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Citation style: Cycoń M., Żmijowska A., Piotrowska-Seget Zofia. (2014). Enhancement of deltamethrin degradation by soil bioaugmentation with two different strains of "Serratia marcescens". "International Journal of Environmental Science and Technology" (Vol. 11, iss. 5 (2014), s. 1305-1316), doi 10.1007/s13762-013-0322-0



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Enhancement of deltamethrin degradation by soil bioaugmentation with two different strains of *Serratia marcescens*

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Received: 29 August 2012/Revised: 11 March 2013/Accepted: 1 May 2013/Published online: 17 May 2013
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Abstract Deltamethrin is one of the most commonly used pyrethroid in agricultural practice in different geographic regions of the world. It is detected in many environments, especially in soil and water, and can exhibit toxic effect to human and other organisms. In this study, we describe two bacterial strains DeI-1 and DeI-2, isolated from soil, and both identified as *Serratia marcescens* based on profile of the fatty acid methyl esters, biochemical test, and 16S RNA gene analysis, which were shown to efficiently degrade deltamethrin. Degradation of deltamethrin in mineral salt medium (50 mg l^{-1}) proceeded by strains DeI-1 or DeI-2 reached the values of 88.3 or 82.8 % after 10 days, and DT50 was 2.8 or 4.0 days, respectively. Bioaugmentation of deltamethrin-contaminated non-sterile soils (100 mg kg^{-1}) with strains DeI-1 or DeI-2 (3×10^6 cells g^{-1} of soil) enhanced the disappearance rate of pyrethroid, and its DT50 was reduced by 44.9, 33.1, 44.4, and 58.2 days or 39.1, 25.8, 35.6, and 46.0 days in sandy, sandy loam, silty loam, and silty soils, respectively, in comparison with non-sterile soils with only indigenous microflora. The three-way ANOVA indicated that DT50 of deltamethrin was significantly ($P < 0.01$) affected by soil type, microflora presence, and inoculum, and the

interaction between these factors. Generally, the lower content of clay and organic carbon in soil, the higher degradation rate of deltamethrin was observed. Obtained results show that both strains of *S. marcescens* may possess potential to be used in bioremediation of deltamethrin-contaminated soils.

Keywords Bacteria · Bioremediation · Degradation kinetics · Soil contamination · Pyrethroid

Introduction

Deltamethrin ((*S*)- α -cyano-3-phenoxybenzyl (1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate) (Fig. 1) is a broad-spectrum insecticide belonging to pyrethroids. They are synthetic compounds that made to mimic the pyrethrins produced by chrysanthemum flowers. Deltamethrin has been applied widely around the world for more than 30 years on various crops including cotton, corn, cereals, soybeans, and vegetables for pests such as mites, ants, weevils, and beetles. As a result of year-long application, it is detected in many environmental matrices, especially in soil and water where it can exhibit toxic effect to target and non-target organisms (Hintzen et al. 2009; You et al. 2009). As extremely lipophilic, deltamethrin easily penetrates the cuticles of insects and acarines, as well as kills insects through ingestion. It acts as neurotoxin, and α -cyano group induces the inhibition of the sodium channel activation gate. This results in prolonged permeability of the nerve to sodium leading to blockage of nerve conduction. Deltamethrin can also affect activity of the chloride and calcium channels, possibly due to their phosphorylation (Burr and Ray 2004).

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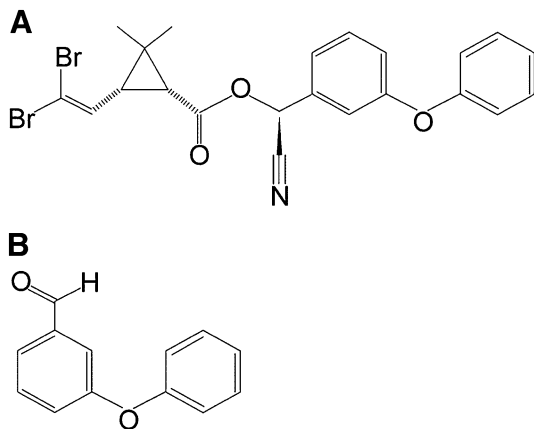


Fig. 1 Chemical structure of deltamethrin (a) and 3-phenoxybenzaldehyde (b)

Deltamethrin as hydrophobic compound has a low mobility in soils. This property causes strong sorption to soil organic matter, and limited its leaching into groundwater (Oudou and Hansen 2002). In soil, deltamethrin undergoes various dissipation processes and is degraded via hydrolysis, photolysis, and microbial activity with half-life ranged from 11 to 72 days, depending on the soil type and oxygen availability (Elliott 1989; WHO 1990). Degradation of deltamethrin is slower under anaerobic or sterile conditions, indicating important role of soil microorganisms in this process (Chapman et al. 1981; Zhang et al. 1984; Grant and Betts 2004). Deltamethrin- and other pyrethroid-degrading bacteria were found in various genera such as *Micrococcus* (Tallur et al. 2008), *Pseudomonas* (Grant et al. 2002; Jilani and Khan 2006; Fulekar 2009), *Spingobium* (Guo et al. 2009), *Ochrobactrum* (Chen et al. 2011a), *Streptomyces* (Chen et al. 2011b, 2012a), *Stenotrophomonas* (Chen et al. 2011c), and *Bacillus* (Chen et al. 2012b). The pathway of deltamethrin degradation as well as its intermediates was described in detail. In the first step, bacteria cleave the carboxylester linkage of deltamethrin resulting in the formation of α -hydroxy-3phenoxy-benzeneacetonitrile and 3-phenoxybenzaldehyde (Fig. 2). The latter compound is further oxidized to 2-hydroxy-4-methoxy benzophenone (Chen et al. 2011b). The formed metabolites are more mobile, and they do not accumulate in soils. Under anaerobic conditions, deltamethrin is metabolized to carboxylic acids that are further subsequently degraded. However, the reduction of 3-phenoxybenzoic acid to 3-phenoxybenzyl alcohol in the flooded soil was also observed (Kaufman and Kayser 1980).

The wide use and repeated applications of deltamethrin and its high affinity to soil particles may lead to soil contamination with this insecticide. Therefore, detailed investigations of microbial degradation of deltamethrin may be

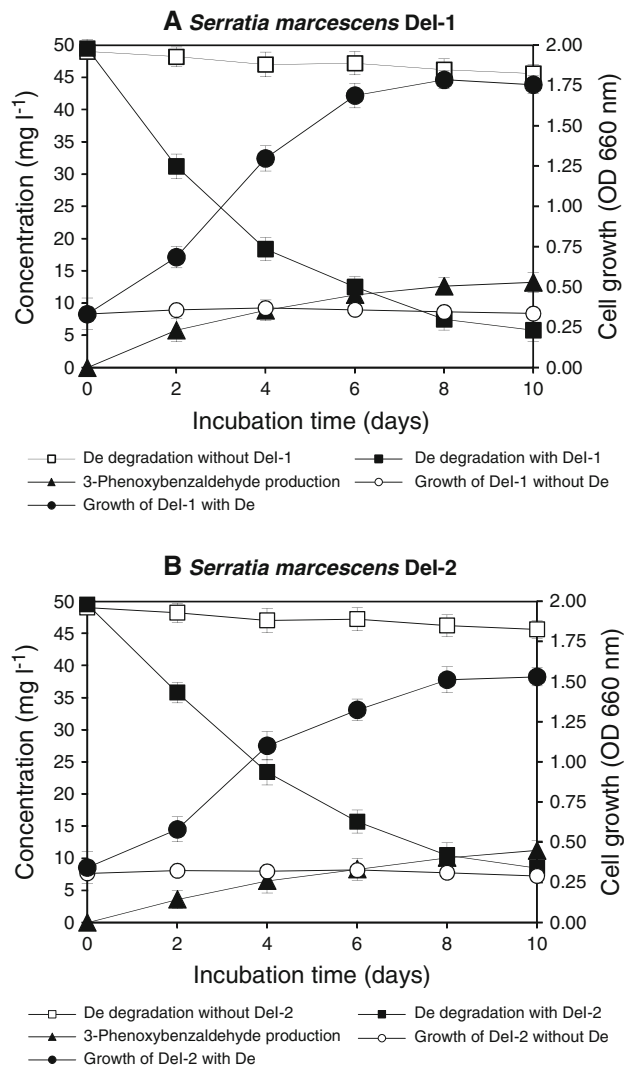


Fig. 2 Degradation of deltamethrin (De) and subsequent production of 3-phenoxybenzaldehyde by strain Del-1 (a) or strain Del-2 (b) in mineral salt medium (MSM). Symbols are the means of three replicates. Error bars represent the standard deviation which was within 5 % of the mean

useful for the development of insecticide degradation strategies using microorganisms and/or enzymes involved in their hydrolysis (Chen et al. 2011b). The aim of the study was to isolate and identify deltamethrin-degrading strains, to investigate their degradation potential in liquid medium and soils, and to assess the biodegradation rates of deltamethrin in soils. Samples of soils differing in their physico-chemical parameters were collected in June 2011 from the top layer (0–20 cm) of five different commercial fields located in Upper Silesia, Southern Poland, that is, loamy sand (49°59'48''N, 18°55'14''E), sandy (50°5'4''N, 18°58'28''E), sandy loam (49°59'93''N, 18°00'15''E), silty loam (49°58'17''N, 18°59'34''E), and silty soils (49°58'5''N, 18°50'29''E).

Materials and methods

Chemicals and media

Certified standards of deltamethrin (98 % chemical purity) and 3-phenoxybenzaldehyde (98 % chemical purity) were purchased from Sigma-Aldrich (Germany), while all other chemicals were of analytical grade and purchased from Merck (Germany). Stock solution of deltamethrin was sterilized by filtration through 0.22- μm -pore-size Millipore membranes and used for the preparation of the insecticide containing media. For the biodegradation studies, mineral salt medium (MSM) was used. The medium contained 2.0 g of $(\text{NH}_4)_2\text{SO}_4$, 0.2 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.001 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1.5 g of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, and 1.5 g of KH_2PO_4 per litre of deionized water. The final pH value was adjusted to 7.2. After autoclaving (121 °C, 20 min) and cooling, the medium was supplemented with a suitable insecticide solution.

Soils

A loamy sand soil treated with deltamethrin annually for 7 years was used for the isolation of deltamethrin-degrading bacteria. The other sampled soils (sandy, sandy loam, silty loam, and silty soils) with no known history of pesticide applications were used for the inoculation biodegradation studies. Detailed physico-chemical properties of the soils are presented in Table 1. Determination of soil parameters was performed according to the methods described in our previous studies (Cycoń et al. 2011, 2012). In the laboratory, the soils were gently air-dried to the point of soil moisture suitable for sieving. After sieving to a maximum particle size of <2 mm, the soils were immediately used in the bacteria isolation and biodegradation experiments.

Table 1 General characteristic of the soils used in the experiment

Parameter	Loamy sand ^a	Sand ^b	Sandy loam ^b	Silty loam ^b	Silt ^b	Method
Sand (2,000–50 μm) (%)	86.0 \pm 3.1	91.0 \pm 4.2	69.0 \pm 3.2	18.0 \pm 2.0	4.0 \pm 0.4	ISO 11277:2009
Silt (<50–2 μm) (%)	11.0 \pm 2.3	6.0 \pm 1.1	21.0 \pm 1.3	76.0 \pm 3.8	85.0 \pm 2.2	ISO 11277:2009
Clay (<2 μm) (%)	3.0 \pm 0.5	3.0 \pm 0.9	10.0 \pm 1.0	6.0 \pm 1.3	11.0 \pm 0.8	ISO 11277:2009
Density g cm^{-3}	1.10 \pm 0.1	1.02 \pm 0.03	1.11 \pm 0.04	1.28 \pm 0.09	1.46 \pm 0.09	ISO 11272:1998
pH _(in water) (1:5)	6.8 \pm 0.2	6.5 \pm 0.4	6.7 \pm 0.2	6.8 \pm 0.3	7.2 \pm 0.2	ISO 10390:2005
CEC ($\text{cmol} + \text{kg}^{-1}$)	7.6 \pm 0.3	3.3 \pm 0.2	8.0 \pm 0.7	11.5 \pm 0.9	22.0 \pm 1.3	ISO 11260:1994
WHC (%)	34.6 \pm 2.1	32.0 \pm 2.2	38.0 \pm 1.9	48.0 \pm 1.5	54.0 \pm 1.6	ISO 14239:1997
C _{org} (%)	1.4 \pm 0.1	1.1 \pm 0.2	1.6 \pm 0.02	2.9 \pm 0.6	3.8 \pm 0.04	ISO 14235:1998
N _{tot} (%)	0.08 \pm 0.02	0.07 \pm 0.01	0.09 \pm 0.02	0.18 \pm 0.03	0.22 \pm 0.03	ISO 11261:1995
MB (mg kg^{-1})	665 \pm 28	600 \pm 23	710 \pm 32	925 \pm 38	1125 \pm 56	ISO 14240-1:1997

CEC cation exchange capacity, WHC water-holding capacity, MB microbial biomass

^a Soil previously treated with deltamethrin and used for the isolation of deltamethrin-degrading bacteria

^b Soils with no known history of previous deltamethrin applications and used for the biodegradation and bioremediation studies with the isolated bacteria. The values are the means of three replicates with the standard deviation which was within 5 % of the mean

Isolation of deltamethrin-degrading bacteria

The enrichment culture technique was used for the isolation of deltamethrin-degrading bacteria. For this purpose, 10 g of commercial agricultural soil (previously treated with deltamethrin) was added to flasks containing 100 ml of MSM supplemented with deltamethrin (50 mg l^{-1}). Samples were incubated for 72 h on a rotary shaker (120 rpm) in a darkened thermostatic chamber maintained at 30 ± 1 °C. After this time, 1 ml of soil suspension was transferred into flasks containing the fresh medium supplemented with the same concentration of deltamethrin and incubated for an additional 72 h under the same conditions. After seven subsequent transfers into the same medium, serial dilution of the flask samples was plated onto MSM agar plates supplemented with deltamethrin (50 mg l^{-1}) for isolation of individual colonies. Isolates exhibiting distinct colonial morphologies were isolated by repeated streaking on the same agar medium.

Identification of bacterial isolates

Isolates were characterized and identified using the fatty acid methyl esters (FAMES), biochemical test, and 16S RNA gene analysis. The biochemical properties of isolates and the substrate utilization patterns were determined by the API 20E Systems (bioMérieux Inc., France) according to the manufacturer's recommendations. The FAME profiles of isolates were analysed by gas chromatography (GC) using the Sherlock Microbial Identification System (MIDI Inc., Newark, USA). The FAMES were extracted from each isolate using the standard and recommended procedure, consisting of saponification, derivatization, extraction, and final base washing. Cellular FAME was separated by Hewlett Packard 6890 GC equipped with a capillary column Ultra 2-HP and flame

ionization detector (FID) using hydrogen as a carrier gas. For the 16S rRNA sequence analysis, the genomic DNA was extracted from the strain collected at the late exponential stage of growth using Genomic DNA Isolation Kit (Qiagen, USA) according to the manufacturer's recommendations. The 16S rRNA genes of *S. marcescens* were amplified using the universal primer pair: 27f and 1492r (Lane 1991) obtained from Sigma-Aldrich (Germany). Amplification was conducted by using a PCR Master Mix Kit (Promega) according to the manufacturer's recommendations, and a PTC-118 Thermal Cycler (BIO-RAD, CA, USA) under the following conditions: (1) an initial denaturation step of 95 °C for 2 min, (2) 30 cycles of denaturation, annealing, and extension (95 °C for 1 min followed by 54 °C for 30 s, with an extension step at 72 °C for 2 min), and (3) a final extension at 72 °C for 5 min. The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, USA) before the amplicons were sequenced. The partial 16S rRNA sequences of strains were compared by the BlastN search analysis. Then, the identification to the species level was determined by the 16S rRNA sequence similarity with that of the prototype strain sequence in the GenBank.

Biodegradation experiment

Inoculum preparation

Two deltamethrin-degrading strains designated as DeI-1 and DeI-2 were used for inoculum preparation. Bacterial strains were cultured in 100-ml Erlenmeyer flasks containing 20 ml of MSM supplemented with deltamethrin at a concentration of 50 mg l⁻¹. At the exponential phase, the bacteria were pelleted by centrifugation (5 min, 10,000g). The pellet was washed twice with 0.85 % of sterile NaCl and then resuspended in NaCl to obtain the bacterial suspension at a concentration of approximately 6 × 10⁸ cells/ml. The cell density (OD 660 nm) was measured using a UV-VIS spectrophotometer (Varian, USA).

Biodegradation of deltamethrin in MSM

The degradation studies were performed in 500-ml Erlenmeyer flasks containing 200 ml of sterile MSM supplemented with deltamethrin as the only source of carbon. The applied amount of insecticide corresponded to a MSM concentration of 50 mg l⁻¹. Next, the medium was inoculated with 1 ml of bacterial suspension giving a final concentration of approximately 3 × 10⁶ cells ml⁻¹. Triplicate samples of MSM with strain DeI-1 or strain DeI-2 as well as insecticide only were used as controls. All samples were incubated on a rotary shaker (120 rpm) in a darkened thermostatic chamber maintained at 30 ± 1 °C. Samples of MSM were periodically removed aseptically for bacterial growth rate and pH

determinations, as well as for chemical analyses to determine deltamethrin concentrations and its main metabolite 3-phenoxybenzaldehyde. The growth of bacterial strains was recorded spectrophotometrically by measuring the OD at 660 nm using a UV-VIS spectrophotometer (Varian, USA), while the pH values of MSM were measured with a glass electrode using a Jenway pH-meter.

Biodegradation of deltamethrin in soils

The degradation experiment with both *S. marcescens* strains was performed in sterile (SS) and non-sterile (nSS) soils. In the first case, soils were sterilized three times by autoclaving for 1 h at 121 °C 1 week prior to the commencement of the experiment to permit the release of the toxic volatile compounds produced. The stock solution of deltamethrin was sprayed on the surface of 500 g of SS and nSS soils by means of a micro-syringe that dispensed very small droplets and ensured thorough mixing. The applied amount of insecticide corresponded to a soil concentration of 100 mg kg⁻¹. Next, 1 ml of bacterial suspension was introduced in triplicate into the soil to give a final bacterial count of approximately 3 × 10⁶ cells g⁻¹ of soil. In order to study the insecticide dissipation rates under abiotic conditions, the triplicate samples of SS without bacteria were kept as controls. Additionally, the same dose of insecticide was applied to nSS, to study the potential of autochthonous microorganisms to degrade deltamethrin. The water content of the soil samples was adjusted to 50 % of the maximum water-holding capacity by the addition of sterile deionized water. All soil samples were incubated in a darkened thermostatic chamber maintained at 30 ± 1 °C. Throughout the incubation period, the sterile deionized water was added to the soil treatments to compensate for any water losses exceeding 5 % of the initial amount added. Samples of soil treatments were periodically removed aseptically for the determination of deltamethrin concentrations and its main metabolite.

Chemical analyses

For deltamethrin concentration determination, 10 ml of MSM or 10 g of soil were taken for analyses. Samples of MSM were filled to volume of 20 ml with deionized water and extracted twice with 10 ml of ethyl acetate on a rotary shaker (30 min), whereas soil samples were extracted with 20 ml of ethyl acetate on a rotary shaker for 1 h. Next, the extracts were dehydrated with anhydrous Na₂SO₄, evaporated to dryness under a stream of N₂ at 45 °C using rotary evaporator (IKA, RV05 Basic, Janke & Kunkel-Ika Labortechnik, Germany), subsequently diluted to a final volume of 10 ml with hexane, and reserved for chromatographic analysis. Concentrations of deltamethrin were determined by gas chromatography (GC) using a Hewlett

Packard 6890 N GC equipped with a capillary column HP-5 (cross-linked phenyl methyl polysiloxane; 30 m, 0.32 mm i.d.; film thickness 0.25 μm) and electron capture detector (ECD) using nitrogen (0.8 ml min^{-1}) as a carrier gas. The obtained data were analysed with ChemStation Software (Rev.A.0.9.03).

For 3-phenoxybenzaldehyde concentration determination, 10 ml of MSM or 10 g of soil were taken for analyses. Samples of MSM and soils were extracted with 50 ml of methanol–dichloromethane mixture (60:40, v/v) after adding 1 ml of HCl on a rotary shaker for 30 min and 1 h, respectively. Next, the extracts were dehydrated with anhydrous Na_2SO_4 , evaporated to dryness under a stream of N_2 at 45 °C using rotary evaporator (IKA, RV05 Basic, Janke & Kunkel-Ika Labortechnik, Germany), subsequently diluted to a final volume of 10 ml with mobile phase, and reserved for chromatographic analysis. Concentrations of 3-phenoxybenzaldehyde were measured by high-performance liquid chromatography (HPLC) using a Varian ProStar System (Varian, Inc., USA) equipped with a UV–VIS detector (ProStar 325), solvent delivery module (ProStar 210), and reverse-phase column (Microsorb-MV 100-5 C18 (250 mm \times 4.6 mm \times 5 μm)). The mobile phase was acetonitrile–universal buffer pH 2.36 (60:40, v/v) injected at a flow rate of 1.0 ml min^{-1} . The obtained data were analysed with the Chromatography Workstation Software (Star, LC WS, version 6.2).

Kinetics and statistics analyses

Disappearance of deltamethrin in MSM or soils was fitted to first-order kinetic model. The rate constant k (day^{-1}) was determined using the algorithm $C_t/C_0 = e^{-kt}$, where C_0 is the amount of insecticide in soil at time zero and C_t is the amount of insecticide in soil at time t (day). Linear regression ($\ln(C_t/C_0)$ of the chemical data and time) was used to calculate the time in which the deltamethrin concentration in the soil was reduced by 50 % (DT50).

Kinetic data of deltamethrin degradation in MSM were analysed by a one-way ANOVA; whereas the data obtained for soil kinetics were analysed by a three-way analysis of variance (ANOVA) to determine the percentage of the variation attributable to the factors type of soil, microflora presence, and bacterial inoculum. The statistical significance of differences in measured data was assessed by a post hoc comparison of the means using the least significant differences (LSD) test. For determination of correlations between disappearance time (DT50) of deltamethrin in sterile (SS) and non-sterile (nSS) soils of different textures inoculated with bacterial strains and the respective soil properties were also calculated the Pearson's correlation coefficients. All statistical analyses were made using the Statistica 9.0 PL software package.

Results and discussion

Isolation and identification of isolates

Using a soil enrichment procedure, two deltamethrin-degrading strains designated as DeI-1 and DeI-2 were screened. After incubations on nutrient agar plates (24 h), the colonies of strain DeI-1 were found to be red in colour, while the colonies of strain DeI-2 were creamy white. The two strains showed a similar biochemical pattern with the exception of urea hydrolysis. Strain DeI-1 exhibited urease activity whereas urease test of strain DeI-2 was negative. The API 20E test identified the isolates as *Serratia marcescens* with 99.8 and 97.0 % similarity for strains DeI-1 and DeI-2, respectively (Table 2). Similarly, profiles of the fatty acids (Table 3) allowed to identify both strains as *S. marcescens*, and the similarity index (SIM) calculated by the MIDI system on the level of 0.876 and 0.762 for DeI-1 and DeI-2, respectively, showed very good matches. SIM is an indication of the confidence with which the isolate is identified. Strains with a SIM level of >0.1 , and especially those with a SIM of ≥ 0.3 , are considered positively identified, whereas strains with a SIM of ≤ 0.1 are considered tentatively identified (Germida and Siciliano 2001). Also, identification based on the analysis of the partial 16S RNA sequence of the two strains showed that they were related to *S. marcescens* with high similarity (99 %) (Table 4). Isolated strains are deposited in culture collection of the Department of Microbiology and Virology, University of Silesia, Poland. Two beta-cypermethrin-degrading strains JC1 and JCN13 belonging to genus *Serratia* that differed in their capability of the red pigment production and urea hydrolysis were also screened by Zhang et al. (2010). Bacteria from genus *Serratia*, known as very metabolically active bacteria, were isolated from various environments contaminated with synthetic pyrethroids (Grant et al. 2002; Zhang et al. 2010).

Biodegradation of deltamethrin in MSM

Culturing bacteria in MSM revealed that both strains were capable of using deltamethrin as the sources of carbon and energy (Fig. 2). However, results showed differences in their growth and the deltamethrin degradation rates in MSM. As indicated by kinetic data, the degradation of deltamethrin proceeded by strain DeI-1 was more effective in comparison with degradation proceeded by strain DeI-2. In the first case, the degradation of deltamethrin reached the values of 88.3 % after 10 days of incubation (Fig. 2a), and this process was characterized by a rate constant of 0.214 day^{-1} , and DT50 was 2.8 days (Table 5). In contrast, in MSM with strain DeI-2 deltamethrin disappeared with a rate constant of 0.176 day^{-1} , resulted in the

Table 2 Physiological and biochemical characteristics of deltamethrin-degrading strains

Physiological and biochemical test	Strain/results		Physiological and biochemical test	Strain/results	
	DeI-1	DeI-2		DeI-1	DeI-2
Gram-staining	–	–	Gelatin liquefaction	+	+
Pigment production	+	–	Glucose utilization	+	+
Arginine hydrolysis	–	–	Mannitol utilization	+	+
Lysine decarboxylation	+	+	Inositol utilization	+	+
Ornithine decarboxylation	+	+	Sorbitol utilization	+	+
Citrate utilization	+	+	Rhamnose utilization	–	–
H ₂ S production	–	–	Sucrose utilization	+	+
Urea hydrolysis	+	–	Melibiose utilization	+	+
Tryptophane deamination	–	–	Amygdalin utilization	+	+
Indole production	–	–	Arabinose utilization	–	–
Voges-Proskauer	+	+	Oxidase production	–	–

+ Positive, – Negative

Table 3 The fatty acid profiles of bacterial strains isolated from deltamethrin-treated soil

Strain DeI-1				Strain DeI-2			
Fatty acid	%	Fatty acid	%	Fatty acid	%	Fatty acid	%
10:0 <i>iso</i>	0.24	14:0 <i>iso</i>	0.16	10:0	0.22	16:1 ω 7c/16:1 ω 6c	13.86
10:0	0.57	14:0	4.32	10:0 3OH	4.38	16:0	30.69
9:0 3OH	0.13	14:0 2OH	2.19	12:0 <i>iso</i>	0.13	17:0 <i>cyclo</i>	11.08
12:0 <i>aldehyde</i>	0.78	16:1 <i>iso</i> I/14:0 3OH	6.43	12:0 <i>anteiso</i>	0.10	17:0	0.42
10:0 3OH	10.86	16:1 ω 7c/16:1 ω 6c	9.39	12:0	1.54	16:0 3OH	0.12
12:0 <i>iso</i>	0.10	16:0	27.17	13:0	0.18	18:1 ω 7c	14.75
12:0 <i>anteiso</i>	0.16	17:0 <i>cyclo</i>	11.95	12:0 2OH	0.86	18:0	0.44
12:0	1.24	17:0	0.54	12:1 3OH	1.61	19:0 <i>cyclo</i> ω 8c	2.55
11:0 3OH	0.25	18:1 ω 7c	12.76	12:0 3OH	1.87	19:0	0.26
12:0 2OH	0.76	18:0	0.56	14:0	4.97		
12:1 3OH	2.92	19:0 <i>cyclo</i> ω 8c	2.28	14:0 2OH	2.36		
12:0 3OH	3.61	20:1 ω 7c	0.11	14:0 3OH/16:1 <i>iso</i> I	7.62		

Table 4 The homology assay results based on 16S RNA sequence for deltamethrin-degrading strains

Strain	Species	Accession number	Similarity (%)
DeI-1	<i>S. marcescens</i> strain HL1	EU371058	99
	<i>S. marcescens</i> strain S418	GQ202220	99
	<i>S. marcescens</i> strain DAP33	EU302858	99
DeI-2	<i>S. marcescens</i> strain N4.1	AY514435	99
	<i>S. marcescens</i> strain N2.4	AY514434	99
	<i>S. marcescens</i> strain N1.14	AY514433	99
	<i>S. marcescens</i> strain LEP9	AB635400	99

degradation of this insecticide at a level of 82.8 % of the initial dose within 10 days (Fig. 2b), giving a higher value of DT50 on the level of 4.0 days (Table 5). In turn, the degradation of deltamethrin in MSM without bacteria was

slight, and 93.1 % of the initial dose of deltamethrin still persisted after 10-day experimental period (Fig. 2).

To the best of our knowledge, there is no information on *S. marcescens* degrading deltamethrin; however, earlier studies showed a high potential of this species to degrade other pesticides belonging to the group of the organophosphorus insecticides (Lakshmi et al. 2008; Cycoń et al. 2009; Abo-Amer 2011). Moreover, some strains from the genus *Serratia* were characterized by a high potential for degradation of other pyrethroids. For example, Zhang et al. (2010) showed that strain JC1 could degrade 92 % beta-cypermethrin within 10 days, while the degradation rate of strain JCN13 reached 89 % after 4 days. Also, Grant et al. (2002) observed that strain *Serratia* closely related to *Serratia plymuthica* degraded cypermethrin or flumethrin by at least 50 % after 20 days of incubation in mineral medium. It has also been reported that some bacteria were found to be capable of degrading a wide spectrum of synthetic

Table 5 Kinetic data of deltamethrin (De) degradation in mineral salt medium (MSM), and in sterile (SS) and non-sterile (nSS) soils of different textures inoculated with strain DeI-1 or strain DeI-2

Medium	Treatments	k (day ⁻¹)	Regression equation	R^2	DT50 (days)
MSM ^a	MSM + De	0.007	$\ln(C_t/C_0) = -0.0069t + 0.0043$	0.9485	99.8 ± 3.2^a
	MSM + De + DeI-1	0.214	$\ln(C_t/C_0) = -0.2198t + 0.0433$	0.9930	2.8 ± 0.7^b
	MSM + De + DeI-2	0.176	$\ln(C_t/C_0) = -0.1841t + 0.0018$	0.9932	4.0 ± 0.7^c
<i>Soils^b</i>					
Sandy soil	SS + De	0.003	$\ln(C_t/C_0) = -0.0030t + 0.0104$	0.9779	234.5 ± 9.8^a
	SS + De + DeI-1	0.021	$\ln(C_t/C_0) = -0.0203t - 0.0730$	0.9854	30.5 ± 1.9^{bc}
	SS + De + DeI-2	0.016	$\ln(C_t/C_0) = -0.0163t - 0.0523$	0.9825	39.3 ± 2.3^c
	nSS + De	0.010	$\ln(C_t/C_0) = -0.0105t + 0.0620$	0.9843	71.9 ± 3.3^d
	nSS + De + DeI-1	0.029	$\ln(C_t/C_0) = -0.0305t + 0.1292$	0.9794	27.0 ± 1.5^b
	nSS + De + DeI-2	0.022	$\ln(C_t/C_0) = -0.0240t + 0.0929$	0.9825	32.8 ± 1.4^e
Sandy loam soil	SS + De	0.002	$\ln(C_t/C_0) = -0.0022t + 0.0005$	0.9848	315.3 ± 9.6^f
	SS + De + DeI-1	0.017	$\ln(C_t/C_0) = -0.0156t - 0.1112$	0.9810	$51.6 \pm 2.8^{g,k}$
	SS + De + DeI-2	0.013	$\ln(C_t/C_0) = -0.0132t - 0.0505$	0.9878	48.7 ± 3.1^g
	nSS + De	0.011	$\ln(C_t/C_0) = -0.0105t + 0.0292$	0.9694	68.8 ± 2.5^d
	nSS + De + DeI-1	0.021	$\ln(C_t/C_0) = -0.0218t + 0.0841$	0.9764	35.7 ± 1.5^h
	nSS + De + DeI-2	0.018	$\ln(C_t/C_0) = -0.0180t + 0.0814$	0.9900	43.0 ± 1.3^i
Silty loam soil	SS + De	0.002	$\ln(C_t/C_0) = -0.0016t + 0.0009$	0.9984	433.8 ± 8.7^j
	SS + De + DeI-1	0.012	$\ln(C_t/C_0) = -0.0126t - 0.0289$	0.9916	52.7 ± 2.1^k
	SS + De + DeI-2	0.011	$\ln(C_t/C_0) = -0.0112t + 0.0135$	0.9953	63.1 ± 3.1^l
	nSS + De	0.008	$\ln(C_t/C_0) = -0.0083t + 0.0243$	0.9923	86.4 ± 3.4^m
	nSS + De + DeI-1	0.016	$\ln(C_t/C_0) = -0.0163t - 0.0081$	0.9737	42.0 ± 1.5^n
	nSS + De + DeI-2	0.013	$\ln(C_t/C_0) = -0.0139t + 0.0136$	0.9770	50.8 ± 2.0^k
Silty soil	SS + De	0.001	$\ln(C_t/C_0) = -0.0013t + 0.0060$	0.9894	537.8 ± 9.7^o
	SS + De + DeI-1	0.011	$\ln(C_t/C_0) = -0.0109t + 0.0060$	0.9962	64.1 ± 2.3^p
	SS + De + DeI-2	0.008	$\ln(C_t/C_0) = -0.0088t - 0.0087$	0.9881	77.8 ± 3.2^q
	nSS + De	0.006	$\ln(C_t/C_0) = -0.0067t + 0.0121$	0.9928	105.3 ± 3.9^r
	nSS + De + DeI-1	0.014	$\ln(C_t/C_0) = -0.0150t + 0.0136$	0.9906	47.1 ± 2.1^s
	nSS + De + DeI-2	0.011	$\ln(C_t/C_0) = -0.0124t + 0.0424$	0.9811	59.3 ± 3.9^p

The values are the means of three replicates with the standard deviation which was within 5 % of the mean

^a Different letters indicate significant differences between treatments ($P < 0.05$, LSD test)

^b Different letters indicate significant differences, considering the effects of bacterial inoculum, microflora presence, and type of soil ($P < 0.05$, LSD test)

pyrethroids. For example, deltamethrin-degrading *Streptomyces aureus* strain HP-S-01 (Chen et al. 2011b) and fenvalerate-degrading *Stenotrophomonas* sp. strain ZS-S-01 (Chen et al. 2011c) were capable of degrading such pyrethroids as fenprothrin, bifenthrin, beta-cypermethrin, cyhalothrin, and permethrin with a wide range of efficacy. For efficient bioremediation of pyrethroid-contaminated environments, it would be advantageous if microorganisms could be used against many pyrethroids. Since their structure is generally similar, it is expected that these organisms would be capable of degrading various insecticides from this group.

As it was revealed by Zhang et al. (2010), there was a relationship between high cell surface hydrophobicity (CSH) and degradation ability of bacteria from genus *Serratia*. The authors showed that strain JCN13 had higher

hydrophobicity and potential to degrade beta-cypermethrin than strain JC1. Differences in the degradation rate of deltamethrin proceeded by strains in our study might be the results of their different CSH. Moreover, a lower rate of deltamethrin degradation in MSM by strain DeI-2 in comparison with strain DeI-1 might be also attributed to its higher sensitivity to 3-phenoxybenzaldehyde, one of the main deltamethrin metabolites known from its toxicity. Chemical analyses showed that the concentration of 3-phenoxybenzaldehyde in MSM inoculated with respective strain increased proportionally to the decrease in the parent compound over the experimental period. After 10 days of incubation, the concentration of 3-phenoxybenzaldehyde in MSM inoculated with strain DeI-1 or DeI-2 was measured at 13.2 or 11.2 mg l⁻¹ of MSM,

respectively (Fig. 2). 3-phenoxybenzaldehyde has been reported as an antimicrobial compound, and therefore could prevent the proliferation of bacteria (Xu et al. 2007; Xia et al. 2008). This is one of the possible reasons for not completely degrading of deltamethrin in MSM by strains DeI-1 and DeI-2. This phenomenon during deltamethrin degradation was also observed earlier by Khan et al. (1988). However, some bacteria from the genus *Streptomyces* (Chen et al. 2011b) and fungi belonging to the genus *Cladosporium* (Chen et al. 2011d) were found to be capable of degrading this compound in mineral medium with high efficiency.

Due to the growth of both strains and the degradation of deltamethrin, a slight decrease in MSM pH from 7.20 to 5.91 and 6.26 for MSM + DeI-1 and MSM + DeI-2, respectively, during 10 days of incubation was observed (data not shown). It has been reported that pH had a marked influence on the deltamethrin and other synthetic pyrethroids degradation by bacterial isolates, and a decreased stability under alkaline conditions was observed for these compounds (Chen et al. 2011b, c).

Biodegradation of deltamethrin in soil

Our study showed that degradation of deltamethrin in non-sterile soils (nSS) with naturally occurring microorganisms was relatively slow. However, autochthonous microflora in all used soils was characterized by a degradation potential of deltamethrin, and during the 84-day experiment, 56.7, 59.8, 48.1, and 41.8 % of the initial dose of deltamethrin was removed (Fig. 3), giving its DT50 of 71.9, 68.8, 86.4, and 105.3 days (Table 5) in sandy, sandy loam, silty loam, and silty soils, respectively. In contrast, significant amounts of deltamethrin, that is, 77.0–89.0 % of the initial dosage, still persisted in sterile soils after 84 days of the experiment (Fig. 3). These results confirm the observations made by Khan et al. (1988) and Gu et al. (2008) showing that microbial degradation is the main mechanism of deltamethrin dissipation in soils whereas its abiotic breakdown is less important.

Studies on other synthetic pyrethroids have shown that microorganisms which can degrade them in culture conditions also perform their degradation in soil (Chen et al. 2011c, 2012b). In our biodegradation experiment, soils

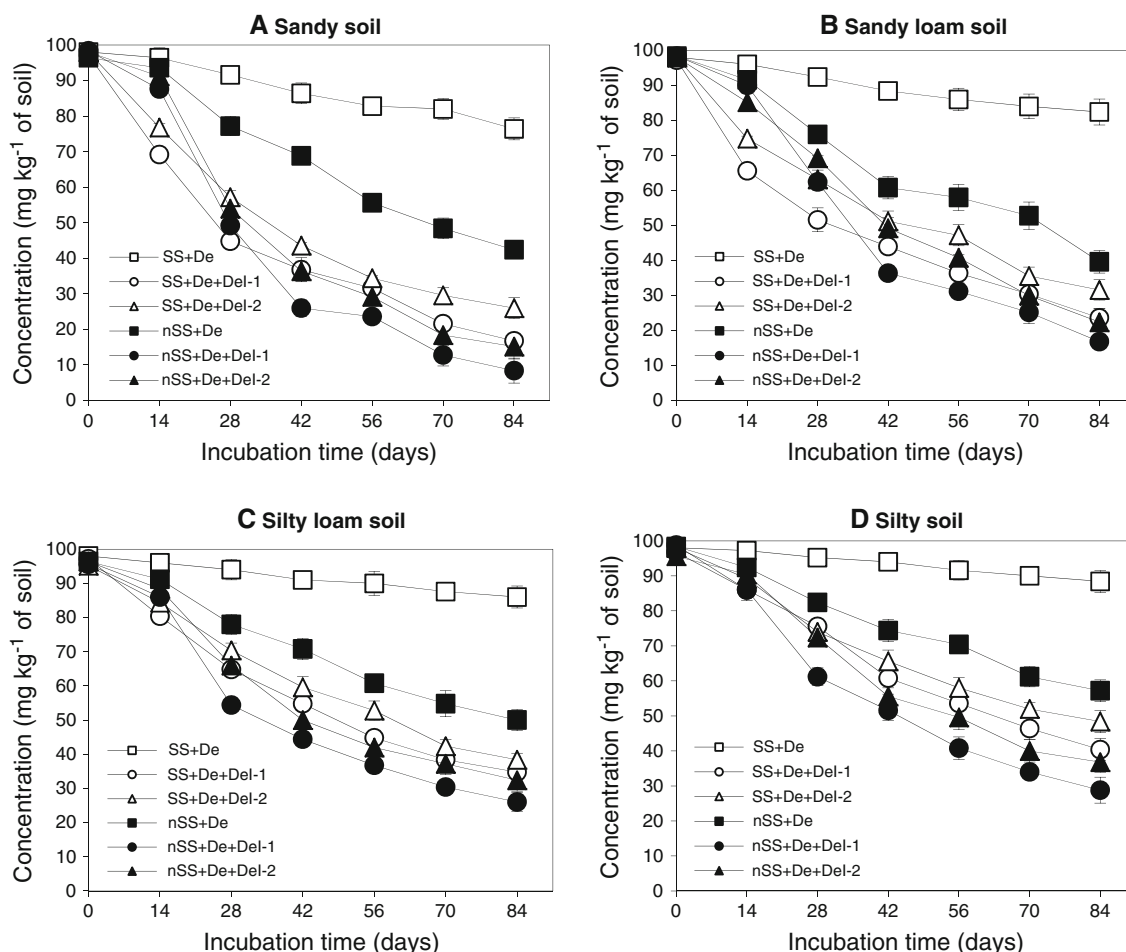


Fig. 3 Degradation dynamics of deltamethrin (De) in sterile (SS) and non-sterile (nSS) soils of different textures inoculated with strain DeI-1 or strain DeI-2. Symbols are the means of three replicates. Error bars represent the standard deviation which was within 5 % of the mean



were inoculated with 3×10^6 bacterial cells g^{-1} of soil, and this inoculum density appeared to be able to eliminate deltamethrin efficiently. As indicated by other experiments, the inoculum size is an important factor determining the efficient biodegradation of the applied pesticides (Comeau et al. 1993; Chen et al. 2011b; Cycoń et al. 2011). Strains DeI-1 or DeI-2 introduced into sterile soils showed a higher degradation potential for deltamethrin removal (16–26 or 5–17 %) than those observed in non-sterile soils with naturally occurring attenuation, and no delay in deltamethrin degradation was observed (Fig. 3; Table 5). Generally, in all experimental treatments, the degradation of deltamethrin proceeded by strain DeI-1 was more effective in comparison with degradation proceeded by strain DeI-2.

The degradation dynamics of deltamethrin in nSS bioaugmented with respective strains indicated that during the first 2 weeks the process was slow and its rate was similar to that observed in non-bioaugmented nSS (Fig. 3). Depending on the soil type, only 10–14 % of the applied dose of deltamethrin was degraded in nSS during 14 days. After this time, the disappearance of pyrethroid increased substantially, giving the final rate of degradation on the level of 61–82 or 70–92 % for soils inoculated with strains DeI-1 or DeI-2, respectively (Fig. 3). Inoculation of non-sterile soils with strains DeI-1 or DeI-2 enhanced the disappearance rates of deltamethrin (Fig. 3), and its DT50 was reduced by 44.9, 33.1, 44.4, and 58.2 days or 39.1, 25.8, 35.6, and 46.0 days in sandy, sandy loam, silty loam, and silty soils, respectively, in comparison with non-sterile soils with only indigenous microflora (Table 5). The observed low rate of degradation during the first 2 weeks might be the result of the necessity of bacterial adaptation to the presence of pyrethroid, adaptation of introduced strain to soil conditions as well as proliferation of indigenous bacteria that can use deltamethrin as an additional source of carbon and energy. This suggestion may be supported by the increase in the numbers of specific group of microorganisms in soil after application of different pyrethroids and other chemicals, which was observed in many studies (Cycoń et al. 2006; Vig et al. 2008; Zhang et al. 2009; Cycoń et al. 2010). The initial delay in deltamethrin degradation may also be explained by the fact that soils used in this study had no contact with pyrethroids and other pesticides for at least 10 years, and the number of bacteria capable of pesticide utilization was very small. The retardation in pesticide dissipation may be also related to the competition of inoculated strains with other microorganisms or antagonistic inhibition via substances synthesized by indigenous microorganisms. Moreover, a relatively high deltamethrin concentration (100 mg kg^{-1} of soil) was also an important factor affecting microbial growth. However, synthetic pyrethroids applied at the recommended field rate are non-toxic to soil microorganism, as these organisms do not have sensitive

targets (Rangaswamy and Venkateswarlu 1993; Widenfalk et al. 2004; Cycoń et al. 2006). The delay in pesticides and other chemicals degradation just after their application into soil indicating the period of time needed for the proliferation of a small population of pesticide-degrading microorganisms to the optimal level required for an effective degradation of the pesticide was also observed in some studies (Mrozik et al. 2010; Chen et al. 2011a, b). The observed enhancement of deltamethrin elimination in our study can be explained by the fact that both strains increased the catabolic potential of soil, and moreover, autochthonous soil microorganisms had a synergistic ability to degrade deltamethrin. This phenomenon was also observed in earlier studies on degradation of other synthetic pyrethroids. For example, an inoculation of soil contaminated with fenvalerate (50 mg kg^{-1} of soil) with *Stenotrophomonas* sp. strain ZS-S-01 increased the rate of pyrethroid dissipation, and its half-life value was eight times lower than for soil without strain (Chen et al. 2011c). Also, Chen et al. (2012a) found that inoculation of beta-cypermethrin-degrading *S. aureus* HP-S-01 into soil with this pyrethroid (50 mg kg^{-1} of soil) resulted in a decrease in beta-cypermethrin at a level of 87.8 % of the initial dose within 10 days of experiment, whereas in the same period, the degradation rate of beta-cypermethrin in non-inoculated soil reached 25.1 %.

As indicated by chemical data, the concentration of 3-phenoxybenzaldehyde, the main metabolite of the degradation of deltamethrin, increased proportionally in all soil treatments to the decrease in the parent compound over the experimental period (Table 6). However, the concentration of this compound in sterile soils inoculated with strains DeI-1 or DeI-2 as well as with naturally occurring microorganisms, and in the same soils bioaugmented with respective strain reached maximum values generally after 56 days, and after this period declined (Table 6). The observed initial increase in the concentration of 3-phenoxybenzaldehyde in soils, followed by its depletion during the soil incubation, might suggest that both indigenous microorganisms and introduced strains had the ability to utilize this compound. As a general degradation product of deltamethrin and other synthetic pyrethroids, 3-phenoxybenzaldehyde has higher mobility than the parent compounds and causes widespread contamination (Xia et al. 2008; Chen et al. 2012b). Moreover, 3-phenoxybenzaldehyde is characterized by a high level of toxicity and classified as an endocrine disrupting chemical (Meecker et al. 2009). Therefore, there is a need to search for cost-effective and highly efficient bioremediation method to eliminate this compound from the soils. Examples of strains capable of degrading of 3-phenoxybenzaldehyde, thus useful for its removal from soil, are deltamethrin-degrading *S. aureus* strain HP-S-01 (Chen et al. 2011b) and fenvalerate-degrading *Stenotrophomonas* sp. strain ZS-S-01 (Chen et al. 2011c).

Table 6 Concentration (mg kg⁻¹ of soil) of 3-phenoxybenzaldehyde determined in sterile (SS) and non-sterile (nSS) soils of different textures inoculated with strain DeI-1 or strain DeI-2 during deltamethrin (De) degradation

Soil	Treatments	Day after treatment						
		0	14	28	42	56	70	84
Sandy soil	SS + De	ND	0.6 ± 0.1	1.3 ± 0.2	2.6 ± 0.6	3.0 ± 0.2	3.2 ± 0.3	4.3 ± 0.4
	SS + De + DeI-1	ND	5.6 ± 0.4	11.1 ± 0.9	12.4 ± 1.1	13.1 ± 1.6	17.2 ± 0.2	16.1 ± 0.9
	SS + De + DeI-2	ND	4.2 ± 0.2	8.7 ± 0.6	10.9 ± 1.0	12.5 ± 1.0	16.1 ± 1.0	14.0 ± 1.3
	nSS + De	ND	0.9 ± 0.1	3.8 ± 0.3	5.8 ± 0.8	9.5 ± 0.8	8.9 ± 0.5	6.4 ± 0.6
	nSS + De + DeI-1	ND	2.2 ± 0.3	9.8 ± 0.2	14.7 ± 0.9	16.8 ± 0.9	13.1 ± 0.7	11.1 ± 0.9
	nSS + De + DeI-2	ND	1.4 ± 0.4	8.7 ± 0.4	11.8 ± 1.0	14.7 ± 0.6	11.8 ± 0.5	9.7 ± 0.6
Sandy loam soil	SS + De	ND	0.4 ± 0.1	1.1 ± 0.5	2.0 ± 0.2	2.4 ± 0.2	2.8 ± 0.1	3.1 ± 0.2
	SS + De + DeI-1	ND	6.3 ± 0.5	9.1 ± 0.6	11.4 ± 0.9	14.8 ± 0.7	13.0 ± 0.5	10.1 ± 0.7
	SS + De + DeI-2	ND	4.6 ± 0.2	8.0 ± 0.8	9.4 ± 0.3	12.1 ± 0.9	10.0 ± 0.5	8.9 ± 0.3
	nSS + De	ND	1.4 ± 0.3	4.2 ± 0.4	7.2 ± 0.6	10.1 ± 0.9	9.7 ± 0.6	7.4 ± 0.6
	nSS + De + DeI-1	ND	1.7 ± 0.5	7.8 ± 0.3	13.1 ± 0.9	15.2 ± 1.0	10.1 ± 0.3	8.7 ± 0.4
	nSS + De + DeI-2	ND	2.6 ± 0.1	5.9 ± 0.6	12.8 ± 1.0	14.1 ± 1.1	11.2 ± 0.7	10.0 ± 0.6
Silty loam soil	SS + De	ND	0.4 ± 0.1	0.9 ± 0.2	1.4 ± 0.4	1.6 ± 0.3	2.2 ± 0.2	2.4 ± 0.3
	SS + De + DeI-1	ND	3.4 ± 0.3	6.9 ± 0.4	9.2 ± 0.9	11.8 ± 1.2	10.2 ± 0.8	9.7 ± 0.7
	SS + De + DeI-2	ND	2.2 ± 0.1	6.0 ± 0.7	7.9 ± 0.5	12.4 ± 0.8	10.9 ± 0.3	10.0 ± 1.0
	nSS + De	ND	1.0 ± 0.2	3.2 ± 0.3	4.7 ± 0.5	8.9 ± 0.6	9.8 ± 0.9	8.1 ± 0.4
	nSS + De + DeI-1	ND	1.9 ± 0.3	9.2 ± 0.2	12.3 ± 1.4	14.3 ± 0.4	13.0 ± 0.6	11.0 ± 0.7
	nSS + De + DeI-2	ND	1.6 ± 0.2	6.8 ± 0.4	10.8 ± 1.0	12.4 ± 0.5	11.2 ± 0.3	9.7 ± 0.3
Silty soil	SS + De	ND	0.2 ± 0.1	0.6 ± 0.5	0.8 ± 0.2	1.1 ± 0.2	1.5 ± 0.1	1.7 ± 0.2
	SS + De + DeI-1	ND	2.6 ± 0.3	5.8 ± 0.2	8.4 ± 0.6	9.9 ± 0.8	11.8 ± 0.6	8.4 ± 0.6
	SS + De + DeI-2	ND	1.8 ± 0.4	6.2 ± 0.4	7.5 ± 0.5	10.4 ± 0.4	12.9 ± 0.3	11.2 ± 0.8
	nSS + De	ND	1.2 ± 0.3	3.4 ± 0.2	4.8 ± 0.2	9.7 ± 0.8	9.4 ± 0.6	8.5 ± 0.2
	nSS + De + DeI-1	ND	2.2 ± 0.2	7.9 ± 0.7	10.1 ± 0.5	12.8 ± 1.4	14.1 ± 0.3	11.3 ± 0.9
	nSS + De + DeI-2	ND	1.0 ± 0.1	5.2 ± 0.4	9.1 ± 0.6	11.4 ± 0.9	13.5 ± 0.9	12.1 ± 1.0

The values are the means of three replicates with the standard deviation which was within 5 % of the mean

ND not determined

Table 7 Multivariate analysis of variance by three-way ANOVA of disappearance time (DT50) of deltamethrin in different soil treatments

Source of variation	df	Sum of squares	Mean squares	Variance explained (%)	F	P
Soil (S)	3	58,123.36	19,374.46	4.6	955.66	<0.01
Microflora (M)	1	203,479.50	203,479.50	16.4	10,036.80	<0.01
Inoculum (I)	2	544,321.90	272,160.90	43.8	13,424.58	<0.01
S × M	3	24,892.04	8,297.35	2.0	409.27	<0.01
S × I	6	45,745.20	7,624.20	3.7	376.07	<0.01
M × I	2	325,368.90	162,684.50	26.2	8,024.55	<0.01
S × M × I	6	38,997.49	6,499.58	3.1	320.59	<0.01

The ability of our strains to degrade 3-phenoxybenzaldehyde should to be proved in both liquid and soil experiments.

Our data indicated that the type of soil had a marked effect on the deltamethrin disappearance, and this was also confirmed by the statistical analyses. The three-way ANOVA indicated that DT50 of deltamethrin were significantly affected by soil type ($P < 0.01$), microflora presence ($P < 0.01$), and inoculum ($P < 0.01$), and the

interaction between these factors was also significant ($P < 0.01$). The inoculum (43.8 %), microflora presence (16.4 %), and the interaction between these factors (26.2 %) explained most of the variance (Table 7). In our study, the highest rate of deltamethrin degradation was observed in non-sterile sandy soil bioaugmented with DeI-1 or DeI-2, in which DT50 reached the value of 27.0 or 32.8 days, respectively, whereas DT50 in non-sterile silty

Table 8 Correlation for deltamethrin (De) between disappearance time (DT50) of its residues in sterile (SS) and non-sterile (nSS) soils of different textures inoculated with strain DeI-1 or strain DeI-2 and the respective soil properties

Treatment	Soil parameter					
	Sand	Silt	Clay	pH	Organic carbon	Microbial biomass
SS + De	-0.989, $P < 0.001$	0.966, $P < 0.001$	0.652, $P = 0.022$	0.961, $P < 0.001$	0.994, $P < 0.001$	0.995, $P < 0.001$
SS + De + DeI-1	-0.849, $P < 0.001$	0.811, $P < 0.001$	0.854, $P < 0.001$	0.904, $P < 0.001$	0.859, $P < 0.001$	0.876, $P < 0.001$
SS + De + DeI-2	-0.962, $P < 0.001$	0.947, $P < 0.001$	0.647, $P = 0.023$	0.959, $P < 0.001$	0.983, $P < 0.001$	0.986, $P < 0.001$
nSS + De	-0.846, $P < 0.001$	0.849, $P < 0.001$	0.401, $P = 0.196$	0.880, $P < 0.001$	0.908, $P < 0.001$	0.909, $P < 0.001$
nSS + De + DeI-1	-0.957, $P < 0.001$	0.936, $P < 0.001$	0.713, $P = 0.009$	0.922, $P < 0.001$	0.951, $P < 0.001$	0.954, $P < 0.001$
nSS + De + DeI-2	-0.952, $P < 0.001$	0.931, $P < 0.001$	0.716, $P = 0.009$	0.944, $P < 0.001$	0.959, $P < 0.005$	0.965, $P < 0.001$

soil was 47.1 or 59.3 days for soil inoculated with DeI-1 or DeI-2, respectively. These results indicated that the degradation of deltamethrin is not only related to individual features of the microorganisms but also to the type of soil. The soils used in our experiment differed in their physico-chemical parameters including amount of organic matter, sand, silt, clay, and microbial biomass (Table 1). Analysis of the Pearson's correlation coefficients indicated that there was a significant positive or negative relationship between DT50 of deltamethrin in different soil treatment and the respective soil properties (Table 8). Generally, the lower content of clay and organic carbon in soil, the higher degradation rate of deltamethrin was obtained. Correlation between dissipation of deltamethrin and soil organic matter contents was observed by Gu et al. (2008). However, in contrast to our results, they found the highest dissipation of deltamethrin in soils characterized with the highest organic matter. The significant differences in the cypermethrin mineralization in soils differing in the sand and silt contents were also reported (Ismail et al. 2012; Fenlon et al. 2011). The observed in our study lower degradation of deltamethrin in silty loam and silty soils though high amount of microbial biomass confirmed that organic matter and clay contents are the major factors controlling insecticide bioavailability (Hong et al. 2007; Gu et al. 2008; Munoz-Leoz et al. 2009). The lipophilic properties of deltamethrin cause its strong tendency to bind various organic and non-organic soil components, and these pyrethroids may persist for long period in soils (Oudou and Hansen 2002). Khan et al. (1988) using deltamethrin labelled with ^{14}C found that its residues, identified as a parent compound and its metabolite, were still present at the end of a 40-month experiment. However, the adsorption and desorption processes are associated with other soil parameters such as pH and water content (Oudou and Hansen 2002). The higher ratio of deltamethrin dissipation observed in sandy soils, that characterized by a low content of organic matter and clay fraction, was connected with a higher availability of insecticide to bacteria as compared to silty soil.

Conclusion

The studies on degradation of pyrethroids are important because the pesticide-degrading bacteria, and their consortia may be an efficient tool for bioremediation of pyrethroid-contaminated environments. Results of our study showed that both strains DeI-1 and DeI-2 have a potential to degrade deltamethrin in liquid medium and various type of soils. The bioaugmentation of all tested soils with strains DeI-1 or DeI-2 increased the soil catabolic activity and significantly enhanced the removal of deltamethrin. These results suggest that the tested strains could be a promising candidate for remediation of soil environments contaminated with deltamethrin.

Acknowledgments This study was performed within the framework of the statutory Project (KNW-1-041/P/1/0) funded by Medical University of Silesia.

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References

- Abo-Amer AE (2011) Biodegradation of diazinon by *Serratia marcescens* DI101 and its use in bioremediation of contaminated environment. *J Microbiol Biotechnol* 21:71–80
- Burr SA, Ray DE (2004) Structure-activity and interaction effects of 14 different pyrethroids on voltage-gated chloride ion channels. *Toxicol Sci* 77:341–346
- Chapman RA, Tu CM, Harris CR, Cole C (1981) Persistence of five pyrethroid insecticides in sterile and natural, mineral and organic soil. *Bull Environ Contam Toxicol* 26:513–519
- Chen S, Yang L, Hu M, Liu J, Zhong G, Yang L (2011a) Biodegradation of beta-cypermethrin and 3-phenoxybenzoic acid by a novel *Ochrobactrum lupini* DG-S-01. *J Hazard Mater* 187:433–440
- Chen S, Lai K, Li Y, Hu M, Zhang Y, Zeng Y (2011b) Biodegradation of deltamethrin and its hydrolysis product 3-phenoxybenzaldehyde by a newly isolated *Streptomyces aureus* strain HP-S-01. *Appl Microbiol Biotechnol* 90:1471–1483



- Chen S, Yang L, Hu M, Liu J (2011c) Biodegradation of fenvalerate and 3-phenoxybenzoic acid by a novel *Stenotrophomonas* sp. strain ZS-S-01 and its use in bioremediation of contaminated soils. *Appl Microbiol Biotechnol* 90:755–767
- Chen S, Hu Q, Hu M, Luo J, Weng Q, Lai K (2011d) Isolation and characterization of a fungus able to degrade pyrethroids and 3-phenoxybenzaldehyde. *Bioresour Technol* 102:8110–8116
- Chen S, Geng P, Xiao Y, Hu M (2012a) Bioremediation of β -cypermethrin an 3-phenoxybenzaldehyde contaminated soils using *Streptomyces aureus* HP-S-01. *Appl Microbiol Biotechnol* 94:505–515
- Chen S, Luo J, Hu M, Lai K, Geng P, Huang H (2012b) Enhancement of cypermethrin degradation by a coculture of *Bacillus cereus* ZH-3 and *Streptomyces aureus* HP-S-01. *Bioresour Technol* 110:97–104
- Comeau Y, Greer CW, Samson R (1993) Role of inoculum preparation and density on the bioremediation of 2,4-D contaminated soil by bioaugmentation. *Appl Microbiol Technol* 38:681–687
- Cycoń M, Piotrowska-Seget Z, Kaczyńska A, Kozdrój J (2006) Microbiological characteristics of a loamy sand soil exposed to tebuconazole and λ -cyhalothrin under laboratory conditions. *Ecotoxicology* 15:639–646
- Cycoń M, Wójcik M, Piotrowska-Seget Z (2009) Biodegradation of the organophosphorus insecticide diazinon by *Serratia* sp. and *Pseudomonas* sp. and their use in bioremediation of contaminated soil. *Chemosphere* 76:494–501
- Cycoń M, Piotrowska-Seget Z, Kozdrój J (2010) Responses of indigenous microorganisms to a fungicidal mixture of mancozeb and dimethomorph added to sandy soils. *Int Biodeterior Biodegrad* 64:316–323
- Cycoń M, Wójcik M, Piotrowska-Seget Z (2011) Biodegradation kinetics of the benzimidazole fungicide thiophanate-methyl by bacteria isolated from loamy sand soil. *Biodegradation* 22:573–583
- Cycoń M, Wójcik M, Borymski S, Piotrowska-Seget Z (2012) A broad-spectrum analysis of the effects of teflubenzuron exposure on the biochemical activities and microbial community structure of soil. *J Environ Manage* 108:27–35
- Elliott M (1989) The pyrethroids: early discovery, recent advances and the future. *Pest Sci* 27:337–351
- Fenlon KA, Andreou K, Jones KC, Semole KT (2011) The extractability and mineralisation of cypermethrin aged in four UK soils. *Chemosphere* 82:187–192
- Fulekar MH (2009) Bioremediation of fenvalerate by *Pseudomonas aeruginosa* in a scale up bioreactor. *Rom Biotechnol Lett* 14:4900–4905
- Germida JJ, Siciliano SD (2001) Taxonomic diversity of bacteria associated with the roots of modern, recent and ancient wheat cultivars. *Biol Fertil Soils* 33:410–415
- Grant RJ, Betts WB (2004) Mineral and carbon usage of two synthetic pyrethroid degrading bacterial isolates. *J Appl Microbiol* 97:656–662
- Grant RJ, Daniell TJ, Betts WB (2002) Isolation and identification of synthetic pyrethroid-degrading bacteria. *J Appl Microbiol* 92:534–540
- Gu X-z, Zhang G-y, Chen L, Dai R-l, Yu Y-c (2008) Persistence and dissipation of synthetic pyrethroid pesticides in red soils from the Yangtze River Delta area. *Environ Geochem Health* 30:67–77
- Guo P, Wang B, Hang B, Li L, Ali SW, He J, Li S (2009) Pyrethroid-degrading *Sphingobium* sp. JZ-2 and the purification and characterization of a novel pyrethroid hydrolase. *Int Biodeterior Biodegrad* 63:1107–1112
- Hintzen EP, Lydy MJ, Belden JB (2009) Occurrence and potential toxicity of pyrethroids and other insecticides in bed sediments of urban stream in central Texas. *Environ Pollut* 157:110–116
- Hong Q, Zhang Z, Hong Y, Lia S (2007) A microcosm study on bioremediation of fenitrothion-contaminated soil using *Burkholderia* sp. FDS-1. *Int Biodeterior Biodegrad* 59:55–61
- Ismail BS, Mazlinda M, Zuriati Z (2012) Effects of temperature, soil moisture content and soil type on the degradation of cypermethrin in two types of Malaysian agricultural soils. *World Appl Sci J* 17:428–432
- Jilani S, Khan MA (2006) Biodegradation of cypermethrin by pseudomonas in a batch activated sludge process. *Int J Environ Sci Tech* 3:371–380
- Kaufman DD, Kayser AJ (1980) Degradation of 14C-cyano-, phenoxy-, and 14C-vinyl-decamethrin in flooded soil. Report USDA-III-12.05.80/A. WHO
- Khan SU, Behki RM, Tapping RI, Akhtar MH (1988) Deltamethrin residues in an organic soil under laboratory conditions and its degradation by a bacterial strain. *J Agric Food Chem* 36:636–638
- Lakshmi CV, Kumar M, Khanna S (2008) Biotransformation of chlorpyrifos and bioremediation of contaminated soil. *Int Biodeterior Biodegrad* 62:204–209
- Lane DJ (1991) 16S/23S rRNA sequencing. Wiley, Chichester, pp 177–203
- Meecker JD, Barr DB, Hauser R (2009) Pyrethroid insecticide metabolites are associated with sperm hormone levels in adult men. *Reprod Toxicol* 27:155–160
- Mrozik A, Cycoń M, Piotrowska-Seget Z (2010) Changes in FAMES profiles of *Pseudomonas* sp. CF600 as a marker of phenol degradation in different soils. *Int Biodeterior Biodegrad* 64:86–96
- Munoz-Leoz B, Garbisu C, Antiguada I, Alonso ML, Alonso RM, Ruiz-Romera E (2009) Deltamethrin degradation and soil microbial activity in a riparian wetland soil. *Soil Sci* 174:220–228
- Oudou HC, Hansen HCB (2002) Sorption of lambda-cyhalothrin, cypermethrin, deltamethrin and fenvalerate to quartz, corundum, kaolinite and montmorillonite. *Chemosphere* 49:1285–1294
- Rangaswamy V, Venkateswarlu K (1993) Ammonification and nitrification in soil, and nitrogen fixation by *Azospirillum* sp. as influenced by cypermethrin and fenvalerate. *Agric Ecosyst Environ* 45:311–317
- Tallur PN, Megadi VB, Ninnekar HZ (2008) Biodegradation of cypermethrin by *Micrococcus* sp. strain CPN 1. *Biodegradation* 19:77–82
- Vig K, Singh DK, Agarwal HC, Dhawan AK, Dureja P (2008) Soil microorganisms in cotton fields sequentially treated with insecticides. *Ecotoxicol Environ Saf* 69:263–276
- WHO (1990) Environmental Health Criteria 97—Deltamethrin. International programme on chemical safety. World Health Organization, Geneva, Switzerland, pp 1–133
- Widenfalk A, Svensson JM, Goedkoop W (2004) Effects of the pesticides captan, deltamethrin, isoproturon, and pirimicarb on the microbial community of a freshwater sediment. *Environ Toxicol Chem* 23:1920–1927
- Xia WJ, Zhou JM, Wang H, Chen XQ (2008) Effect of nitrogen on the degradation of cypermethrin and its metabolite 3-phenoxybenzoic acid in soil. *Pedosphere* 18:638–644
- Xu YX, Sun JQ, Li XH, Li SP, Chen Y (2007) Study on cooperating degradation of cypermethrin and 3-phenoxybenzoic acid by two bacteria strains. *Acta Microbiol Sin* 47:834–837
- You J, Brennan A, Lydy MJ (2009) Bioavailability and biotransformation of sediment-associated pyrethroid insecticides in *Lumbricus variegatus*. *Chemosphere* 75:1477–1482
- Zhang LZ, Khan SU, Akhtar MH, Ivarson KC (1984) Persistence, degradation, and distribution of deltamethrin in an organic soil under laboratory conditions. *J Agric Food Chem* 32:1207–1211
- Zhang B, Bai Z, Hoefel D, Tang L, Wang X, Li B, Li Z, Zhuang G (2009) The impacts of cypermethrin pesticide application on the non-target microbial community of the pepper plant phyllosphere. *Sci Total Environ* 407:1915–1922
- Zhang C, Jia L, Wang S, Qu J, Li K, Xu L, Shi Y, Yan Y (2010) Biodegradation of beta- cypermethrin by two *Serratia* spp. with different cell surface hydrophobicity. *Bioresour Technol* 101:3423–3429