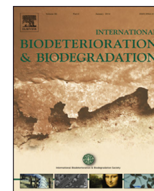
International Biodeterioration & Biodegradation 96 (2014) 80–86

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod



Aerobic removal of methoxychlor by a native *Streptomyces* strain: Identification of intermediate metabolites

Natalia Bourguignon [a, 1], María S. Fuentes [a, 1], Claudia S. Benimeli [d, e], Sergio A. Cuozzo [a, b, *], María J. Amoroso [a, c, e]

[a] Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET), Avenida Belgrano y Pasaje Caseros, 4000 Tucumán, Argentina
[b] Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán, Miguel Lillo 205, 4000 Tucumán, Argentina
[c] Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 471, 4000 Tucumán, Argentina
[d] Unidad de Administración Territorial, Centro Científico Tecnológico, CONICET-Tucumán, Crisóstomo Álvarez 722, 4000 Tucumán, Argentina
[e] Universidad del Norte Santo Tomás de Aquino (UNSTA), 9 de Julio 165, 4000 Tucumán, Argentina

A R T I C L E   I N F O

Article history:
Received 30 May 2014
Received in revised form
23 September 2014
Accepted 25 September 2014
Available online

Keywords:
Bioremediation
Streptomyces
Methoxychlor
Biodegradation
Metabolites

A B S T R A C T

Streptomyces strains isolated from pesticide-contaminated sediments of Santiago del Estero, Argentina were able to grow in the presence of 1.66 mg L^{-1} of methoxychlor (MTX). *Streptomyces* sp. A14 showed the best growth in the presence of MTX in culture medium at 30 °C and pH 7. When soil microcosms were contaminated with MTX and inoculated with *Streptomyces* sp. A14, a decrease in MTX was detected in both concentrations assayed (8.33and 16.60 mg kg^{-1}). This actinobacterium was able to remove the pesticide, reaching its maximum removal percentages (40% and 76%) after 28 days of incubation. Methoxychlor was gradually converted into 1,1-dichloro-2,2-bis(4-methoxyphenyl)ethane, 1,1-dichloro-2,2-bis(4-methoxyphenyl)ethylene, 1-chloro-2,2-bis(4-methoxyphenyl)ethane, and 2,2-bis(4-hidroxyphenyl)acetonitrile, indicating that MTX is dominantly degraded by dechlorination, dehydrogenation and CN-replacement, resulting in the production of several major degradation products. This is an interesting proposal as an alternative method for soil cleanup. This is the first report of methoxychlor degradation using a native *Streptomyces* strain under aerobic conditions.

© 2014 Elsevier Ltd. All rights reserved.

Introduction

Methoxychlor [2,2-bis(*p*-methoxyphenyl)-1,1,1-trichloroethane] (MTX) is an organochlorine pesticide that was developed for use as a replacement for DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane]. DDT has been internationally prohibited since 1970s due to its high toxicity (Stuchal et al., 2006). MTX is structurally similar to DDT and it was primarily used against various species of insects that attack field crops, trees, vegetables, fruits, gardens, stored grain, livestock, and domestic pets (ATSDR, 1994). MTX is persistent in soils and its residues are found to be present even after 18 months of post-treatment with microbes that scavenge MTX (Golovleva et al., 1984). The use of MTX was banned worldwide in 2004 because of failure to register with the U.S. Environmental Protection Agency (EPA) (Stuchal et al., 2006); however several sites still exist around the world where soils and sediments are highly polluted with this compound. These places include areas of (former) pesticide manufacturing and formulation, water courses used to receive discharges from these factories, obsolete pesticide storage locations and others (Baczynski, 2012). Globally, humans and domestic animals are exposed to MTX through the extensive usage of this chemical and through consumption of agricultural products (Stuchal et al., 2006).

Despite its relatively low level of toxicity and short half-life there is a considerable amount of concern regarding MTX exposure because it is an endocrine disrupting chemical (EDC). EDCs produce their effects by mimicking, antagonizing, or altering levels of endogenous steroids (androgens or estradiol) via changes in their rates of synthesis or metabolism and/or expression or action at the receptor targets (Frye et al., 2012).

Since harmful effects of MTX on health are well known (Guo et al., 2013), it is imperative to develop methods to remove it from the environment. One of the strategies that have been adopted is bioremediation using microorganisms with the ability to degrade pesticides. MTX metabolism in higher organisms has been well investigated in mammals, birds, fish, and bivalves (Masuda

* Corresponding author. Avenida Belgrano y Pasaje Caseros, T40001MVB Tucumán, Argentina. Tel.: +54 381 4344888.
E-mail address: scuozzo@proimi.org.ar (S.A. Cuozzo).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ibiod.2014.09.016
0964-8305/© 2014 Elsevier Ltd. All rights reserved.

et al., 2011), and MTX biodegradation has also been described for certain fungi and bacteria that are able to use this kind of pesticide under aerobic or anaerobic conditions (Fogel et al., 1982; Lee et al., 2006; Satsuma et al., 2012). However, little information is available on MTX biotransformation by Gram-positive microorganisms such as actinobacteria (Fuentes et al., 2010, 2013; Lal et al., 2010). These microorganisms have been shown to have great potential for biodegradation of toxic organic compounds, and several studies have demonstrated the ability of different genera of actinobacteria to degrade pesticides including lindane, chlordane, MTX, chlorpyrifos, diuron, and pentachlorophenol (Fuentes et al., 2010; Briceño et al., 2012).

It has also been determined that environmental factors such as temperature, pH, and salinity have a strong influence on microbial activity, as well as on the bioavailability of target chemicals. Therefore, the optimization of these parameters is necessary in order to achieve substantial degradation of pollutants. In this context, the aim of the present work is to determine the optimal conditions for MTX removal by indigenous *Streptomyces* strains, to characterize their potential degradation in soil microcosm, and to investigate the production of metabolites from MTX.

Materials and methods

Chemicals

Methoxychlor [2,2-bis(*p*-methoxyphenyl)-1,1,1-trichloroethane] (99.8% pure) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Solvents were of pesticide grade, and all other chemicals used throughout the study were of analytical grade and were purchased from standard manufacturers.

Microorganisms and culture media

Four actinobacteria strains (*Streptomyces* spp. A3, A6, A12,A14) previously isolated from a contaminated environment in Santiago del Estero, Argentina and identified as belonging to the genus *Streptomyces* were selected because of their ability to grow in the presence of MTX as a carbon source and remove MTX from the culture medium (Fuentes et al., 2010). Also, two strain were used: *Streptomyces coelicolor* A3 (2), obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ), and *Streptomyces* sp. M7, previously isolated in our laboratory from sediment samples contaminated with heavy metals and pesticides (Benimeli et al., 2003).

All removal assays were carried out in Minimal Medium (MM), which contained (g L^{-1}): $(NH_4)_2SO_4$, 2.00; K_2HPO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.20; and $FeSO_4 \cdot 7H_2O$, 0.01 (Hopwood et al., 1985). The pH of the medium was adjusted to 7.0 prior to sterilization. All media were sterilized by autoclaving at 121 °C for 20 min.

A stock solution of MTX dissolved in acetone was poured into the sterile glass flasks and air-dried. Then autoclaved MM was added aseptically, according to the requirements of each test. The MM with pesticide was allowed to stand overnight to promote the dissolution of the MTX.

Batch cultures of Streptomyces spp. strains in minimal medium supplemented with methoxychlor

Spore suspensions of the six actinobacterium strains (150 μL) were inoculated in 125 mL flasks containing 30 mL of MM supplemented with MTX (1.66, 8.30, and 16.60 mg L^{-1}) as the carbon source. All cultures were incubated on a rotatory shaker (200 rpm) at 30 °C, for 7 days. Supernatant samples of centrifuged cultures (9900 × *g*, 30 min, 4 °C) were used to determine residual MTX by gas chromatography. Biomass was estimated after centrifugation by washing the pellets with 25 mM Tris–EDTA buffer (pH 8.0) and drying to constant weight at 105 °C.

Inoculated MM without MTX and supplemented with glucose 1 mg L^{-1} as carbon source (designed as "biotic control") and non-inoculated MM with MTX (designed as "abiotic control") samples were also included. All experiments were carried out in triplicate; results are given as the means from the three tests.

Soil microcosms: condition and inoculation

For soil microcosms, surface soil samples (5–15 cm depth) were taken from an experimental site northwest of San Miguel de Tucumán, Argentina. Samples were kept at10–15 °C in the dark and used in the next days. Prior to use, soil was air-dried, lightly ground using mortar and pestle, and finally sieved through a 1-mm sieve. Such preparation was found necessary to improve homogeneity on the pollutant distribution.

Glass pots were filled with 200 g of soil at 20% moisture (dry weight base) and kept for 36 h at room temperature so that water in the soil was balanced. Soil samples were sterilized (three successive sterilizations at 121 °C, 1 h each one, with 24 h in between) and the soil humidity was adjusted with sterile water or MTX to a final pesticide concentration of 8.33, and 16.60 mg kg^{-1} wet weight (w/w). Sterility was checked for each set of sterilized soil pots by the enumeration of bacteria (CFU g^{-1}) (Benimeli et al., 2008).

Before being inoculated on soil, *Streptomyces* sp. A14 was precultured in Erlenmeyer flasks containing Tryptic Soy Broth (TSB) 50 mL at 30 °C on a rotatory shaker (100 rpm) for approximately 3–4 days. Pots with MTX were inoculated with precultured strain using a microbial concentration of 2 g kg^{-1} of soil (designed as "assay") (Benimeli et al., 2008). Soil, inoculum and MTX were mixed thoroughly to ensure a uniform distribution. Inoculated soil samples without MTX (designed as "biotic control") and non-inoculated soil pots spiked with MTX (designed as "abiotic control") were also used. Soil pots were incubated at 30 °C for 4 weeks and the soil humidity was monitored regularly twice a week. Samples were taken once a week for determining residual MTX concentration by GC and microbial growth by the enumeration of bacteria (CFU kg^{-1}). The growth of *Streptomyces* sp. A14 was measured as CFU kg^{-1} by transferring 1 g of soil from each pot into a sterile flask, containing 9 mL of a sterile solution of sodium hexametaphosphate (1.66 g L^{-1}, pH 7) according to the method used by Benimeli et al. (2008). Soil was vortexed for 10 min. Serial 10-fold dilutions in sodium dihydrogenophosphate (0.05 M, pH 7) were made and plated in triplicate on starch casein agar. The plates were incubated at 30 °C for 72 h.

Colorimetric assay for dechlorination activity

Cell-free supernatant samples were immediately used for indirect determination of the release of chloride ions using a modification of the procedure described in Phillips et al. (2001), in which Phenol Red Sodium Salt is added to 1 mL of supernatant at a 1:10 ratio as a pH indicator. A color change from red through orange to yellow caused by the presence of chloride in the supernatant is indicative of dechlorination of MTX, and therefore represents a positive result. Culture medium with a pH indicator was used as a blank. Chloride concentrations were determined colorimetrically at 540 nm using a Beckman spectrophotometer and compared with standard HCl solutions. A decrease in optical density at 540 nm ($\Delta A_{540\ nm}$) was indicative of microbial dechlorination activity (Cuozzo et al., 2012).

Download English Version:

https://daneshyari.com/en/article/6289225

Download Persian Version:

https://daneshyari.com/article/6289225

Daneshyari.com