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Influence of lactic acid bacteria on stereoselective degradation of theta-cypermethrin

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Abstract

The purpose of this study was to investigate the influence of four kinds of *Lactic acid bacteria* (LAB) on stereoselective degradation of *theta*-cypermethrin (CYP), including *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus delbrueckii*, and *Streptococcus thermophilus*. An effective analytical method for (±)-*theta*-CYP in medium was developed by high-performance liquid chromatography with cellulose tris-(3,5-dimethylphenylcarbamate) chiral stationary phase. *theta*-Cypermethrin was spiked to LAB medium with different inoculation rates and sampled at 0, 2, 8, 24, 36, 48, 72, 120, 168, and 240 hours. The results showed that LAB influenced the half-lives and enantiomer fractions of *theta*-CYP enantiomers, which lead a closer degradation rate between the 2 stereoisomers, and no obvious difference was found among 4 LABs. Besides, the stereoselective degradation of *theta*-CYP was closely related to pH. The lower the pH (pH of 3, 5, 7, and 9), the lower the enantiomer fraction (from 4.88 to 6.69). At pH of 3, 7, and 9, significant differences of half-lives between enantiomers were observed. (−)-*theta*-Cypermethrin decreased faster than (+)-*theta*-CYP under pH of 3, while opposite results were indicated under pH of 7 and 9. Moreover, the acidic condition contributed to the higher chiral configuration stability of (±)-*theta*-CYP. (+)-Enantiomer was influenced by pH in a greater degree than (−)-enantiomer.

KEYWORDS

degradation, enantiomer fraction, enantioselectivity, lactic acid bacteria, theta-cypermethrin

1 | INTRODUCTION

In recent decades, the use of pesticides plays a key role in agriculture production and harvest quality, which brings many benefits. However, pesticide exposure was related to a variety of human health effects.[1-3] Nowadays, great attention has been focused on chiral pollutants, the biological transformation and environmental fate of which can be stereoselective.[4,5] The enantiomers of the chiral pesticides show different activities in biological systems because of their differential interaction with enzymes or other naturally occurring chiral molecules.[6-8]

Pyrethroids, a class of chiral insecticides, used to control a wide range of pests in rural and urban areas. Cypermethrin (CYP) [(*RS*)-α-cyano-3-phenoxybenayl (1*RS*)-*cis*,*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate] [CAS Registry No. 52315-07-8] is one of the most common type II synthetic pyrethroid insecticides, which makes up more than 50% of the total pyrethroid market in China. It affects human health adversely by interfering with the endocrine system and referred as endocrine-disrupting chemicals. Epidemiological and experimental evidences have revealed that exposure to CYP is a key risk factor for Parkinson's disease.[9-11] Cypermethrin contains 8 optical

isomers, with 2 *cis* diastereomers of *cis*A (1*R*-*cis*-αR + 1*S*-*cis*-αS) and *cis*B (1*S*-*cis*-αR + 1*R*-*cis*-αS), and 2 *trans* diastereomers of *trans*A (1*R*-*trans*-αR + 1*S*-*trans*-αS) and *trans*B (1*R*-*trans*-αS + 1*S*-*trans*-αR). Different biological activities were found between these enantiomers. *cis*B and *trans*B are two pairs of diastereomers that have highly insecticidal effects, whereas the rest of the diastereomers have low insecticidal activity.[12,13] The *trans*B is also called *theta*-CYP [CAS Registry No. 71697-59-1], which was developed by Chinoin form Hungary.[14] In *theta*-CYP, *1R-trans* isomer was substantially more toxic than the *1S-trans* enantiomer.[15,16] Previous study found that (+)-*theta*-CYP converted to the (−)-*theta*-CYP in rats, which was likely the result of biotic interactions.[17]

Lactic acid bacteria (LAB) as the natural intestinal microbiota of most animals is one of the most common microorganisms applied in fermented food production, which shall be in a viable state, active and still present in product through the end of shelf life.[18-20] Recently, LAB strains have been examined for their potential to reduce exposure to toxins.[21] Moreover, some LAB and commercial yogurt starters, eg, *Lactobacillus brevis*, *Lactobacillus plantarum*, and *Lactobacillus delbrueckii* subsp. *bulgaricus*, had acceleration effects on pesticide degradation.[22-26] The enzymes or other naturally occurring chiral molecules in LAB may lead to enantioselectivity in degradation of chiral pesticides. However, no sufficient information is available to show the influence of LAB on enantioselective degradation.

This is the first study to investigate the influence of LAB on dissipation of pesticide on chiral level. In the present study, *theta*-CYP was added to deMan Rogosa Sharpe (MRS) liquid medium, which was commonly used for cultivation of fastidious LAB interference in bacteriocin purification.[27] The medium was inoculated with four strains of symbiotic starter cultures including *Lactobacillus plantarum* (LP), *Lactobacillus casei* (LC), *Lactobacillus delbrueckii* (LB), and *Streptococcus thermophilus* (ST) and cultured for separate times. The residual *theta*-CYP was extracted and quantified by using a high-performance liquid chromatography (HPLC). The dissipation kinetics with different inoculations was evaluated. To investigate the influence of LAB on enantioselective degradation, the enantiomer fractions (EFs) of *theta*-CYP at different culture times were calculated and compared. In addition, the correlation between pH and enantioselectivity was investigated in this study.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

Analytical standard of *theta*-CYP (purity > 92.0%) was used. 2-Propanol and n-hexane were of chromatography purity and purchased from Mallinckrodt Baker (NJ, USA). Analytical grade of yeast extract and tryptone were purchased from Oxoid (Hants, UK). Analytical grade of sodium acetate trihydrate and D-glucose were purchased from Xilong Scientific (Guangdong, China). Analytical grade of Tween-80 was purchased from Beijing Solarbio Science & Technology (Beijing, China). Analytical grade of acetonitrile, acetone, sodium hydroxide, hydrochloric acid, sodium chloride, petroleum ether, ammonium citrate, magnesium sulfate, manganese sulfate monohydrate, di-potassium hydrogen phosphate trihydrate, and beef extract were purchased from Sinopharm Chemical Reagent (Beijing, China). Petroleum ether was distilled before use and fractioned at 60 to 70°C was collected. The Florisil solid phase extraction (SPE) column (1 g/6 mL) was purchased from ANPEL Laboratory Technologies (Shanghai, China).

Four LABs named LP, LC, LB, and ST were obtained from China General Microbiological Culture Collection Center (Beijing, China). According to previous literature, these four kinds of LAB were typical stains used in fermentation. Moreover, some of them had accelerating effects on pesticide degradation. [23-26,28,29]

2.2 | Culture medium

The composition of MRS liquid medium used was D-glucose 20 g, tryptone 10 g, beef extract 5 g, sodium acetate trihydrate 5 g, yeast extract 4 g, di-potassium hydrogen phosphate trihydrate 2 g, ammonium citrate 2 g, magnesium sulfate 0.2 g, manganese sulfate monohydrate 0.05 g, Tween-80 1 mL, and distilled water 1000 mL. The medium was sterilized by autoclaving at 121°C for 20 minutes and cooled to ambient temperature.

2.3 | Resuscitation of strains

The lyophilized strains were individually rehydrated in 1 mL of sterilized rehydration medium with subsequent 5% (vol/vol) inoculation into MRS liquid medium, then cultured at optimum temperature for 24 hours (30°C for LC and 37°C for the others). To ensure the purity and viability of LABs, the strains were subcultured in MRS liquid medium thrice before further application.

2.4 | Sample preparation

theta-Cypermethrin was added to sterilized MRS liquid medium at level of 10 mg/kg. Then, the medium was shaken vigorously for 1 minute to ensure pesticide distribution and subpacked in 50-mL centrifuge tube. One of four strains was inoculated into the medium at a level of 0.5% and 5.0%(vol/vol), and then cultured at optimum

temperature (30°C for LC and 37°C for the others) for 0, 2, 8, 24, 36, 48, 72, 120, 168, and 240 hours. The pH was determined, and the growth of bacteria was measured by absorbance values at OD_{600} by using a UV752 ultraviolet spectrophotometer from Youke (Shanghai, China). Control sample was also prepared with the same procedure but without strain inoculation. Each treatment consisted of 3 replicates.

2.5 | Influence of PH on enantioselective degradation

theta-Cypermethrin was added to sterilized MRS liquid medium at level of 10 mg/kg. Then, the pH of medium was adjusted to 3, 5, 7, and 9 with hydrochloric acid or sodium hydrate (1 mol/L), determined by Sartorius PB-10 pH meter (Gottingen, Germany). After that, the medium was shaken vigorously for 1 minute to ensure pesticide distribution and subpacked in 50-mL centrifuge tube (10 mL for each one), cultured at 37°C for 0, 8, 20, 72, and 96 hours. The pH value of the medium was measured after sampling time to ensure no change of pH occurred during incubation time. Each treatment consisted of 3 replicates.

2.6 | Extraction and purification

The MRS liquid medium sample (10.00 ± 0.01 g) was extracted with 20 mL of acetonitrile by vigorously shaking for 1 minute. Afterward, 6 g of sodium chloride was added and the tube was shaken vigorously for 1 minute, then centrifuged at relative centrifugal force at 1610×*g*. A portion (10 mL) of supernatant was evaporated to near dryness by using a vacuum rotary evaporator at 35°C.

For the cleanup, the Florisil SPE cartridge was previously conditioned with 5 mL of petroleum ether/acetone (9:1, vol/vol). The concentrated extracts were dissolved in 5-mL petroleum ether/acetone (9:1, vol/vol) and transferred to the cartridge, then eluted through the column with 10 mL of petroleum ether/acetone (9:1, vol/vol). The eluents were collected and evaporated to near dryness at 35°C. The residues were redissolved in 2.5 mL of n-hexane/2-propanol (9:1, vol/vol) and filtered by using a 0.22-μm nylon syringe filter into auto-sampler vial for HPLC analysis.

2.7 | High-performance liquid chromatography analysis

The determination of *theta*-CYP was performed by using the Agilent 1200 HPLC (Agilent Technologies, CA, USA) equipped with ultraviolet detector (UV). The enantiomers of *theta*-CYP were separated on a Daicel Chiralcel OD-H column (250 × 4.6 mm, 5 μm) (cellulose *tris*-3,5-dimethylphenylcarbamate). The mobile phase was a mixture of n-hexane/2-propanol (97:3, vol/vol) with flow rate of 1 mL/min. The injection volume was 20 μL. Ultraviolet detection was conducted at 230 nm at room temperature.

2.8 | Statistical analyses

All experiments and analyses were carried out 3 times. The data were expressed as mean ± standard deviation (SD). Generally, the dissipation of chiral pesticides hypothetically follows first-order kinetics, which expressed as below[30]:

$$C_t = C_0 e^{-kt} \quad (1)$$

where C_t is the concentration of pesticide residue (mg/kg) at the time t (day), C_0 is the initial concentration after application (mg/kg), and k is the degradation rate constant (d^{-1}). The half-life ($t_{1/2}$) is defined as the time required for the pesticide residue level to fall to half of the initial residue level after application and was calculated from the k value:

$$t_{1/2} = \frac{\ln 2}{k} \quad (2)$$

The EF was used as a measurement of stereoselective dissipations of *theta*-CYP. This descriptor was proposed as a more meaningful representation of graphical data than the conventional enantiomeric ratio and more easily employed in mathematical fate expressions.[31] Enantiomer fraction was defined as the following equation:

$$\text{EF} = \text{peak areas of the } \frac{(-)}{[(-)+(+)]} \quad (3)$$

where (−) and (+) are the first and second eluting enantiomers. A racemic EF = 0.50, whereas preferential degradation of the (+) or (−) yields EF <0.50 and >0.50, respectively.

Data were statistically evaluated by one-way ANOVA analysis with SPSS version 20.0 from IBM (NY, USA) and OriginPro 8.0 (MA, USA). When significant differences were found between groups ($P < .05$), the t test and Duncan test were used to determine the differences among means.

3 | RESULTS AND DISCUSSION

3.1 | Optimization and validation of analytical methodology

The stereoselective separation of *theta*-CYP was investigated on 2 chiral columns, including Chiralcel OD-H (250 × 4.6 mm, 5 μm) and cellulose-4 (250 × 4.6 mm, 5 μm). Furthermore, the mobile phase was optimized with different ratio (90:10, 95:5 and 97:3, vol/vol) of n-hexane and 2-propanol. The result showed that, under the HPLC condition in which the mobile phase was a mixture of n-hexane/2-propanol (97:3, vol/vol) with flow rate of 1 mL/min, the baseline chiral separations for *theta*-CYP were obtained by using Chiralcel OD-H, which was not achieved on cellulose-4. These results agree with previous report of Ta.[32] As shown in Figure 1, the enantiomers were separated entirely, with no interference peaks eluted at retention times in any sample. The identification of enantiomeric elution orders was conducted in previous research.[33] On cellulose-tris (3,5-dimethylphenylcarbamate) chiral stationary phase, the first eluted enantiomer on hexane/isopropanol showed a negative optical signal, while the second one showed a positive optical signal. Correspondingly, the first and second eluted enantiomers were (−)-*theta*-CYP and (+)-*theta*-CYP.

In the cleanup procedure, both Florisil and aminopropyl-bonded SPE cartridges (1 g/6 mL) with different elution solvent were optimized. The elution solvents for Florisil SPE cartridges were different ratio (9:1, 8:2 and 7:3, vol/vol) of petroleum ether and acetone, while acetonitrile and toluene (3:1 and 1:0, vol/vol) for aminopropyl bonded SPE cartridges. The results showed that good recoveries were obtained with these cleanup procedures, while petroleum ether and acetone (9:1, vol/vol) achieved the minimum interference of impurity during determination.

The limit of quantification of method was 1 mg/kg. Linear calibration curves were obtained over each *theta*-CYP enantiomer concentration ranging from 0.5 to 50 mg/L. The linear calibration curves of (−)-*theta*-CYP and (+)-*theta*-CYP were $y = 33.827x + 7.3667$ ($R^2 = 0.9999$) and $y = 32.288x + 4.7737$ ($R^2 = 0.9999$), respectively. The recoveries of both enantiomers ranged from 86% ± 2.1% to 97% ± 5.8%, which are summarized in Table 1 (n = 5). The recoveries and reproducibility of recovery results confirmed that the method is sufficiently reliable for analysis in this study.[34]

3.2 | Influence of *theta*-cypermethrin on lactic acid bacteria resuscitation

Different kinds of resuscitation medium consist of several ingredients, which may lead to diversity of activation and sensitivity.[35] To investigate the influence of pesticide on resuscitation, both pesticide medium and nonpesticide medium were prepared in activation of LB. The pesticide medium was fortified with 10 mg/L of *theta*-CYP. After resuscitation, the LB was inoculated in medium to investigate the fate of pesticide. The result showed that there was no significant difference on growth of LAB, which revealed that *theta*-CYP had no influence on LAB

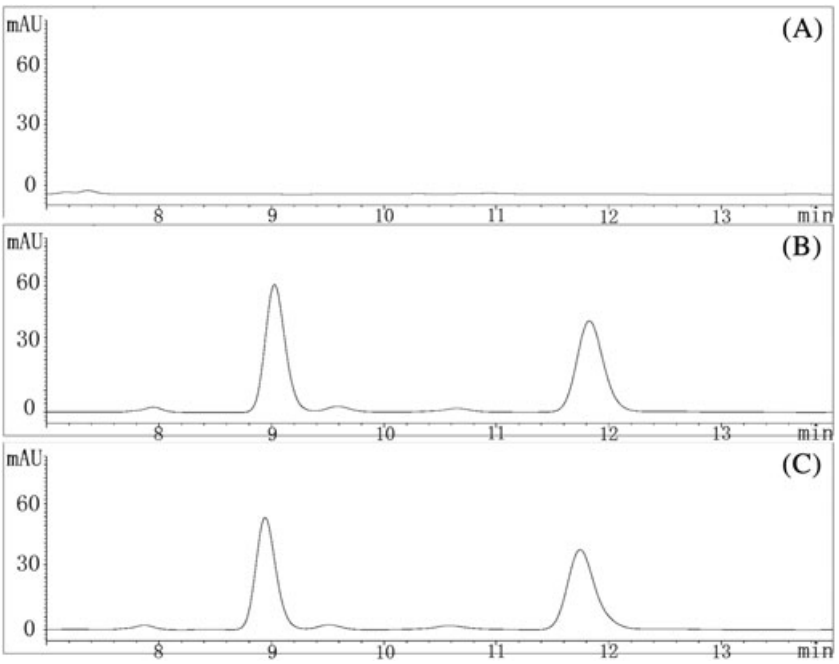

FIGURE 1 High-performance liquid chromatography (HPLC) chromatograms of *theta*-cypermethrin enantiomers. A, Extract from medium sample without *theta*-cypermethrin. B, Standard sample of 20 mg/L in n-hexane. C, Medium spiked with *theta*-cypermethrin at 10 mg/kg

TABLE 1 Recoveries and relative standard deviation (RSD) of each enantiomer in culture medium (n = 5)

Spiked Level (mg/kg)	(−)-*theta*-Cypermethrin		(+)-*theta*-Cypermethrin	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
1	93	4.1	97	5.8
10	86	2.1	91	2.7
20	90	3.1	92	3.0

resuscitation. Hence, nonpesticide medium was used for resuscitation of strains in this study.

3.3 | The growth curve of lactic acid bacteria and PH in medium

Biomass in the culture medium was determined by cell density expressed as optical absorbance (OD_{600}). As shown in Figure 2, for LP, LC, and ST, the OD_{600} increased rapidly within 24 hours and peaked at 36 hours, then it remained steady. But LB was an exception, which showed a decrease after 36 hours. This suggested that the growth of LAB was most active in first 36 hours.

To investigate the influence of inoculation on growth of LABs, each of them was inoculated into the medium at a level of 0.5% and 5.0% (vol/vol). In the first 8 hours, the biomass of 5.0% inoculation was more than 0.5% inoculation. As for ST, the 5.0% inoculation held a higher total bacterium count than 0.5% inoculation. For the other 3 kinds of LABs, the biomass of 2 inoculations was at the same level. The pH of medium with LAB decreased from 7 to 5 after 24 hours and kept unchanged for the rest time, while the control group remained 7.

3.4 | Enantioselective dissipation of *theta*-cypermethrin in medium

Assuming that the degradation of both stereoisomers followed first-order kinetics, the dissipation regressive equation, correlation coefficient (r), and half-lives were shown in Table 2, which reveal a good fit. There was steady decrease of both stereoisomers. The half-lives of theta-CYP ranged from 4.1 to 6.1 days. In controlled groups, the half-life of (−)-*theta*-CYP versus (+)-*theta*-CYP differed as 5.7, 6.4, 4.8, and 5.0 days versus 5.2, 5.7, 4.1, and 4.4 days. While in groups with LAB, no matter how much the inoculation rate was, the half-lives of 2 stereoisomers got closer. The value (half-life ratio (−)/(+) at same inoculation and LAB) was shown in Table 2. The ratio under the inoculation 0.5% or 5.0% was 1 or much closer to 1 than the inoculation rate 0%. The data showed that the LAB inoculation inhibits the enantioselective degradation of *theta*-CYP.

When it comes to EF values, the effect of LAB was more obvious, which was shown in Figure 3. At the beginning of sampling (0 h), the EF values of 3 groups (control group and 2 groups spiked with LAB) were close to 0.50. There was no significant change of EF values in groups with LAB within 10 days, while the EF values of the control group increased steadily with time. On the 10th

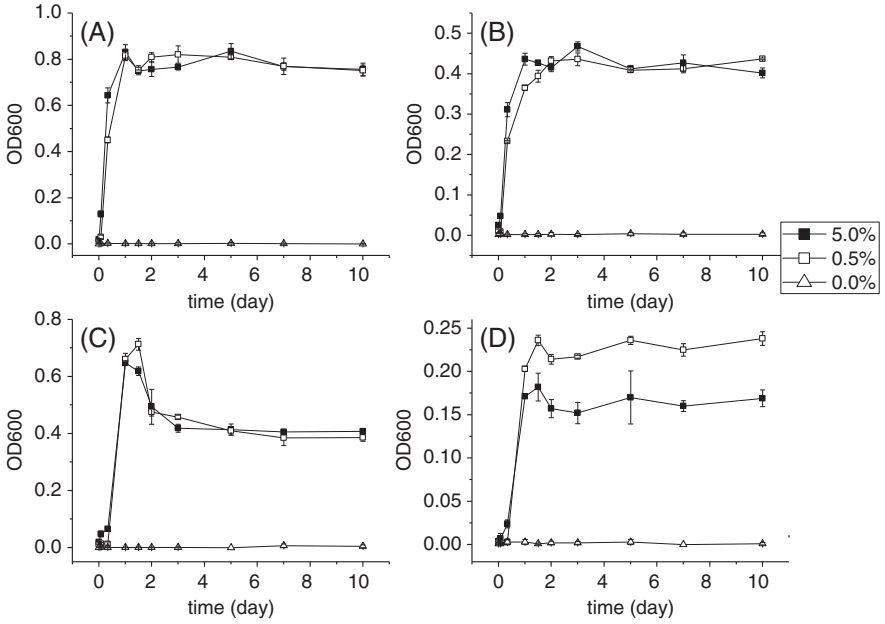

FIGURE 2 The OD_{600} of medium during 240 hours. A, *Lactobacillus plantarum* (LP); B, *Lactobacillus casei* (LC); C, *Lactobacillus delbrueckii* (LB); D, *Streptococcus thermophilus* (ST) (n = 3)

TABLE 2 The dissipation regressive equation, correlation coefficient (*r*), and half-lives of each enantiomer in culture medium (n = 3)

Lactic acid bacteria (LAB)	Enantiomers	Inoculation Rate (vol/vol)	Dissipation Regressive Equation	Correlation Coefficient (*r*)	Half-Lives (day)	Half-Life Ratio (−)/(+) at Same Inoculation and LAB
Lactobacillus plantarum	(−)	0.0%	$y = 7.4381e^{-0.121x}$	−0.9442	5.7	1.10
		0.5%	$y = 6.7838e^{-0.114x}$	−0.9284	6.1	1.00
		5.0%	$y = 7.2962e^{-0.115x}$	−0.9362	6.1	1.00
	(+)	0.0%	$y = 7.6724e^{-0.133x}$	−0.9470	5.2	
		0.5%	$y = 7.0276e^{-0.113x}$	−0.9324	6.1	
		5.0%	$y = 7.6023e^{-0.113x}$	−0.9348	6.1	
Lactobacillus casei	(−)	0.0%	$y = 6.61e^{-0.109x}$	−0.9626	6.4	1.12
		0.5%	$y = 5.9644e^{-0.134x}$	−0.9315	5.2	1.02
		5.0%	$y = 6.134e^{-0.118x}$	−0.9447	5.9	1.02
	(+)	0.0%	$y = 6.5168e^{-0.121x}$	−0.9643	5.7	
		0.5%	$y = 5.9727e^{-0.135x}$	−0.9354	5.1	
		5.0%	$y = 6.1344e^{-0.119x}$	−0.9537	5.8	
Lactobacillus delbrueckii	(−)	0.0%	$y = 8.0463e^{-0.143x}$	−0.9411	4.8	1.17
		0.5%	$y = 6.9227e^{-0.134x}$	−0.9159	5.2	0.98
		5.0%	$y = 7.6926e^{-0.15x}$	−0.9372	4.6	1.05
	(+)	0.0%	$y = 7.7288e^{-0.17x}$	−0.9382	4.1	
		0.5%	$y = 6.9442e^{-0.13x}$	−0.9218	5.3	
		5.0%	$y = 8.0602e^{-0.156x}$	−0.9586	4.4	
Streptococcus thermophilus	(−)	0.0%	$y = 7.6151e^{-0.14x}$	−0.9373	5.0	1.14
		0.5%	$y = 7.1622e^{-0.146x}$	−0.9236	4.7	1.00
		5.0%	$y = 7.5611e^{-0.138x}$	−0.9459	5.0	1.00
	(+)	0.0%	$y = 7.4903e^{-0.158x}$	−0.9422	4.4	
		0.5%	$y = 7.0075e^{-0.147x}$	−0.9197	4.7	
		5.0%	$y = 7.4691e^{-0.139x}$	−0.9458	5.0	

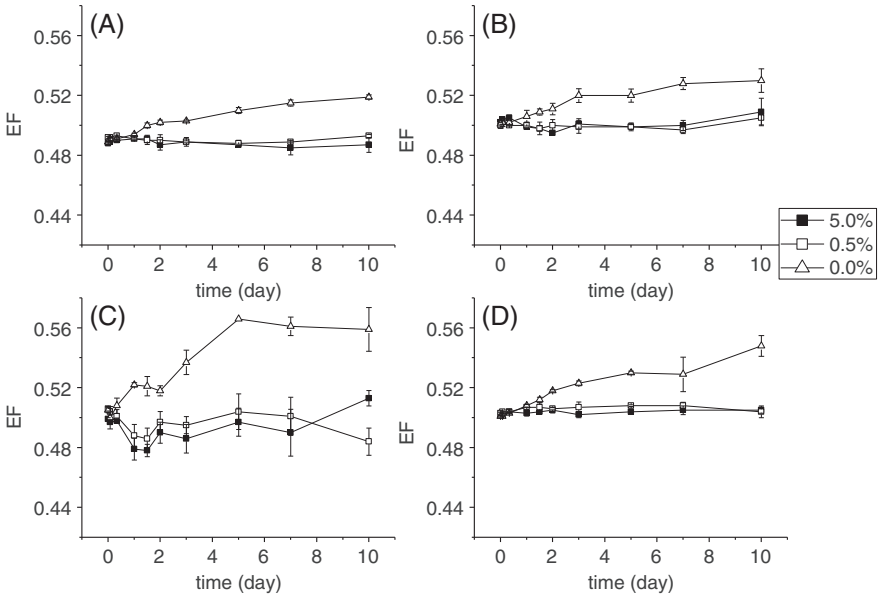

FIGURE 3 The enantiomer fraction of *theta*-cypermethrin under different inoculation rates. A, *Lactobacillus plantarum* (LP); B, *Lactobacillus casei* (LC); C, *Lactobacillus delbrueckii* (LB); D, *Streptococcus thermophilus* (ST) (n = 3)

day, for LP, LC, LB, and ST, the EF values of control group reached 0.52, 0.53, 0.56, and 0.55, respectively, indicating that preferential degradation of (+)-*theta*-CYP was observed in these groups. The declined trend was similar with previous study that (+)-*theta*-CYP degraded faster than the antipode in rats and soil.[17,36] On the contrary, all the EF values of groups with LAB were about 0.50 or even less than 0.50. Moreover, EF values were not influenced by the inoculation rate (0.5% and 5.0%).

Bo et al showed that the degradation of 6 organophosphorus pesticides (OPPs) in milk during yoghurt processing was enhanced by starters.[37] Zhao et al investigated the degradation of 7 OPPs in milk inoculated with 1 strain of *Lactobacillus* spp. including *Lactobacillus bulgaricus*, *Lactobacillus paracasei*, and *L. plantarum* and found that the selected *Lactobacillus* spp. exhibited acceleration on OPP degradation totally.[25] Zhang et al found that the inoculated *L. plantarum* strain in planted corn had accelerating effect on chlorpyrifos and phorate degradation during the storage.[23] Another study reported that yeast *Saccharomyces cerevisiae* and LP showed ability for significant reduction of pirimiphos methyl, ie, chlorpyrifos methyl in wheat during fermentation.[24] Islam et al isolated chlorpyrifos-degrading *L. brevis* WCP902 during kimchi fermentation and cloned a gene encoding organophosphorus hydrolase enzyme (OpdB).[22] Zhou applied LAB and yogurt starters to study 9 OPPs and got a conclusion that LAB of higher phosphatase production has more potential to decrease OPPs in fermented foods.[26] The present paper provides supplementary information of LAB biodegradation on pyrethroids other than OPPs. Compared with OPPs in these mentioned studies, *theta*-CYP was relatively insensitive to LAB.

The pH value clearly affects the enantioselectivity of the primary degradation of *cis*-epoxiconazole and metalaxyl in previous literature.[38] In this experiment, LABs fermented carbohydrates chiefly into lactic acid, which led to acidic condition. Meanwhile, the groups without LABs were in neutral condition. Besides, no significant correlation was obtained between the logarithmic phase of LABs and degradation rates. Based on these results, we made an assumption that the pH may play an important role in the stereoselective dissipation of *theta*-CYP. To verify this hypothesis, the correlation between pH and enantioselectivity was investigated in this study.

3.5 | Influence of PH on enantioselective degradation of *theta*-cypermethrin

The 2 enantiomers gradually decreased in medium of all treatments. The half-lives under different pH were shown in Table 3. The change of EF during incubation time was shown in Figure 4.

For all the treatments, the EF value ranged from 0.502 to 0.527 at 0 hour. However, different degradation trends of the 2 enantiomers were observed under different pH. As shown in Figure 4, during the incubation time, there was an obvious increase of EF under pH of 9, slighter increase under pH of 7, no change under pH of 5, and slighter decrease under pH of 3. At 96 hours of the experiment, the EF value arrived at 4.88, 5.05, 5.35, and 6.69 for pH of 3, 5, 7, and 9, respectively. The statistical analysis of half-lives (*t* test) was also performed, and the result in Table 3 suggested that there was substantial enantioselectivity on the dissipation of (±)-*theta*-CYP under pH of 3 ($P = .005$), 7 ($P = .024$), and 9 ($P = .005$), while no significant difference of the 2 enantiomers was observed under pH of 5 ($P = .912$). The result is the same as that of experiment with LABs as described in section 3.4, which showed enantioselectivity in neutral condition and no enantioselectivity in acidic condition. In this study, (−)-*theta*-CYP decreased faster than (+)-*theta*-CYP under pH of 3, while opposite results were obtained under pH of 7 and 9. It is said that (−)-*theta*-CYP was preferentially degraded with pH lower than 5, while (+)-*theta*-CYP was preferentially degraded with pH higher than 5. Comparing pH 5 with the middle of the neutral,

TABLE 3 Half-lives of two enantiomers under different pH (n = 3)

pH Value	(−)-Enantiomers	(+)-Enantiomers
3	69.0 ± 0.5[a]	78.4 ± 0.6[a]
5	57.8 ± 5.4[b]	58.3 ± 6.3[b]
7	58.9 ± 2.5[b]	52.8 ± 1.7[b]
9	48.1 ± 3.5[c]	35.2 ± 1.9[c]

Values with the different letters are significantly different ($P < .05$).

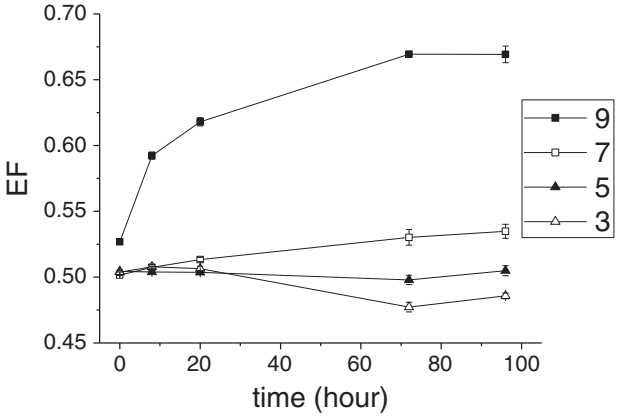

FIGURE 4 The enantiomer fraction of *theta*-cypermethrin under pH of 3, 5, 7, and 9 (n = 3)

the lower the pH and the lower the EF, the higher the pH and the higher the EF. This indicated that the relative stability of 2 enantiomers was closely related to pH. Therefore, the assumption we made is confirmed by the result of this experiment. It is worth noting that an opposite result was obtained in a previous study. Yang and Ji investigated the stereoselective degradation of *beta*-CYP in soils with different pH values, which consists of *alpha*-CYP and *theta*-CYP. They found that degradation occurred the most quickly in soil with pH of 5.8, followed by 7.1, and was slowest in 8.5.[36] It can be assumed that the opposite preferences in stereoselective degradation of *theta*-CYP may be caused by different types of microorganisms in soil and this study. Meanwhile, the characteristics of soil, such as organic matter, particle size, and humidity, could also influence the enantioselective behaviors.

For each enantiomer, Duncan analysis showed that the half-lives under pH 3 were significantly longer than other 3 treatments, and pH 9 led to the shortest one, while no distinct difference was found between pH 5 and 7. As shown in Table 3, the half-lives of (±)-*theta*-CYP decreased with the increase of pH value. In other words, compared with alkaline condition, acidic condition contributed to more stability of (±)-*theta*-CYP. Moreover, the (+)-*theta*-CYP was influenced by pH in a greater degree than the other enantiomer. As we know, the degradation rate of pesticide was influenced by many factors, 1 of which is pH. The enantioselectivity of *theta*-CYP under different pH is confirmed in this paper, which is of great significance for the further evaluated.

4 | CONCLUSION

In this study, a validated chiral analytical method for *theta*-CYP in LAB medium was developed by HPLC. The chiral resolution parameters and sample preparation procedure were optimized systematically. Then, the method was successfully applied to study of the enantioselective dissipation of *theta*-CYP in the medium. The (−)-stereoisomer degraded slower than (+)-stereoisomer without LAB, while the degradation rate got closer with LAB, which indicated that the stereoselective degradation was inhibited by LAB. The results of correlation between pH and enantioselectivity showed that the lower the pH, the lower the EF. It can be concluded that (−)-*theta*-CYP was preferentially degraded with pH lower than 5, while (+)-*theta*-CYP was preferentially degraded with pH higher than 5. Compared with alkaline condition, the acidic condition leads to higher stability of (±)-*theta*-CYP. Moreover, the (+)-*theta*-CYP was influenced by pH in a greater degree than the (−)-*theta*-CYP. The research will provide a more thorough understanding of influence of LAB on stereoselective dissipation of *theta*-CYP and could contribute to the risk assessment of *theta*-CYP in fermented food from the perspective of chirality.

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