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Biodegradation of the synthetic pyrethroid insecticide α-cypermethrin by *Stenotrophomonas maltophilia* OG2

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Abstract: A novel gram-negative bacterium, OG2, was isolated from the body microflora of cockroaches (*Blatta orientalis*). Based on morphological, biochemical, and 16S ribosomal DNA sequence analysis, the isolated strain OG2 was identified as *Stenotrophomonas maltophilia*. This bacterial strain was screened for its α-cypermethrin-degrading potential with minimal salt medium (MSM). The α-cypermethrin degradation and utilization ability of the isolated organism was verified. Bacterial growth was measured by optical density in the presence of the pesticide at different concentrations (50–200 mg/L). *S. maltophilia* OG2 utilized α-cypermethrin as the sole carbon source for growth, and α-cypermethrin degradation increased when MSM was supplemented with glucose. In the absence and presence of glucose, α-cypermethrin (100 mg/L) degradation efficiency of OG2 was 69.9% and 81.3%, respectively. Analysis of the degradation products indicated that *S. maltophilia* OG2 converted α-cypermethrin to 3-phenoxybenzoic acid, 3-phenoxybenzaldehyde, phenol, and muconic acid. Medium composition had considerable influence on the types of metabolic products. According to the results, *S. maltophilia* OG2 may have potential use in the bioremediation of cypermethrin-contaminated environments.

Key words: α-Cypermethrin, biodegradation, *Stenotrophomonas maltophilia*

1. Introduction

Synthetic pyrethroids (SPs) such as cyfluthrin, cypermethrin, deltamethrin, fenvalerate, and permethrin have been widely used to control insect pests for public health in agriculture, houses, and gardens throughout the world (Chen et al., 2011a). SPs generally have lower toxicity for living systems than organochlorines or organophosphate insecticides (Katsuda, 1999). Studies have demonstrated that SPs might have teratogenic, mutagenic, neurotoxicity, and endocrine disruption effects (Shafer et al., 2005). SPs are especially toxic to aquatic organisms (Bradbury and Coats, 1989). Total average annual use of SPs is estimated at nearly 1.5 billion dollars and accounts for 17% of global insecticide sales (Khambay and Jewess, 2005). Due to increasing use of pesticides, their level is increasing in the environment, and ultimately these pesticides enter the food chain (Weston et al., 2011). α-Cypermethrin is widely used against a broad range of insects (especially Lepidoptera, Coleoptera, and Hemiptera) in food and nonfood crops (Diao et al., 2011). Its abundant use poses a threat to environmental quality and public health.

α-Cypermethrin is degraded by photochemical and biological processes. Maloney et al. (1988) studied the microbial transformation of α-cypermethrin by *Pseudomonas fluorescens*, *Achromobacter* sp., and *Bacillus cereus*. Various cypermethrin-degrading microorganisms such as *Pseudomonas* sp. (Grant et al., 2002; Jilani and Khan, 2006), *Micrococcus* sp. CPN1 (Tallur et al., 2008), *Serratia* sp. (Grant et al., 2002; Zhang et al., 2010), and *Ochrobactrum lupini* DG-S-01 (Chen et al., 2011a) have been reported. Recently, *Stenotrophomonas maltophilia* was used for biodegradation of keratin (Jeong et al., 2010), alkane (Hassanshahian et al., 2013), toluene, benzene, ethylbenzene, xylene (Lee et al., 2002), pesticides (Dubey and Fulekar, 2012), and phenol (Basak et al., 2014).

Invertebrates host numerous microorganisms with interactions ranging from symbiosis to pathogenesis. Cockroach (Insecta: Blattodea) species live in warehouses, kitchens, cellars, and hospitals. They have been researched as transmitters and spreaders of pathogenic bacteria in many areas (Pai et al., 2005; Elgderi et al., 2006). SPs are intensively used for cockroach management/control due to their forceful and low mammalian toxicity. Cockroaches have evolved a resistance to different insecticides (Valles et

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al., 2000). No information is available about the microflora of cockroaches living in an environment with pesticides. Cockroaches are generally exposed to many pesticides and other organic pollutants. Therefore, the chance of isolating novel microorganisms capable of degrading the different hazardous materials present in insecticides from insect microflora is very high. The present study investigates α-cypermethrin biodegradation using *S. maltophilia* OG2 isolated from *Blatta orientalis*.

2. Materials and methods

2.1. Chemicals

α-Cypermethrin, 3-phenoxybenzoic acid, phenol, 3-phenoxybenzaldehyde, and muconic acid were purchased from Sigma Chemical Co. (St Louis, MO, USA). The stock α-cypermethrin solution was prepared in acetone and used for all the experiments.

2.2. Isolation of α-cypermethrin biodegrading microorganism

Cockroaches (*Blatta orientalis* Linnaeus 1758; Dictyoptera) were collected from a cow barn contaminated with pyrethroid and chlorinated organochlorine pesticide in Samsun, Turkey. The insects were surface-sterilized using 70% ethanol for 1 min and crushed in a sterile mortar containing 10 mL of 0.85% (w/v) saline solution (Okay et al., 2013). Dilutions of 10^{-4} of each sample (1 mL) were suspended in 5.0 mL of liquid minimal salt medium (MSM) containing 100 mg/L α-cypermethrin in a 250-mL Erlenmeyer flask and incubated at 30 °C with shaking (150 rpm). The MSM was autoclaved at 121 °C for 20 min, after which it was aseptically spiked with α-cypermethrin dissolved in acetone to yield a final concentration of 100 mg/L. The MSM consisted of (g/L): $(NH_4)_2SO_4$, 2; MgSO4.7H2O, 0.2; $CaCl_2.2H_2O$, 0.01; $FeSO_4.7H_2O$, 0.001; $Na_2HPO_4.12H_2O$, 1.5; and KH_2PO_4, 1.5. It was used for both the pure and mixed culture studies (Chen et al., 2011a, 2012a). The trace element solution prepared for MSM contained (mg/L): $MnCl_2.4H_2O$, 198; $ZnCl_2$, 136; $CuCl_2.2H_2O$, 171; $CoCl_2.6H_2O$, 24; and $NiCl_2.6H_2O$, 24 (Siddique et al., 2003). After 7 days, 0.2 mL of each culture was reinoculated into new α-cypermethrin MSM medium and further incubated at 30 °C for 7 days. This subculture was repeated under the same culture conditions, and then an aliquot (0.1 mL) from each culture was applied to solid α-cypermethrin MSM for isolation of single colonies.

2.3. Identification of α-cypermethrin biodegrader microorganisms

Analysis of 16S rDNA was performed for the taxonomic characterization of the isolated strain. The genomic DNA was extracted from pure culture using the DNA Mini Prep Kit (QIAGEN), and 16S rDNA was PCR-amplified using universal primers 27F: 5′-AGAGTTTGATCMTGGCTCAG-3′ and 1492R: 5′-ACGGCTACCTTGTTACGACTT-3′. The universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-CGGYTACCTTGTTACGACTT-3′) were used to amplify the 16S rDNA. A typical reaction mixture in each PCR tube for 25 μL of total volume contained 1 μL of the appropriate dilutions of DNA, 5 μL of 10X PCR reaction buffer, 0.5 μL of Taq DNA polymerase, 0.5 μL of dNTPs, 2.5 μL of $MgCl_2$, and 0.5 μL of each primer. The mixture was brought to 13 μL with sterile deionized, distilled water. The PCR was run for 30 cycles with the following thermal profile: denaturation at 94 °C for 30 s, primer annealing at 57 °C for 45 s, and extension at 72 °C for 90 s. The final cycle included an extension for 7 min at 72 °C. PCR products were cleaned and sequenced by REFGEN (Ankara, Turkey). The isolate identification was verified by the analysis of the 16S ribosomal DNA sequence, which was compared with the National Center for Biotechnology Information (NCBI) database using the online BLAST search (http://www.ncbi.nlm.nih.gov/BLAST).

2.4. Preparation of bacterial inoculum for biodegradation studies

S. maltophilia OG2 was grown in nutrient broth (Merck) to the midlog phase of growth (30 °C, 150 rpm, 24 h). The cells were then centrifuged at 5000 rpm for 10 min, washed twice with sterile 0.85% saline solution, and diluted with sterile water to a uniform optical density (OD_{600}) of 0.50.

2.5. Optimum concentration of α-cypermethrin for *S. maltophilia* OG2

Different concentrations (50, 100, 150, and 200 mg/L) of α-cypermethrin were prepared in 100 mL of MSM in 250-mL conical flasks, and 1 mL of inoculum (OD_{600} 0.5) was added. The flasks were shaken at 150 rpm at 30 °C for 10 days.

2.6. Effect of glucose supplementation on the biodegradation of α-cypermethrin

Growth of the bacterial strain was measured by optical density in the absence and presence of glucose (1 g/L) as a supplementary carbon source in addition to α-cypermethrin (100 mg/L) in MSM for 10 days. Growth was also seen in medium supplemented with glucose as a carbon source without α-cypermethrin. Samples withdrawn at intervals of 3, 5, and 10 days were also analyzed for residual α-cypermethrin concentration using HPLC.

2.7. Analytical methods

α-Cypermethrin and its degradation products, 3-phenoxybenzoic acid, phenol, 3-phenoxybenzaldehyde, and muconic acid, were analyzed by HPLC. In the cultures α-cypermethrin was extracted by the addition of an equal volume of ethyl acetate. The sample was dehydrated by passing it through anhydrous Na_2SO_4 and then concentrated with a rotary evaporator. Samples were analyzed by HPLC using a SUPELCOSIL C_{18} DB column (250 × 4.6

mm, 5 µm) with a acetonitrile:water (85:15) mobile phase. The solutes were detected using a UV-Vis detector at 235 nm (Chen et al., 2011a). The compounds were identified by comparison of HPLC retention times to those of authentic standards. Retention times for α-cypermethrin, 3-phenoxybenzoic acid, phenol, 3-phenoxybenzaldehyde, and cis,cis-muconic acid under these analytical conditions were 4.603, 2.119, 8.298, 2.273, and 3.573 min, respectively. The optical densities of the culture media were determined spectrophotometrically by measuring the absorbance at 600 nm.

3. Results and discussion

3.1. Isolation and identification of α-cypermethrin-biodegrading bacteria

Several interactions (pathogenic, symbiotic, and vectoring) can be distinguished between insects and bacteria (Dillon and Dillon, 2004; Ozdal et al., 2012). The present study represents the isolation and identification of a bacterial strain capable of degrading α-cypermethrin as the carbon source and taken from the microflora of cockroaches living in a pesticide-contaminated environment.

A bacterium was isolated from the body microflora of cockroaches through repetitive enrichment culture and successive transfer using α-cypermethrin MSM. Classical tests showed that it was a gram-negative, aerobic, oxidase-negative, catalase- and nitrate-reduction-positive, rod-shaped, bright-yellow pigmented, and mobile organism. Finally, a 902-bp 16S ribosomal DNA sequence of the strain was BLAST-searched (http://www.ncbi.nlm.nih.gov/BLAST) and aligned with *S. maltophilia* sequences. The sequence was deposited in GenBank with the accession number KC453991. The isolate was named *S. maltophilia* strain OG2. A phylogenetic tree was composed, as shown in Figure 1, with the selected 16S rDNA sequences using the neighbor-joining method. This is the first report of α-cypermethrin-degrading bacteria isolated from *B. orientalis*.

3.2. Optimum concentration of α-cypermethrin for *S. maltophilia* OG2

Different concentrations (50, 100, 150, and 200 mg/L) of α-cypermethrin were tested in order to determine the toxic dose for the isolated bacterial strain. Maximum bacterial growth was obtained at an initial concentration of 100 mg/L. α-Cypermethrin concentrations higher than 100 mg/L had a negative effect on bacterial growth (Figure 2).

3.3. Growth kinetics of *S. maltophilia* OG2 in medium supplemented with glucose as the carbon source

Significant differences were obtained in different media (containing only α-cypermethrin, α-cypermethrin + glucose, and only glucose in MSM) in terms of bacterial growth and biodegradation. Maximum bacterial growth was obtained with the addition of glucose (Figure 3). After 10 days of incubation, the bacterial growth in the medium containing only α-cypermethrin (without glucose in MSM) was 0.66 (OD_{600}), and it increased up to 1.6 (OD_{600})

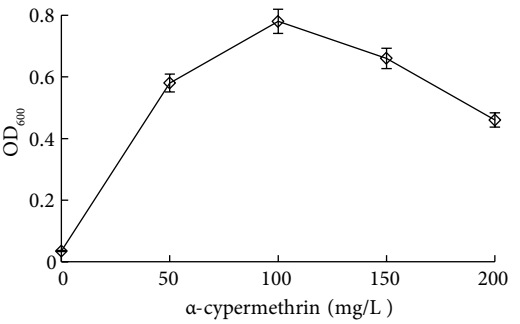

Figure 2. Effect of the different concentrations of α-cypermethrin on the growth of *S. maltophilia* OG2.

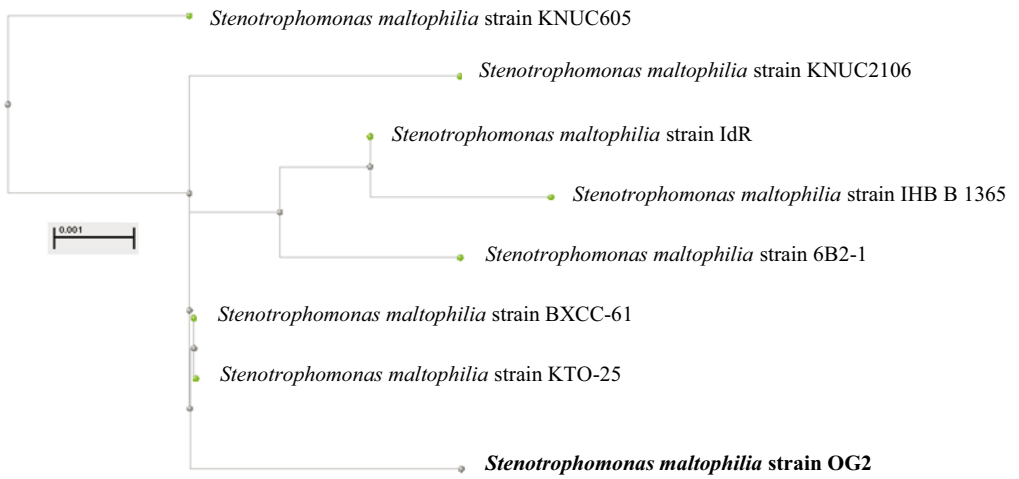

Figure 1. Phylogenetic tree based on the 16S rDNA sequences of strain OG2 and related species.

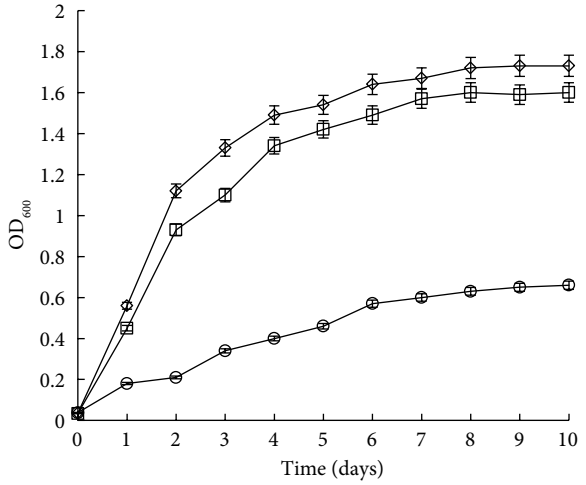

Figure 3. Effects of glucose addition on the growth of *S. maltophilia* OG2, i.e. MSM + glucose (◊), MSM + glucose + α-cypermethrin (□), MSM + α-cypermethrin (○).

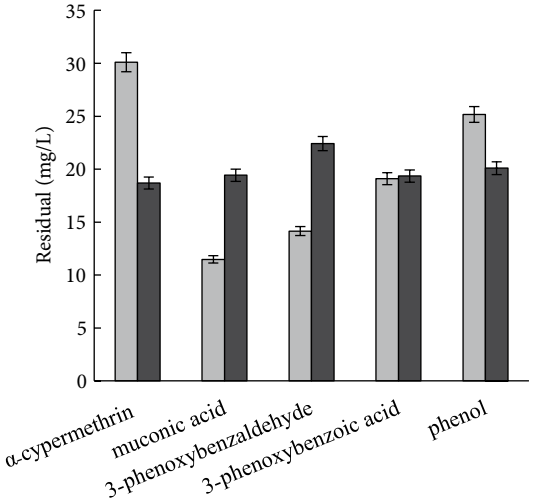

Figure 4. Biodegradation of α-cypermethrin (100 mg/L) in the presence and absence of glucose and its metabolites at 10 days.
Degradation in MSM supplemented with α-cypermethrin as the sole carbon source: gray.
Degradation in MSM supplemented with glucose as an additional source of carbon: black.

when glucose was added. It is quite likely that carbon sources stimulated the growth (Kumar and Philip, 2006), and the presence of α-cypermethrin in the medium had a toxic effect on growth.

3.4. Degradation of α-cypermethrin by *S. maltophilia* OG2 in batch culture

The addition of glucose increased biodegradation of α-cypermethrin. In the absence of glucose, the α-cypermethrin degradation efficiency of *S. maltophilia* OG2 was 69.9% at the end of 10 days. The addition of 1 g/L of glucose increased α-cypermethrin degradation efficiency to 81.3% (Figure 4), which corresponds to an increase of 16.3%. Many researchers reported that the efficiency of pesticide biodegradation increased following the addition of extra carbon sources (Kumar and Philip, 2006; Chen et al., 2011a, 2012b). These results suggested that the increase in biodegradation was dependent on the increase in cell concentration, as reported in another study (Pino and Penuela, 2011). These results were in agreement with the results of the present study.

As seen in Figure 3, the rate of biodegradation products increased in the presence of glucose. Although phenol was the major metabolite in the absence of glucose, 3-phenoxybenzoic acid was the major metabolite in the presence of glucose. As seen in Figure 5, 3-phenoxybenzoic acid, phenol, and 3-phenoxybenzaldehyde are intermediates in the biodegradation of α-cypermethrin by *S. maltophilia* OG2. In the control flasks the degradation rate of α-cypermethrin was about 15.87% after 10 days of incubation (Figure 5). *S. maltophilia* strain OG2 metabolized 3-phenoxybenzaldehyde to 3-phenoxybenzoic acid and oxidized phenol to muconic acid through an ortho-cleavage pathway similar to that reported for *Micrococcus* sp. strain CPN 1 (Tallur et al., 2008). Muconic acid, which is the final product, was found in both cultures. According to the results of research to date, pyrethroid-degrading microorganisms tended to transform pyrethroids by hydrolysis to yield 3-phenoxybenzoic acid, and 3-phenoxybenzoic

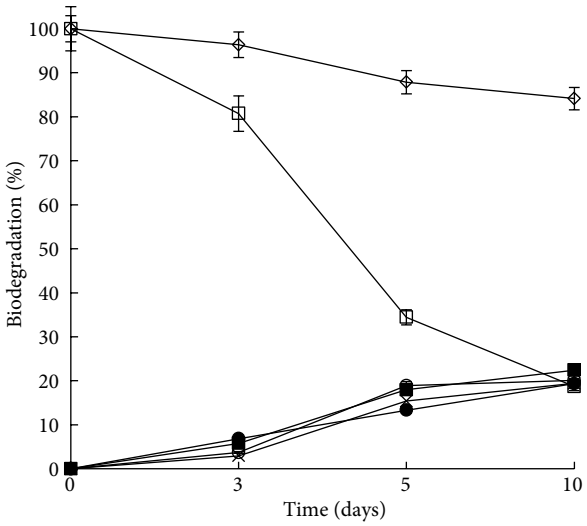

Figure 5. Biodegradation of α-cypermethrin and its metabolites in MBS + α-cypermethrin (100 mg/L) + glucose (0.1%), i.e. control (◊); α-cypermethrin (□); muconic acid (×); 3-phenoxybenzoic acid (●); 3-phenoxybenzaldehyde (▪); phenol (○).

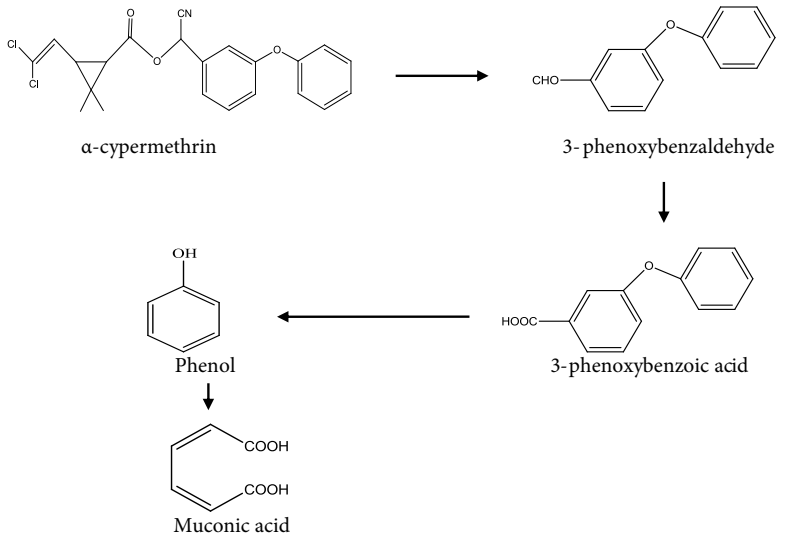

Figure 6. Proposed pathway of α-cypermethrin biodegradation for *S. maltophilia* OG2 (Tallur et al., 2008).

acid is the major metabolite of pyrethroids, with the exception of cyfluthrin (Laffin et al., 2010). Furthermore, 3-phenoxybenzoic acid is classified as an endocrine-disrupting chemical due to its antiestrogenic activity (Tyler et al., 2000). These results showed that *S. maltophilia* OG2 could degrade toxic metabolites such as 3-phenoxybenzoic acid and phenol. Tallur et al. (2008) proposed the pathway of α-cypermethrin biodegradation, as seen in Figure 6, and we can speculate that the pathway of α-cypermethrin biodegradation in *S. maltophilia* OG2 is similar. On the other hand, *Stenotrophomonas* species were reported to degrade diverse compounds including organochlorine pesticides such as endosulfan (Barragain-Huerta et al., 2007; Kumar et al., 2007), a wide range of pyrethroids (Chen et al., 2011b), organophosphates, and 4-substituted phenols (Liu et al., 2009). Zhang et al. (2010) reported that *Serratia* sp. JC1 and JCN13 could degrade 92% and 89% β-cypermethrin (100 mg/L) within 10 days, respectively. *Ochrobactrum lupini* DG-S-01 degraded β-cypermethrin (50 mg/L) by over 90% within 5 days (Chen et al., 2011a). Chen et al. (2011b) reported that *Stenotrophomonas* sp. ZS-S-01 could rapidly degrade a wide range of pyrethroids such as fenvalerate, deltamethrin, β-cypermethrin (about 86%, 50 mg/L), β-cyfluthrin, and cyhalothrin. Chen et al. (2012b) reported that cypermethrin (50 mg/L) was completely degraded in a coculture of *Bacillus cereus* ZH-3 and *Streptomyces aureus* HP-S-01 within 72 h. However, there are rare reports of α-cypermethrin-degrading strains. To the best of our knowledge, this is the first report on *S. maltophilia* OG2 capable of α-cypermethrin degradation.

In this study, we obtained a new bacterial isolate, *S. maltophilia* OG2, which can degrade α-cypermethrin successfully. Pollution by pyrethroids has become a very important problem in pesticide-treated areas. Currently, one of the major environmental problems is pesticide contamination caused by activities related to agricultural applications. Different mechanical and chemical methods are rarely used to remove pesticides from contaminated fields due to limited efficiency and the expense. Biodegradation is an encouraging process for the treatment of pesticide-contaminated areas due to the rapid and complete mineralization of pesticides. An isolated bacterial strain might be useful for bioremediation of α-cypermethrin-polluted soil and water environments.

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