WCP904[g]
cadusafos (CS)	95a[a]	82ab	90b	84c	83abc
chlorpyrifos (CP)	94bc	20c	24abc	20ef	16cd
coumaphos (CM)	93hi	75hi	74b	64efg	78bc
diazinon (DZ)	96cd	28gh	56c	41ac	22a
dyfonate (DF)	95i	95i	69c	96bd	98b
ethoprophos (EP)	97a	87j	89cd	93b	86h
fenamiphos (FA)	97b	67ac	63hi	54cd	59i
methylparathion (MPT)	94ab	21d	33j	18i	21d
parathion (PT)	93c	18a	35g	20hi	14d

[a] The strains of WCP907, WCP902, WCP931, and WCP904 growth on MSMN medium at day 9 contained in the different OP. [b] Values indicate the means of three replications ($n = 3$). A p value <0.05 was considered significant. [c] *Escherichia coli* DH5α for negative control. [d] *Leuconostoc mesenteroides* WCP907. [e] *Lactobacillus brevis* WCP902. [f] *Lactobacillus plantarum* WCP931. [g] *Lactobacillus sakei* WCP904.

PT as a carbon source. These compounds have the diethyl (or dimethyl) phosphorothionate side chains, with a phosphotriester bond in all compounds, which may explain their degradation. Recently, Yang et al. (*3*) and Li et al. (*8*) reported that CP-degrading bacteria could use CP as a source of phosphorus and carbon. Kertesz et al. (*27*) suggested that the conditions under which the environmental isolates are enriched are crucial in selecting for strains not only with the desired degrading enzyme systems but also with specific regulation mechanisms for the degradation pathways.

Their activity against a range of OP compounds suggests that *Le. mesenteroides* WCP907, *Lb. brevis* WCP902, *Lb. plantarum* WCP931, and *Lb. sakei* WCP904 may express a versatile enzymatic pathway that might be used for the remediation of highly toxic organophosphate neurotoxic agents. The majority of OP are esters of phosphoric acid and can therefore be hydrolzyed and detoxified by carboxlyesterase and phosphotriesterase (*28*−*30*). These enzymes are remarkable for their extremely broad substrate profiles. They can catalyze the hydrolysis of many neurotoxic agents and OP insecticides (*28*). For example, hydrolysis of OP compounds by carboxlyesterase/phosphotriesterase reduces mammalian toxicity by several orders of magnitude.

Although this study used raw, pesticide-free vegetables, many investigators previously reported that the fruits, crops, and vegetables were contaminated by the various pesticides (*2*, *17*, *31*). Kimchi is an important fermented food for Korean people and is becoming a favorite food even among non-Koreans. The worldwide concern on the safety aspects of foods also calls for the assurance of safe fermented foods. With regard to the safety of pesticide residues in kimchi, the residue levels in raw materials as well as the fate of the residue in fermentation processes must be considered (*18*).

In conclusion, these results suggest that the fermented kimchi meets the minimal residue criteria for food safety due to OP degradation by kimchi microorganisms such as *Le. mesenteroides*, *Lb. brevis*, *Lb. plantarum*, and *Lb. sakei*, rather than by effects of pH and other abiotics. The full genome sequences of *Le. mesenteroides*, *Lb. brevis*, *Lb. plantarum*, and *Lb. sakei* are available (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/) and will facilitate our further studies aimed at discovering the enzymes and pathways responsible for OP degradation by these strains.

LITERATURE CITED

(1) Singh, B. K.; Walker, K. Microbial degradation of organophosphours compounds. *FEMS Microbiol. Rev.* **2006**, *30*, 428–471.
(2) Yang, Z. Y.; Kolosova, A. Y.; Shim, W. B.; Chung, D. H. Development of monoclonal antibodies against pirimiphos-methyl and their application to IC-ELISA. *J. Agric. Food Chem.* **2006**, *54*, 4551–4556.
(3) Yang, C.; Liu, N.; Guo, X.; Qiao, C. Cloning of *mpd* gene from a chlorpyrifos-degrading bacterium and use of this strain in bioremediation of contaminated soil. *FEMS Microbiol. Lett.* **2006**, *265*, 118–125.
(4) Lu, J.; Wu, L.; Newman, J.; Faber, B.; Gan, J. Degradation of pesticides in nursery recycling pond waters. *J. Agric. Food Chem.* **2006**, *54*, 2658–2663.
(5) Singh, B. K.; Walker, A.; Morgan, A. W.; Wright, D. J. Effects of soil pH on the biodegradation of chlorpyrifos and isolation of a chlorpyrifos-degrading bacterium. *Appl. Environ. Microbiol.* **2003**, *69*, 5198–5206.

(6) Singh, B. K.; Walker, A.; Morgan, A. W.; Wright, D. J. Biodegradation of chlorpyrifos by *Enterobacter* strain B-14 and its use in bioremediation of contaminated soils. *Appl. Environ. Microbiol.* **2004**, *70*, 4855–4863.
(7) Yang, L.; Zhao, Y. H.; Zhang, B. X.; Yang, C. H.; Zhang, X. Isolation and characterization of a chlorpyrifos and 3,5,6-trichloro-2-pyridinol degrading bacterium. *FEMS Microbiol Lett*. **2005**, *251*, 67–73.
(8) Li, X.; He, J.; Li, S. Isolation of a chlorpyrifos-degrading bacterium *Sphingomonas* sp. strain Dsp-2, and cloning of the *mpd* gene. *Res. Microbiol*. **2007**, *158*, 143–149.
(9) Randhawa, M. A.; Anjum, F. M.; Ahmed, A.; Randhawa, M. S. Field incuured dchlorpyrifos and 3,5,6-trichloro-2-pyridinol residues in fresh and processed vegetables. *Food Chem*. **2007**, *103*, 1016–1023.
(10) Thrasher, J. D.; Madison, R.; Broughton, A. Immunological abnormalities in humans exposed to chlorpyrifos preliminary observations. *Arch. Environ. Health* **1993**, *48*, 89–93.
(11) Lee, W. J.; Blair, A.; Hoppin, J. A.; Lubin, J. H.; Rusiecki, J. A. Cancer incidence among pesticide applicators exposed to chlorpyrifos in the agricultural health study. *J. Natl. Cancer. Inst*. **2004**, *96*, 1781–1789.
(12) Park, W. P. Effect of starch sources on *yulmoo kimchi* fermentation. *Food Biotechnol.* **1995**, *4*, 98–100.
(13) Lee, M. Y.; Lee, K. Y.; Kim, S. D. Additional effect of calcium acetates prepared from ash of Black snail and vinegars on the quality of *mul-kimchi*. *Food Sci. Biotechnol*. **2004**, *13*, 289–296.
(14) Codex Alimentarius Commission (Codex); Codex standard for *kimchi*; Codex Stan 223; Food and Agriculture Organization of the United Nations: Rome, Italy, 2001.
(15) Kim, M. J.; Chun, J. S. Bacterial community structure in *kimchi*, a Korean fermented vegetable food, as revealed by 16S rRNA gene analysis. *Int. J. Food Microbiol*. **2005**, *103*, 91–96.
(16) Lee, J. S.; Heo, G. Y; Lee, J. W.; Oh, Y. J.; Park, J. A; Park, Y. H; Park, Y. R.; Ahn, J. S. Analysis of *kimchi* microflora using denaturing gradient gel electrophoresis. *Int. J. Food Microbiol*. **2005**, *102*, 143–150.
(17) Cho, J. H.; Lee, D. Y.; Yang, C. N.; Jeon, J. I.; Kim, J. H.; Han, H. U. Microbial population dynamics of *kimchi*, a fermented cabbage product. *FEMS Microbiol. Lett*. **2006**, *257*, 262–267.
(18) Lee, M. G.; Lee, S. R. Elimination of insecticide EPN residues during rice cooking and *kimchi* preparation. *Food Biotechnol.* **1997**, *6*, 39–43.
(19) Yun, S. J. The change of residual chlorpyrifos during fermentation *kimchi*. *Korean J. Food Sci. Technol.* **1989**, *21*, 590–594.
(20) Park, W. J.; Joo, L. A.; Kim, J. E. Removal of organophosphorus pesticides during making and fermentation of *kimchi*. *J. Food Hyg. Safety* **2002**, *17*, 87–93.
(21) Cho, K. M.; Seo, W. T. Bacterial diversity in a Korean traditional soybean fermented foods (*doenjang* and *ganjang*) by 16S rRNA gene sequence analysis. *Food Sci. Biotechnol*. **2007**, *16*, 320–324.
(22) Cullington, J. E.; Walker, A. Rapid biodegradation of diuron and other phenylurea herbicides by a soil bacterium. *Soil Biol. Biochem*. **1999**, *31*, 677–686.
(23) Rake, K. D.; Coats, J. R.; Titus, K. R. Degradation of chlorpyrifos and its hydrolysis products, 3,5,6-trichloro-2-pyridinol, in soil. *J. Environ. Sci. Health B* **1988**, *23*, 527–539.
(24) Nam, S. M.; Lee, H. R; Lee, J. M. Removal efficiency of residual pesticides during processing of Perilla Jangachi preparation. *Korean J. Food Cul.* **2003**, *18*, 562–568.
(25) Cui, Z. L.; Li, S. P.; Fu, G. P. Isolation of methyl parathion-degrading strain M6 and cloning of methyl parathion hydrolase gene. *Appl. Environ. Microbiol*. **2001**, *67*, 4922–4925.
(26) Rosenberg, A.; Alexander, M. Microbial cleavage of various organophosphorus insecticides. *Appl. Environ. Microbiol*. **1979**, *37*, 886–891.
(27) Kertesz, M.; Cook, A. M.; Leisinger, T. Microbial metabolism of sulfur and phosphorus-contating xenobiotcs. *FEMS Microbiol. Rev*. **1994**, *15*, 195–215.
(28) Dumans, D. P.; Durst, H. D.; Landis, W. G.; Raushel, F. M.; Wild, J. R. Inactivation of organophosphorus nerve agents by the phosphotriesterase from *Pseudomonas diminuta*. *Arch. Biochem. Biphys*. **1990**, *227*, 155–159.
(29) Huang, J.; Qiao, C. L.; Li, X.; Xing, J. M. Cloning and fusion expression of detoxifying gene in *Escherichia coli*. *Acta Genetica. Sinca* **2001**, *28*, 583–588.
(30) Weston, D. P.; Amweg, E. L. Whole-sediment toxicity identification evaluation tools for pyrethroid insecticides: ii. Esterase addition. *Environ. Toxicol. Chem*. **2007**, *26*, 2397–2404.
(31) Choi, G. Y.; Kim, J. H.; Jeong, Y. M.; Seo, H. Y.; Shim, S. L.; Kim, K. S. Characteristic of decomposition of residual pesticides on diazinon and endosulfan in young radish. *J. Korean Soc. Appl. Biol. Chem*. **2004**, *47*, 238–243.

Received for review August 4, 2008. Revised manuscript received December 28, 2008. Accepted December 30, 2008. This work was supported by a grant (No. 08-C2-15-002) from the Academic-Industrial Cooperative Research Program of the Small & Medium Business Administration (SMBA), Korea. R.K.M. is supported by scholarships from the BK21 Program, Ministry of Education & Human Resources Development, Korea.

JF803649Z