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Biodegradation and bioremediation potential of diazinon-degrading *Serratia marcescens* to remove other organophosphorus pesticides from soils

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ABSTRACT

The ability of diazinon-degrading *Serratia marcescens* to remove organophosphorus pesticides (OPPs), i.e. chlorpyrifos (CP), fenitrothion (FT), and parathion (PT) was studied in a mineral salt medium (MSM) and in three soils of different characteristics. This strain was capable of using all insecticides at concentration of 50 mg/l as the only carbon source when grown in MSM, and 58.9%, 70.5%, and 82.5% of the initial dosage of CP, FT, and PT, respectively was degraded within 14 days. The biodegradation experiment showed that autochthonous microflora in all soils was characterized by a degradation potential of all tested OPPs; however, the initial lag phases for degradation of CP and FT, especially in sandy soil, were observed. During the 42-day experiment, 45.3%, 61.4% and 72.5% of the initial dose of CP, FT, and PT, respectively, was removed in sandy soil whereas the degradation of CP, FT, and PT in the same period, in sandy loam and silty soils reached 61.4%, 79.7% and 64.2%, and 68.9%, 81.0% and 63.6%, respectively. *S. marcescens* introduced into sterile soils showed a higher degradation potential (5–13%) for OPPs removal than those observed in non-sterile soil with naturally occurring attenuation. Inoculation of non-sterile soils with *S. marcescens* enhanced the disappearance rates of all insecticides, and DT50 for CP, FT, and PT was reduced by 20.7, 11.3 and 13.0 days, and 11.9, 7.0 and 8.1 days, and 9.7, 14.5 and 12.6 days in sandy, sandy loam, and silty soils, respectively, in comparison with non-sterile soils with only indigenous microflora. This ability of *S. marcescens* makes it a suitable strain for bioremediation of soils contaminated with OPPs.

1. Introduction

The organophosphorus pesticides (OPPs) are an important group of pesticides used extensively all over the world for more than sixty years. About 100 OPPs account for ~38% of the total pesticide usage. Some of them are chlorpyrifos (CP) (*O,O*-diethyl-*O*-3,5,6-trichloro-2-pyridyl phosphorothioate), fenitrothion (FT) (*O,O*-dimethyl-*O*-4-nitro-m-tolyl phosphorothioate) and parathion (PT) (*O,O*-diethyl-*O*-4-nitrophenyl phosphorothioate). They belong to a broad spectrum of systemic phosphorothioate esters intended to control a range of agricultural pests, and to a lesser extent for indoor use. The OPPs share the same general chemical structure, therefore their mechanisms of toxicity are similar. They block the activity of acetylcholine esterase that results in the inhibition of acetylcholine breakdown in synapses. The accumulation of acetylcholine in synapses causes convulsion, paralysis and death of insects and mammals (Cáceres et al., 2010). These insecticides are considered to be highly toxic to non-target invertebrates and vertebrates including mammals. Therefore, the use of FT and PT have been banned in European countries. However, these OPPs are still used in agricultural practice in different geographic regions of the world. Due to their toxicity and excessive use in agriculture the OP insecticides are a cause of environmental concern, and their fate has been studied extensively. It has been reported that the application of CP, FT or PT lead to soil, groundwater and surface water contamination. By a direct application or spray drift these insecticides reach the soil where they undergo various dissipation processes such as microbial degradation, chemical hydrolysis, photolysis, volatilization, leaching, and surface runoff. In addition, insecticides could also dissipate through adsorption and bound-residue formation with time. The contribution made by each of these processes to the overall dissipation depends upon the type of

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soil, its physico-chemical parameters such as temperature, moisture, organic matter content, and composition of microbial communities (Karpouzas and Walker, 2000; Hong et al., 2007).

The ability of microorganisms to degrade OPPs is considered to be the primary way of removing these compounds from soils. A range of phylogenetically distinct microorganisms capable of degrading OPPs by co-metabolism or by using them as a source of carbon and phosphorus have been isolated from different contaminated soils around the world. Moreover, it has been observed that repeated application of some OPPs enhanced their biodegradation (Singh et al., 2003). Studies on microbial degradation of the OPPs are useful for the development of bioremediation strategies defined as the process whereby toxic organic compounds are degraded to an innocuous state, or to levels below concentration limits established by regulatory authorities. Bioremediation, which involves the use of microbes to detoxify and degrade pollutants, has received increased attention as an effective biotechnological approach to clean up insecticide-polluted soils (Singh et al., 2006; Chen et al., 2011a).

In our previous study, the screening of degrading strains by the enrichment procedure from soil contaminated with the OPP diazinon allowed us to distinguish three isolates, namely *Serratia marcescens*, *Serratia liquefaciens* and *Pseudomonas* sp., which exhibited a high degrading activity towards diazinon (Cycoń et al., 2009). Moreover, this was the first report on the degradation of diazinon by *S. marcescens*, and our results indicated that this strain may be useful for efficient removal of diazinon from contaminated soils. There are many reports related to the degradation of the OPPs by bacteria from the *Pseudomonas* genus (Deshpande et al., 2001; Foster et al., 2004; Lakshmi et al., 2008; Abo-Amer, 2011). Moreover, it has also been found that bacteria from the *Burkholderia*, *Alteromonas*, *Arthrobacter*, *Variovorax*, and *Bacillus* genera can degrade chlorpyrifos (Ohshiro et al., 1996; Singh et al., 2003), fenitrothion (Hayatsu et al., 2000; Kim et al., 2009; Anwar et al., 2009), and parathion (Rani and Lalithakumari, 1994; Togo et al., 2006). Apart from single bacteria a few consortia with the ability to metabolize OPPs and syntrophic associations involving the degradation of fenitrothion have also been reported (Singh and Walker, 2006; Katsuyama et al., 2009). Only a few papers pointed to the degradation potential of *S. marcescens* for the elimination of OPPs (Lakshmi et al., 2008; Abo-Amer, 2011). Hence, in the present study we have focused on the degradation potential of the previously isolated diazinon-degrading *S. marcescens* to find out whether this strain is suitable for bioremediation of soils contaminated with other OPPs.

2. Materials and methods

2.1. Chemicals and media

Analytical standards of CP (99.5% purity), FT (99.8% purity), and PT (99.9% purity) and their main metabolites, i.e. 3,5,6-trichloro-2-pyridinol (TCP) (99.1% purity), 3-methyl-4-nitrophenol (MNP) (99.3% purity), and *p*-nitrophenol (PNP) (99.0% purity), respectively, were purchased from Sigma–Aldrich (Germany), while all other chemicals were of analytical grade and purchased from Merck (Germany). Acetone stock solutions of insecticides were 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 $(NH_4)_2SO_4$, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.01 g of $CaCl_2 \cdot 2H_2O$, 0.001 g of $FeSO_4 \cdot 7H_2O$, 1.5 g of $Na_2HPO_4 \cdot 12H_2O$, and 1.5 g of KH_2PO_4 per litre of deionised 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.

2.2. Soils

Composite soil samples collected from the top layer (0–20 cm) of commercial fields located in Upper Silesia, southern Poland and classified as sandy, sandy loam and silty soils with no known history of previous OPPs applications were used in this experiment. Detailed physico-chemical properties of the soils and methods of their determination are presented in Table 1.

2.3. Bacterial strain and its identification

A bacterial strain, isolated from the soil contaminated with diazinon by using an enrichment culture technique and identified by MIDI-FAME profiling as *S. marcescens* was used in this experiment (Cycoń et al., 2009). In addition, in this study the strain was identified on the basis of the 16S rRNA sequence analysis. For this purpose, 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: (i) an initial denaturation step of 95 °C for 2 min, (ii) 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 (iii) 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 sequence of the strain was compared by the BlastN search analysis (http://www.blast.ncbi.nlm.nih.gov/). 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 (http://www.ncbi.nlm.nih.gov/genbank).

Table 1
General characteristics of soils used in the biodegradation experiment.

Parameter	Soil texture classification (FAO and USDA System)			Method
	Sand	Sandy loam	Silt	
Sand (2000-50 μm) (%)	91.0 ± 4.2	69.0 ± 3.2	4.0 ± 0.4	ISO 11277:2009
Silt (<50-2 μm) (%)	6.0 ± 1.1	21.0 ± 1.3	85.0 ± 2.2	ISO 11277:2009
Clay (<2 μm) (%)	3.0 ± 0.9	10.0 ± 1.0	11.0 ± 0.8	ISO 11277:2009
Density g cm^{-3}	1.02 ± 0.03	1.11 ± 0.04	1.46 ± 0.09	ISO 11272:1998
$pH_{(in\ water)}$ (1:5)	6.5 ± 0.4	6.9 ± 0.2	7.2 ± 0.2	ISO 10390:2005
Cation exchange capacity (CEC) (cmol+/kg)	3.3 ± 0.2	8.0 ± 0.7	22.0 ± 1.3	ISO 11260:1994
Water-holding capacity WHC (%)[d]	32.0 ± 2.2	38.0 ± 1.9	54.0 ± 1.6	ISO 14239:1997
C_{org} (%)	1.1 ± 0.2	2.3 ± 0.02	3.8 ± 0.04	ISO 14235:1998
N_{tot} (%)	0.07 ± 0.01	0.14 ± 0.02	0.22 ± 0.03	ISO 11261:1995
Microbial biomass MB (mg/kg)	600 ± 23	980 ± 32	1125 ± 56	ISO 14240-1:1997

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