



Possibility of Using Bacteria-Destructors for Cypermethrin Degradation

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

From soils artificially contaminated with cypermethrin, 3 isolates were obtained that were resistant to cypermethrin concentrations of 40 mg/kg soil, and their morphological, cultural and biochemical properties were studied. On the basis of the obtained strains, a bacterial consortium was developed, which consists of the cultures of *Pseudomonas* sp., *Bacillus* sp. and *Ochrobactrum* sp. Laboratory experiments on the decomposition of cypermethrin showed the effectiveness of this consortium, within 28 days the decomposition of cypermethrin (40 mg/kg) was 100%.

Keywords: *Biodegradation; bacterial consortium; pseudomonas; ochrobactrum; Bacillus; pesticides; cypermethrin.*

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1. INTRODUCTION

Contamination of agricultural soils with pesticides is a serious environmental problem. Despite the fact that recently new generations of pesticides, which are less toxic and decompose faster, have begun to be used in most cases, the problem remains open. One of the new generation pesticides is cypermethrin [α -Cyano-3 phenoxybenzyl ester of 3- (2,2-dichloro-vinyl) - 2,2 dimethylcyclopropanecarboxylic acid], a synthetic pyrethroid insecticide. Pyrethroids are insecticides of a wide spectrum of action [1] and affect the immune, nervous, gastrointestinal systems and also cause hematological effects [2]. In nature, chrysanthemum flowers are a natural source of pyrethroid insecticides [1,3,4,5]. Excessive and widespread use [6,7] as well as high doses of pyrethroids suppress the functioning of the chloride ion channel driven by gamma-aminobutyric acid (GABA) [8]. Bacteria and fungi have a high potential for biodegradation of a wide range of pyrethroids [5,9,10,11,12]. Cypermethrin is a type II peritroid [1,13,14]; it was first synthesized in 1974 [15]. In comparison with type I peritroids, cypermethrin contains a cyano group, it is this group that enhances their insecticidal properties [13,14]. It is used in agriculture and private household plots for the control of harmful insects, as well as in the practice of medical, sanitary and household disinfection to control harmful and synanthropic insects, including for the control of ants and cockroaches [16,17]. Cypermethrin, like other pesticides, has a toxic effect on mammals [18]. In many people, it causes allergic skin reactions and eye irritation [19,20,21], as well as disrupts the endocrine system [22]. Intensive use of cypermethrin can cause environmental damage and adverse effects on human health and the environment [23,24,25]. The most effective way to reduce the amount of xenobiotics, including cypermethrin, is currently microbial degradation [18]. There is evidence that some microorganisms belonging to pp. *Pseudomonas* [26,27,28], *Micrococcus* [29], *Serratia* [21,27], *Streptomyces* [16] and *Ochrobactrum* [18] degrade cypermethrin. The most effective use of microbial degradation involves the use of not one species, but consortia consisting of various microorganisms [30,31]. Currently, there are several methods for obtaining bacteria-destroyers of xenobiotics, among which the most common are the method of enrichment culture and genetic methods. Almost all known bacteria-destroyers are isolated by means of

enrichment cultures from cultivated soils, sewage and activated sludge.

2. MATERIALS AND METHODS

2.1 Characteristics of Soils

In the work, we used the gray soil of a farm field, on which pesticides (cypermethrin, chlorpyrifos, etc.) were treated for many years. Soil samples were taken from a depth of about 0-15 cm. Soil samples were preliminarily cleaned of large inclusions, sifted through a stainless steel sieve with a diameter of 2 mm and dried under laboratory conditions. Physicochemical characteristics are presented in Table 1.

2.2 Chemicals and Media

Cypermethrin standard (97% purity), chromatographic grade acetone and all other chemicals and reagents used were analytical grade and commercially available. Stock solutions (20 mg / kg; 40 mg / kg;) were prepared with acetone. To improve the dissolution of cypermethrin in a liquid medium, emulsifiers (Tween - 80) were added. We used a Metler toledo pH meter. For growing microorganisms used nutrient media MPA, MPB (HiMedia Pvt Ltd Mumbai, India), Mineral salt medium (MSM) (pH 6.8-7.0), containing (g / l): K_2HPO_4 -1.5; KH_2PO_4 -0.5; NaCl 0.5; $(NH_4)_2SO_4$ 0.5; $MgSO_4$.

7 H_2O - 0.2 and 1 ml of a solution of trace elements. The solution of trace elements consisted of (g / l): H_3BO_3 - 5.0; $Na_2MoO_4 \times 2H_2O$ -5.0; $MnSO_4 \times 4H_2O$ 3.0; KI - 0.5; NaBr - 0.5; $ZnSO_4 \times 7H_2O$ - 0.2; $Al_2(SO_4)_3 \times 18H_2O$ - 0.3.

2.3 Isolation of Bacteria Resistant to Cypermethrin

Soil samples (1 kg) were additionally contaminated with cypermethrin and left for 1 month at 30°C. After a month, 10 g of soil was introduced into Erlenmeyer flasks (250 ml) containing nutrient broth with the addition of cypermethrin and incubated for 48 hours on a rotary shaker at 30°C and a rotation speed of 150 rpm. On the 5th day, the dilutions were added to plates with nutrient agar containing cypermethrin (10 mg / L). The grown individual colonies were subcultured in plates with mineral salt medium supplemented with higher concentrations of cypermethrin.

Table 1. Physical and chemical characteristics of soil

Humus	N (NO ³⁻)	N (NH ₄ ⁺)	P ₂ O ₅	Cl ⁻	SO ₄ ²⁻	pH	Humidity
1,3	38,310	2,211	22,454	137,731	200,231	7,56	13,6

2.4 Identification of Isolates

The obtained isolates were identified on the basis of morphological-cultural and physiological-biochemical properties.

2.5 Getting a Consortium for the Degradation of Cypermethrin

A bacterial consortium of microorganisms that degrades cypermethrin was developed using of selected isolates. Culture isolates were mixed in equal proportions to obtain the final suspension and 1 ml and added to sterile MCM medium containing cypermethrin and incubated.

2.6 Method of Gas Chromatographic Analysis

The study was performed on Agilent 8890B Split / Splitless Gas Chromatograph used with Agilent 5977B Series GC / MSD in SIM, SCAN, and Electron Impact (EI) Ionization modes. The following has been given conditions for the analysis:

Gas chromatograph analysis parameters. Analytical column HP-5ms Ultra Inert 30 mx 250 µm x 0.25 µm Injection volume 1 µL, Injection mode Split-free Evaporator temperature 280°C UI Liner, splitless, single taper, fiberglass Spray gasket Gold plated, Ultra Inert with washer Carrier gas: Hydrogen, constant flow=1.2 ml / min Thermostat program 60°C for 1 minute, then 40°C / min to 170°C, then 10°C / min to 310°C, then hold for 2 minutes The temperature in the transport line is 280°C. MS conditions: Delay to eliminate solvent effects 3.5 minutes, Data collection mode SIM, SCAN, Amplification factor 1.00, Source temperature 250°C, Quadrupole temperature 150°C.

2.7 Biodegradation of Pesticides in Soil

Studies on the degradation of cypermethrin by a bacterial consortium were carried out on sterile soil. The soil was sterilized by autoclaving for 30 min at 121°C. Cypermethrin (solution in acetone) was added to the soil samples (100 g) to a final concentration of 40 mg / kg. The initially prepared solution was added to a small part (15-20 g) of soil, which was then mixed with the

remaining amount of soil after evaporation of the solvent. Soil samples were inoculated with a consortium of microorganisms and incubated at 30°C for 28 days. The tests were carried out in triplicate. Uninoculated sterile soil with cypermethrin served as a control.

2.8 Calculation of Biodegradation

The decomposition of cypermethrin was calculated using the following formula [32]:

$$X\% = \frac{C_{ck}-C_x}{C_{ck}} \times 100$$

where: X is the decomposition of the pesticide; C_x - pesticide concentration (mg / kg) in the medium with microorganisms that decompose cypermethrin; C_c is the concentration of cypermethrin (mg / kg) in a medium that does not contain microorganisms.

3. RESULTS AND DISCUSSION

During the research, more than 17 isolates were isolated, capable of growing on a medium with 10 mg / kg of cypermethrin. By the method of a gradual increase in concentration, three most effective isolates (№. 3, 6, and 11) were selected for further studies, capable of growing at a cypermethrin concentration of up to 40 mg / kg of soil. Based on the study of morphological-cultural and physiological-biochemical properties, these isolates were identified up to the genus: no. 3 - *Ocrobactrum sp.*, №. 6 - *Pseudomonas sp.* and №. 11-*Bacillus sp.* (Table 2).

The *Ocrobactrum sp.* - single movable sticks with rounded ends. Cells are approximately 0.6-1.2 to 2 µm in length, gram-negative, non-spore-forming, aerobic. Catalase-positive, oxidase-negative, indole, hydrogen sulfide do not form; does not thin gelatin; milk does not change, alkalizes; does not restore nitrates. Assimilates from carbon sources: glucose, sucrose, galactose, mannose, raffinose, starch, sorbitol, glycerin. On MPA forms white smooth shiny colonies, uniformly rising profile, smooth edge, diameter 2.0-2.5 mm. Capable of oxidizing petroleum hydrocarbons. It grows within the temperature range of 20-30 ° C, at a pH of 6.8-7.2. Non-pathogenic (Fig. 1).

Table 2. Some morpho-cultural and physiological-biochemical properties of the isolated cultures

Signs	Strain		
	<i>Ochrobactrum sp. 3</i>	<i>Pseudomonas sp.6</i>	<i>Bacillus sp. 11</i>
Morphology	round, smooth, shiny	rounded, slightly	smooth, convex, shiny
Colonies on MPA medium	convex, opaque white colonies, d = 2.0-2.5 mm	convex, smooth, shiny, with a smooth edge, d = 2-2.6 mm	colonies, white-gray, d = 3-4 mm
Gram stain	Negative	Negative	Positive
Dispute formation	-	-	+
Relationship to oxygen	Aerobe	Aerobe	Aerobe
Temperature, ° C	30-37	30-37	30-37
pH	6,8-7,2	6,8-7,0	6,8-7,5
Oxidase activity	negative	positive	positive
Catalase activity	positive	positive	positive
Hydrolysis of gelatin	-	-	+

Note: "-" - presence / absence of a feature

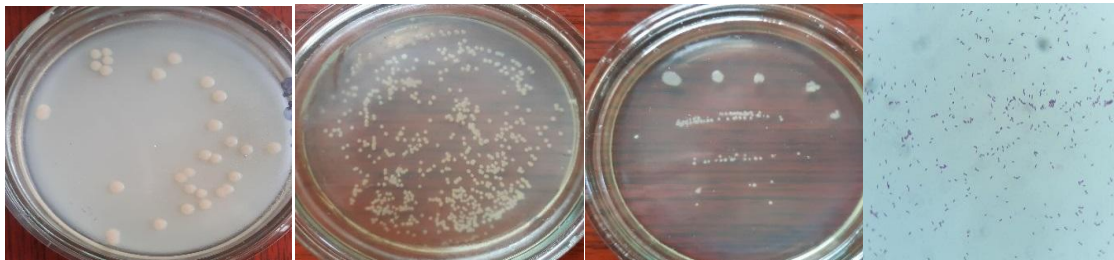


Fig. 1. Growth on MSM medium with cypermethrin (1) and Gram staining of daily culture (2) (magnification x1000)

The *Bacillus sp.* - cells are rod-shaped, size 2-3x0.5-0.6 microns, single or connected in chains, form spores, gram-positive, aerobic. On MPA forms dense, whitish-gray, round, small, wrinkled colonies. Optimum growth at pH 6.8-7.5. A film and sediment are formed in the nutrient broth (Fig. 2).

The *Pseudomonas sp.* - cells are rod-shaped, 1-1.2x0.5 microns in size, single or connected in short chains, gram-negative. Do not form a dispute. On MPA it forms rounded colonies, 2-6 mm in diameter, slightly convex, smooth, shiny, with an even edge; they do not form pigment. pH 6.8-7.0.

Based on these three strains, a cypermethrin-degrading bacterial consortium was developed.

3.1 Biodegradation of Pesticides in Soil

In the process of biodegradation of pesticides, destructive microorganisms use the substrate as the only source of energy. Due to the biochemical potential of microorganisms, the

original pesticide molecule is degraded to simpler compounds. Laboratory experiments on the biodegradation of cypermethrin were carried out for 28 days on sterile soils with the addition of both a bacterial consortium and separately one strain of *Ochrobactrum sp.*

To maintain the moisture content (60%) every 3-4 days the test soil samples were irrigated with sterile distilled water. Soil samples were taken every week for chromatographic analysis. The results of chromatographic analyzes show that the concentration of the pesticide in the variants with the addition of both the consortium and a separate strain of *Ochrobactrum sp.* over time, it decreases and on the 28th day it drops to 0, while in the control variant it remains practically at the same level (Fig. 3).

It should be noted that the use of the consortium proceeds at a higher rate, so on the 28th day, when using both a separate strain and a consortium, the content of cypermethrin from the soil reaches zero values, but when using the consortium, the degradation indicators in the

dynamics of the process are higher, this indicates that the bacterial consortium degrades the parent molecules faster than a single strain (Table 3).

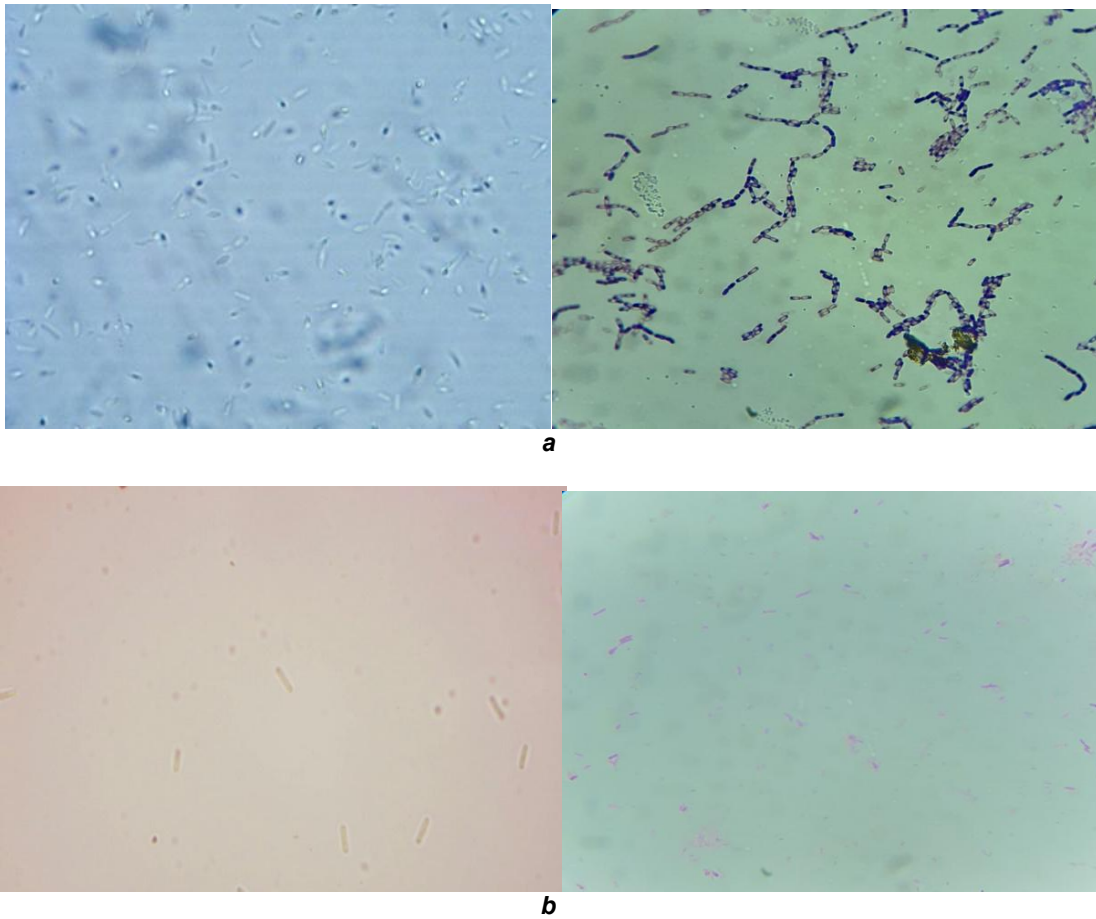
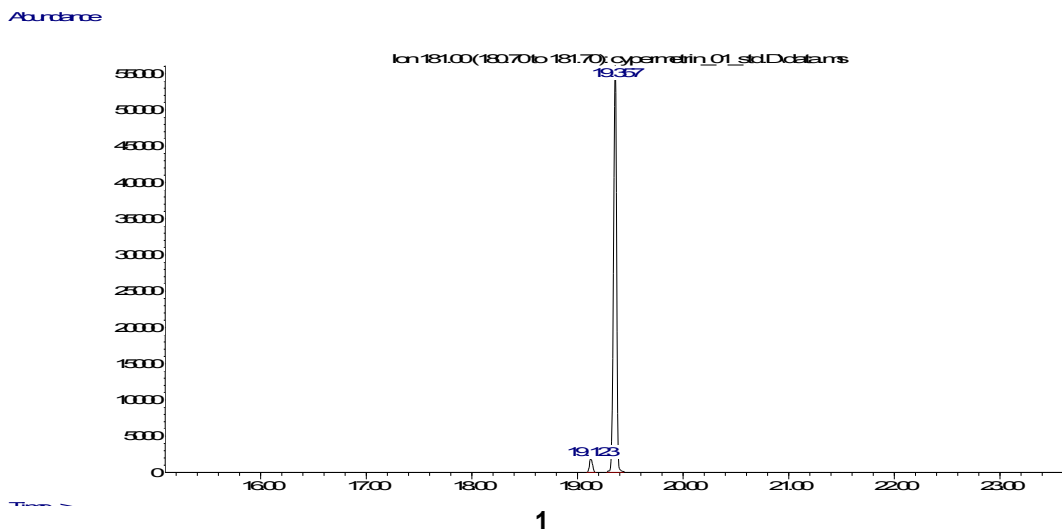
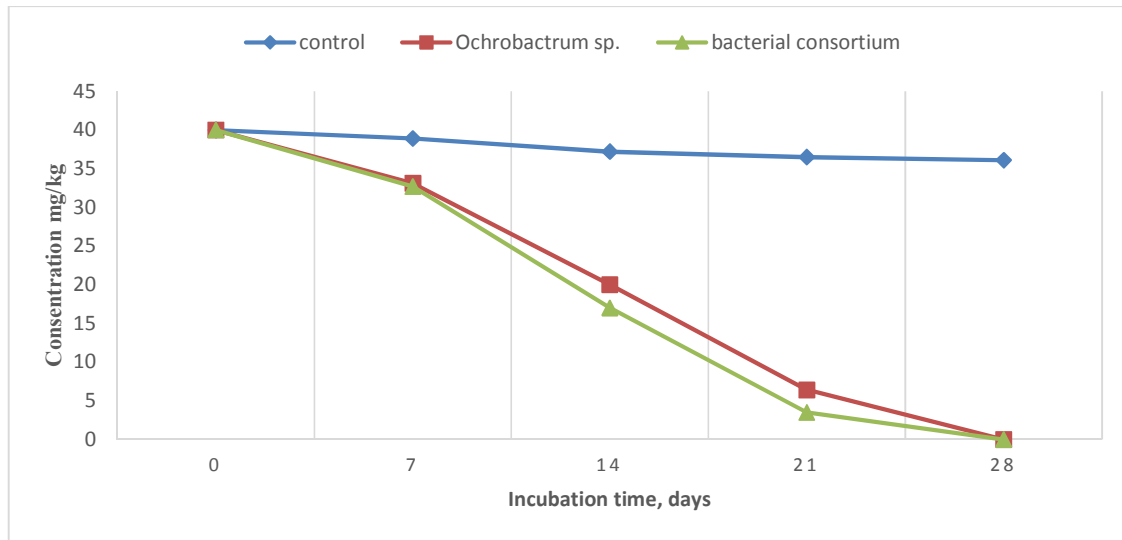


Fig. 2. Micrographs and Gram staining of cypermethrin-resistant strains: a- *Bacillus* sp., B- *Pseudomonas* sp. (sw.x1000)





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Fig. 3. Fig. 1- Standard of cypermethrin; 2 - Degradation dynamics of cypermethrin by bacterial consortium and *Ochrobactrum sp*

Table 3. Degradation of cypermethrin during 28 days %

Sample	40 mg/kg				
	Days				
	0	7	14	21	28
Control	0	2,75	7	8,7	9,75
<i>Ochrobactrum sp.</i>	0	17,2	50	84	100
<i>Bacterial consortium</i>	0	18,25	57,5	91,25	100

These data are consistent with the known information that it is more efficient to use a microbial consortium for the decomposition of organic substances than a single strain [30].

Results of laboratory studies on bioremediation of agricultural soils contaminated with the pesticide cypermethrin. Conducted using different concentrations of cypermethrin (up to 40 mg/kg soil) showed that the consortium of microorganisms created by us completely decomposes cypermethrin.

4. CONCLUSION

Soil pollution with pesticides, in addition to harm to humans and the environment, also leads to a deterioration in the vital activity of soil microorganisms, which significantly reduces the ability of soils to self-purify due to the functioning of natural crops. At the same time, the biodegradation of pesticides by microorganisms is the cheapest, most effective and environmentally friendly way to remove toxic

pollutants from the environment [32,33]. It is also known that for some microorganisms pesticides, as well as their decay products, are deadly, while others have the unique ability to use pesticides as a single source of carbon. Searching for and isolating such microorganisms is a difficult method, but it is important for remediation. Various microorganisms live in the soil, but not all can grow and multiply in a pesticide environment, because for most microorganisms both the initial structure of pesticides and their decay products are very toxic. As a result of our research, active strains of microorganisms were obtained, on the basis of which a consortium was created, capable of completely destroying such a pesticide as cypermethrin in a short period. This opens up great prospects for the use of selected microorganisms for bioremediation of soils contaminated with pesticides. It should also be noted that the use of a microbial consortium is more effective than a single strain, because in this case, various microorganisms resistant to cypermethrin are involved, which, due to their biochemical characteristics, can also decompose

intermediate compounds formed during the decomposition of cypermethrin.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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