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Research Article

Isolation and identification of the pyrethroid insecticide deltamethrin degrading bacteria from insects

Özlem Gür Özdal[1*], Ömer Faruk Algur[2]

[1] Science Faculty, Department of Biology, Ataturk University, Erzurum, Turkey (ORCID: 0000-0003-3460-3638)
[2] Science Faculty, Department of Biology, Ataturk University, Erzurum, Turkey (ORCID: 0000-0001-6447-3454)

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Abstract

Many studies have showed that the pesticide residues in the environment increase day by day because of their continuous use. Pesticides can degrade chemically, physically and biologically. Biodegradation is an eco-friendly, inexpensive and highly effective approach compared to other methods. Bacteria are the most commonly used biological agents in biodegradation studies. Widespread use of pyrethroid pesticides such as deltamethrin causes pollution of environment. A total of 14 bacterial isolates were isolated from insects (*Poecilimon tauricola*, *Locusta migratoria*, *Gryllus bimaculatus* and *Forficula auricularia*) living in pesticide contaminated environments. These bacterial isolates were identified and characterized as *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Bacillus atrophaeus*, *Acinetobacter lwoffii*, *Rhodococcus coprophilus*, *Brevundimonas vesicularis*, *Pseudomonas syringae*, *Yersinia frederiksenii*, *Bacillus licheniformis*, *Enterobacter intermedius* and *Serratia marcescens* based on biochemical and morphological properties and fatty acid profiles. As a result, these bacterial isolates can be used for the remove of deltamethrin at various environments.

Keywords: Bacteria, Biodegradation, Deltamethrin, Isolation

Böceklerden Piretroid İnsektisit Deltametrin Yıkıcı Bakterilerin İzolasyonu ve Tanımlanması

Öz

Birçok çalışma, sürekli kullanımları nedeniyle ortamdaki pestisit kalıntılarının her geçen gün arttığını göstermiştir. Pestisitler kimyasal, fiziksel ve biyolojik olarak parçanabilirler. Biyodegradasyon, diğer yöntemlere kıyasla çevre dostu, ucuz ve oldukça etkili bir yaklaşımdır. Biyodegradasyon çalışmalarında bakteriler en sık kullanılan biyolojik ajanlardır. Deltametrin gibi piretroid pestisitlerin yaygın kullanımı çevrenin kirlenmesine neden olmaktadır. Pestisit kontamine ortamlarda yaşayan böceklerden (*Poecilimon tauricola*, *Locusta migratoria*, *Gryllus bimaculatus* ve *Forficula auricularia*) toplam 14 bakteri izolatı izole edilmiştir. Bu bakteri izolatları, biyokimyasal ve morfolojik özellikleri ve yağ asidi profillerine dayanarak *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Bacillus atrophaeus*, *Acinetobacter lwoffii*, *Rhodococcus coprophilus*, *Brevundimonas vesicularis*, *Pseudomonas syringae*, *Yersinia frederiksenii*, *Bacillus licheniformis*, *Enterobacter intermedius* ve *Serratia marcescens* olarak tanımlanmış ve karakterize edilmiştir. Sonuç olarak, bu bakteri izolatları çeşitli ortamlarda deltametrinin parçaalanması için kullanılabilir.

Anahtar Kelimeler: Bakteriler, Biyodegradasyon, Deltametrin, İzolasyon

[*] Sorumlu Yazar: Ataturk University, Department of Biology, Erzurum, Turkey, ORCID: 0000-0003-3460-3638, ozlemgur55@gmail.com

1. Introduction

Natural or synthetic pesticides (organochlorine, organophosphate, carbamate, pyrethroids) are widely used to control unwanted pests. Pyrethroids account for about one-fifth of the global agrochemical market. Pyrethroids have potent neurotoxic activity against insects and low toxicity to animals. With the permanent use of pyrethroid worldwide, its residue has become a problem to animals, including humans**.** Pyrethroid insecticides, e.g., cyphenothrin, fenvalerate, esfenvalerate, deltamethrin, cypermethrin, cyhalothrin, fluvarinate, tralomethrin, cycloprothrin, acrinathrinallethrin, imiprothrin, permethrin and fenpropathrin are used in agriculture, animal health, home, and garden pest control throughout the world (Cycoń and Piotrowska-Seget, 2016; Zhang et al., 2016; Hao et al., 2018).

Pesticides are degraded into simpler and often less toxic chemicals in various ways such as chemical reactions, photodegradation and biodegradation. Biodegradation is an environment friendly, cheap and high efficiency approach compared to other methods. Bacteria and fungi with high enzyme (transferases, isomerases, ligases and hydrolases especially esterases, peroxidases and oxygenases) activity are used in biodegradation studies (Ortiz-Hernández et al., 2013; Ozdal et al., 2017).

Deltamethrin ($C_{22}H_{19}Br_2NO_3$) is a broad-spectrum insecticide belonging to pyrethroids (Figure 1). Deltamethrin is widely used in agriculture because of its low cost, persistence, stability and low toxicity to mammals. It is used for the control of pests such as mosquitoes, cockroaches, flies, ants and fleas due to effective at very low concentrations (Hao et al., 2018; Lu et al., 2019)

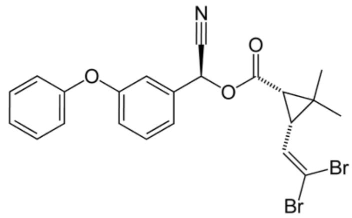

Figure 1. Chemical structure of deltamethrin

Microflora in the digestive tract of insect species is being investigated. The nutrient-rich digestive tract of insects is an appropriate growth environment for these microorganisms. The bacterial flora in the digestive tract of the insect has a very variable and broad enzymatic potential. Insect gut bacterial isolates have been demonstrated to break down many compounds such as pesticide (Ozdal et al., 2016a, b). Insect intestines provide a suitable medium for gene transfer between bacteria. Microorganisms can adapt to new environments by acquiring different features with horizontal gene transfer, conjugative plasmid and simple mutations to different environmental conditions (Pietri et al., 2018; Ramakrishnan et al., 2019). In this context, it is highly possible to isolate pesticide resistant microorganisms from insect intestines.

In many insect groups, resistance to pesticides occurs as a result of the use of pesticides. The intestinal flora of insects, which are observed to be resistant to pesticides, is very rich in bacteria that can be used in the biodegradation of pesticides. The purpose of this study was to isolate the bacteria capable of degrading deltamethrin from different insects.

2. Materials and Methods

2.1. Chemicals

Deltamethrin and other chemicals used in the study were of analytical purity and were obtained from Sigma and the media were obtained from Merck and Difco.

2.2. Preparation of media and solutions used in the study

Carbon-free mineral salt medium (MSM) was used for isolation of deltamethrin degrading bacteria. The medium contained 2.0 g of $(NH_4)_2SO_4$, 0.2 g of $MgSO_4$ $7H_2O$, 0.01 g of $CaCl_2$ $2H_2O$, 0.001 g of $FeSO_4$ $7H_2O$, 1.5 g of Na_2H-PO_4 $12H_2O$, and 1.5 g of KH_2PO_4 per litre of deionized water (Cycoń et al., 2014). The final pH value was adjusted to 7.2. After autoclaving (121 °C, 15 min) and cooling, the medium was supplemented with 100 mg/L deltamethrin.

2.3. Insects used in the study

The insects belonging to Orthoptera and Dermaptera were collected from different regions during the spring-summer period and species identification Prof. Dr. Orhan Erman. Bacteria that can use deltamethrin as a carbon source were isolated from insects.

2.4. Isolation of Deltamethrin Degrading Bacteria

The insect samples were subjected to surface sterilization with 70% ethyl alcohol for 3 minutes and after alcohol removal with sterile physiological water (SFS), homogenized by crushing in a sterile mortar with SFS (Okay et al., 2013). Serial dilutions of homogenate were prepared and 0.1 mL of liquid was inoculated to liquid minimal medium containing 100 mg/L of deltamethrin. After one week, 1 ml of each culture was re-inoculated into new deltamethrin-MSM medium and further incubated at 30°C and 150 rpm for 7 days. This subculture was repeated under the same culture conditions, and then an aliquot (0.2 ml) from each culture was applied to solid deltamethrin-MSM for isolation of single colonies. Colonies of different character were isolated by transferring to Tryptic Soy Agar plates and stored on slant agar at + 4 ° C.

2.5. Identification of isolates

Deltamethrin degrading bacteria were identified using morphological, cultural, biochemical properties (Gram, cell shape, endospores, movement, catalase, oxidase) (Harley and Prescott, 2002) and fatty acid profiles (Kotan et al., 2006).

3. Results and Discussion

There is a close relationship between bacteria and other living things. Therefore, insect microflora enables us to find new and biotechnological microorganisms. *Serratia marcescens* MO-1 isolated from grasshopper (*Poecilimon tauricola*) has both chitinase activity (Okay et al., 2013) and the ability to produce prodigiosin pigment (Kurbanoglu et al., 2015) which has antimicrobial and anticancer properties. Also, *Pseudomonas aeruginosa* OG1 isolated from cockroaches (*Blatta orientalis*) can produce pyocyanin, a pigment of biotechnological importance (Ozdal, 2019). Insects can change their ecological and physiological properties thanks to symbiotic bacteria (Pietri and Liang, 2018). Kikuchi et al., (2012) indicated that bacteria of the genus *Burkholderia* develop resistance against the fenitrothion (organophosphate pesticide) in the bean bug (*Riptortus pedestris*). Chlorpyrifos and fipronil resistant strains of diamond back moth (*Plutella xylostella*) have higher levels of Lactobacillales, Pseudomonadales and Xanthomonadales compared to susceptible insects (Xia et al., 2013). *Stenotrophomonas maltophilia* OG-2, isolated from the intestine of the cockroach (*Blatta orientalis*), can degrade both α-endosulfan (Ozdal et al., 2017) and synthetic pyrethroid α-cypermethrin (Gur et al., 2014).

In this study, insects belonging to Orthoptera and Dermaptera were collected from the areas where insecticides were used (Table 1). As a result of isolations, 14 bacteria were isolated on solid medium containing deltamethrin. Table 1 lists the insect species and isolate groups from which the isolates were obtained.

Table 1 Insect species from which isolates were obtained

Strain Group	Insect Name	Place of collection	Family	Order
DPT	*Poecilimon tauricola* (Ramme 1951)	Erzurum	Tettigoniidae	Orthoptera
DLM	*Locusta migratoria* (Linnaeus 1758)	İzmir	Acrididae	Orthoptera
DGB	*Gryllus bimaculatus* (De Geer 1773)	Antalya	Gryllidae	Orthoptera
DFA	*Forficula auricularia* (Linnaeus 1758)	Samsun	Forficulidae	Dermaptera

A total of 14 bacterial isolates were isolated from medium containing deltamethrin based on visible colony differences. Among all these 14 deltamethrin degrading bacterial isolates, 3 were Gram-positive rods and 11 were Gram-negative rods. Total 2 isolates indicated positive results for endospore. All the isolates were catalase positive. Of these isolates, 3 were oxidase positive, 10 were motile and 3 were urease positive (Table 2).

Table 2 Cultural, morphological and biochemical properties of isolates

Isolate code	Gram	Cell shape	Endospor	Motile	Catalase	Oxidase	Urease
DGB1	-	Rod	-	+	+	+	-
DLM1	+	Rod	-	-	+	-	+
DPT1	+	Rod	+	+	+	-	-
DPT2	-	Rod	-	+	+	-	-
DLM2	-	Rod	-	-	+	-	+
DFA1	+	Rod	+	+	+	-	-
DPT3	-	Rod	-	+	+	-	-
DPT4	-	Rod	-	+	+	-	-
DPT5	-	Rod	-	+	+	+	-
DLM3	-	Rod	-	+	+	-	+
DFA2	-	Rod	-	+	+	-	-
DGB2	-	Rod	-	+	+	+	-
DGB3	-	Rod	-	-	+	-	-
DLM4	-	Rod	-	-	+	-	-

Table 3 Fatty acid profiles of bacteria isolated from insects

Fatty acids	**Bacteria**													
	DGB1	**DGB2**	**DGB3**	**DFA1**	**DFA2**	**DPT1**	**DPT2**	**DPT3**	**DPT4**	**DPT5**	**DLM1**	**DLM2**	**DLM3**	**DLM4**
10:0		0.32			0.62				0.30	0.26		0.47	0.21	
10:0 3OH		3.14			0.16		3.3		10.12	3.18			2.2	
12:0		4.3	6.37		2.67		4.9	5.7	1.13	4.2		6.4	5.09	9.8
12:0 2OH		4.9					4.7	0.4	0.42	4.7			4.29	
12:0 3OH	2.4		4.92		2.82		4.3		2.3			0.4		3.2
13:0 iso					0.41									
14:0 iso				1.1	0.67	1.1								
14:0	3.05	0.63			3.80			6.3	4.7	0.58	1.9	1.7	0.65	
14:0 3ÔH								5.57						
15:0 iso				19.1	33.67	12.47								
15:0 Antesio				40.8	12.10	50.34								
15:0	3.38							2.1			2.8	0.2		
16:0 iso				4.7	1.10	3.3								
16:1 w7c								29.76	12.1		6.2			
16:1 w9c					3.58									
16:1 w11c				2.2										
16:0	20.7	24.8	12.5	5.1	9.05	2.74	26.4	29.99	27.3	25.2	23.2	37.2	25.66	19.44
16:0 10-methyl											14.4			
17:1 w8c	2.2		0.93											
17:0 cyclo		0.85					2.3	3.2	13.7	1		2	0.48	
17:0	1.1	0.13						1.9	0.44	0.15	1.9	0.1		
17:1 iso w10c														
17:0 iso				5.9	3.11	5.7	0.3					0.1		
17:0 antesio				15.8	0.41	16.1								
17:0 10-methyl											2.4			
17:0 w Cyclo 7-8												29.1		
18:1 w7c	52.7	42.4	5.85		0.84		13.7	11.7	14.2	41.3		2.1	44.14	3.45
18:1 w9c			37.21		1.62						2.3			21.67
18:0	0.4	0.38	2.33		0.22		2.3	0.46	0.3	0.44		1.1	0.64	
18:0 10-methyl											6.7			
19:0												0.7		
16:1 w7c/15:0 iso 2OH	6.8	16.66	26.45		10.96		30.6			17.43		3.6	18.29	39.6
17:1 w8c	2.2	0.22								0.20	0.8			
14:0 3OH/16:1 iso												9.4		

According to the results of MIS analysis, fatty acid profiles of the isolates are summarized in Table 3. As a result of MIS analysis, 8 different genera and 13 different species of bacterial isolates were identified. Isolates based on morphological, biochemical and fatty acid data were identified as *Acinetobacter lwoffii* (DLM4), *Pseudomonas aeruginosa* (DPT5, DBG2), *Stenotrophomonas maltophilia* (DFA2), *Bacillus licheniformis* (DFA1), DLM1), *Bacillus atrophaeus* (DPT1), *Pseudomonas syringae* (DPT2), *Yersinia frederiksenii* (DLM2), *Enterobacter intermedius* (DPT3), *Serratia marcescens* (DPT4) and *Flavimonas oryzihabitans* (DLM3). The genera of the isolated bacteria were mainly identified as *Pseudomonas* and *Bacillus*. The highest bacterial diversity was observed in *Poecilimon tauricola* (5) and *Locusta migratoria* (4).

Table 4 Deltamethrin degrading microorganisms isolated from different environments

Strain	**Source**	**Reference**
Streptomyces aureus HP-S-01	Activated sludge	Chen et al., 2011
Bacillus cereus Y1	Deltametrin contaninated soil	Zhang et al., 2016
Lysinibacillus fusiformis ZJ6	Soil	Hao et al., 2018
Acinetobacter calcoaceticus MCm5	Pyrethroid contaninated soil	Akbar et al., 2015a
Brevibacillus parabrevis FCm9		
Sphingomonas sp. RCm6		
Bacillus megaterium JCm2	Pyrethroid contaninated soil	Akbar et al., 2015b
Rhodococcus sp. JCm5		
Ochrobactrum anthropi JCm1		
Pseudomonas aeruginosa JQ-41	Pyrethroid contaninated soil	Song et al., 2015
Serratia marcescens DeI-1, DeI-2	Deltamethrin treated soil	Cycoń et al., 2014
Acinetobacter baumannii ZH-14	Sewage sludge	Zhan et al., 2018

Many different bacteria have been isolated and characterized with their ability to degradation various pesticides. In previous studies, bacteria capable of degrading deltamethrin were mostly isolated from agricultural areas where intensive pesticides were used. However, the potential of these microorganisms to degrade deltamethrin has been confirmed for some bacteria of the genera *Acinetobacter*, *Bacillus*, *Brevibacillus*, *Pseudomonas*, *Serratia*, *Rhodococcus* (Table 4). Song et al., (2015) studied the deltamethrin biodegradation with *Pseudomonas aeruginosa* JQ-41 strain isolated from the pyrethroid contaminated soil. In another study, *Acinetobacter calcoaceticus* MCm5 was used in biodegradation of deltamethrin (Akbar et al., 2015a). Similar bacteria were isolated in this study. When Table 4 is analyzed, it is seen that the bacteria used in deltamethrin degradation are generally isolated from soil and sludge. All bacteria obtained from this study were isolated from insect flora. In addition, it has been determined that new species may be effective in deltamethrin biodegradation.

The bacteria isolated in this study can undoubtedly be used in biodegradation studies. As seen in Table 5, different strains of the species isolated in this study have been reported to have been used for the degradation of many different pesticides. It has been determined that insects are important source for the isolation of bacteria that break down pesticides.

Table 5 Comparison of the use of the isolated species in the degradation of different pesticides

Bacteria	Pesticide	References
Acinetobacter lwoffii	Endosulfan	Ozdal et al., 2016b
	Atrazine	Yang et al., 2017
Pseudomonas aeruginosa	Endosulfan	Ozdal et al., 2016b
	Fenvalerate	Fulekar, 2009
	Acephate, dimethoate, parathion, chlorpyrifos, malathion	Ramu and Seetharaman, 2014
	chlorpyrifos and dichlorvos	Gaonkar et al., 2019
Stenotrophomonas maltophilia	α-endosulfan, α-cypermethrin	Gur et al., 2014; Ozdal et al., 2017
	Diazinon	Pourbabaee et al., 2018
Bacillus licheniformis	Fenvalerate	Tang et al., 2018
	β-cyclodextrin, β-cypermethrin	Zhao et al., 2015
Bacillus atrophaeus	α-Endosulfan	Ozdal et al., 2016b
Pseudomonas syringae		
Yersinia frederiksenii	Permethrin	Lee et al., 2004
Enterobacter intermedius		
Serratia marcescens	DDT	Neerja, Grewal et al., 2016
Flavimonas oryzihabitans	DDT	Barragan-Huerta et al., 2007

4. Conlusion

Strains of *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Bacillus atrophaeus*, *Acinetobacter lwoffii*, *Rhodococcus coprophilus*, *Brevundimonas vesicularis*, *Pseudomonas syringae*, *Yersinia frederiksenii*, *Bacillus licheniformis*, *Enterobacter intermedius* and *Serratia marcescens*, able to use deltamethrin as the only carbon source, were isolated from *Poecilimon tauricola*, *Locusta migratoria*, *Gryllus bimaculatus* and *Forficula auricularia*. Pesticide resistant insect microbiota has been shown to be a rich source for isolation of microbes that can degradation pesticides and a promising tool for biotechnological discovery in bioemediation programs. In order to find new biocatalysts in the degradation of pesticides, isolation can be made from insects that can live in pesticide environments. As a result, it can be said that isolated deltamethrin degrading microorganisms can be used in the treatment studies in the dirty areas of this insecticide. However, optimization studies are also needed to make biodegradation highly efficient and feasible.

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