ELSEVIER

International Biodeterioration & Biodegradation 58 (2006) 196–202

INTERNATIONAL BIODETERIORATION & BIODEGRADATION

www.elsevier.com/locate/ibiod

Biodegradation of the herbicide diuron by streptomycetes isolated from soil

M.A. Castillo[a,*], N. Felis[a], P. Aragón[b], G. Cuesta[a], C. Sabater[a]

[a]*Biotechnology Department, Polytechnic University of Valencia, Camino de Vera, 14, 46022 Valencia, Spain*
[b]*Chemistry Department, Polytechnic University of Valencia, Camino de Vera, 14, 46022 Valencia, Spain*

Received 30 June 2005; received in revised form 21 April 2006; accepted 21 June 2006
Available online 22 August 2006

Abstract

The diuron degrading activity of 17 streptomycete strains, obtained from agricultural and non-agricultural soils, was determined in the laboratory. All strains were identified as *Streptomyces* sp. by phenotypic characteristics and PCR-based assays. The strains were cultivated in liquid medium with diuron ($4\,mg\,L^{-1}$) at 25 °C for 15 days. Biodegradation activity was determined by high-performance liquid chromatography. The results indicated that all strains were able to degrade diuron, but to different amounts. Twelve strains degraded the herbicide by up to 50% and four of them by up to 70%. Strain A7-9, belonging to *S. albidoflavus* cluster, was the most efficient organism in the degradation of diuron, achieving 95% degradation after five days of incubation and no herbicide remained after 10 days. Overall, the strains isolated from agricultural soils exhibited higher degradation percentages and rates than those isolated from non-agricultural soils. Given the high degradation activity observed here, the streptomycete strains show a good potential for bioremediation of soils contaminated with diuron.
© 2006 Elsevier Ltd. All rights reserved.

Keywords: Herbicide; Diuron; Actinomycetes; Streptomycetes; Soil

1. Introduction

Microorganisms play a significant role in the transformation and degradation of pesticides. Even the most persistent pesticides can be metabolized to some extent by microbial cultures, either by utilization of the compounds as sources of energy or nutrients, or by cometabolism with other substrates supporting microbial growth. This last process is probably the most widespread mechanism for pesticide biodegradation. Complete mineralization of pesticides or their transformation to non-toxic products is desirable, but it is more likely to be carried out by consortia of microorganisms than single isolates.

There is growing concern over the potential for contamination of soils, surface and groundwater by the repeated use of herbicides. Among the herbicides used to prevent the growth of undesirable plants, phenylurea derivatives have been widely used since their discovery in the early 1950s. One of them, diuron (3-(3,4-dichlorophenyl)-1, 1-dimethylurea), is employed widely for weed control in non-crop areas, such as roads, railways tracks, and paths, and to control weeds in a range of tree crops, such as, pear, apple, forestry, ornamental trees and pineapples, sugar cane, cotton, alfalfa and wheat (Tomlin, 2003). It kills plants by blocking electron transport at photosystem II, thus inhibiting photosynthesis.

Diuron is applied to soil, where it tends to accumulate because of its low solubility in water. DT50 in soils varies from 90–180 days (Hill et al., 1955; Tomlin, 2003); other sources indicate that it can range from one month to one year (Field et al., 2003), although values of greater than 3000 days have been reported (Madhum and Freed, 1987). The variability in calculated DT50s is likely to be due to differences in soil composition and other conditions (Gaillardon, 1996; Rouchaud et al., 2000). Degradation increases moderately in soils treated with diuron for many years. In one experiment, where soils were treated at a rate of $3\,kg\,ha^{-1}\,year^{-1}$ for 12 years, the half-life decreased by 50% over this period, suggesting that the soil microbial community was becoming increasingly able to degrade it (Rouchaud et al., 2000).

*Corresponding author. Tel.: +34 963877423; fax: +34 963877429.
E-mail address: mcastill@btc.upv.es (M.A. Castillo).

0964-8305/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ibiod.2006.06.020

Much data have been collected on the direct and indirect effects of diuron on target and non-target organisms, and diuron is suspected to be genotoxic (Canna-Michaelidou and Nicolau, 1996). The US Environmental Protection Agency (EPA) has classified diuron as a 'known/likely' carcinogen since 1997. There is conflicting evidence on whether diuron can cause mutations. The National Institute for Occupational Safety and Health in the US categorized diuron as a mutagen, based on old studies (Seiler, 1978). Its use in Europe is being reviewed.

Several reports considered microbial degradation the main mechanism for dissipation of diuron from soil (Sheets, 1964; Geissbühler et al., 1973), as well as in aquatic environments (Ellis and Camper, 1982; Howard, 1991). In a number of these studies, soil fungi have shown a high degradative activity against phenylureas, with occasional ability to degrade diuron (Weinberger and Bollag, 1972; Tillmanns et al., 1978; Vroumusia et al., 1996; Tixier et al., 2000), but only a few studies have found degradative activity in bacteria (Shelton et al., 1996; Esposito et al., 1998; Cullington and Walker, 1999; Tixier et al., 2001, 2002; Widehem et al., 2002). The main degradation products and pathways of diuron degradation are reviewed by Giacomazzi and Cochet (2004).

Among bacteria, actinomycetes have a considerable potential for the biotransformation and biodegradation of xenobiotics. De Schirijver and De Mot (1999) described degradation by actinomycetes of pesticides with widely different chemical structures, including organochlorines, *s*-triazines, carbamates, organophosphates, sulphonylureas and phenylureas. Other studies (Alvarez, 2003) showed that various species of actinomycetes exhibited a high capacity to degrade hydrocarbons.

Few studies have been conducted to assess the ability of actinomycetes to degrade or transform diuron. Only little data are reported about specific strains. Shelton et al. (1996) studied the ability of *Streptomyces* (PS1/5) to metabolize a variety of structurally different herbicides, including diuron, which was degraded by 19% after 7 days. Esposito et al. (1998) found one unique strain with a good potential for diuron degradation. More recently, other authors described *Arthrobacter* strains which have been able to degrade diuron (Tixier et al., 2001, 2002; Widehem et al., 2002).

The metabolic diversity of actinomycetes and their particular growth characteristics (invasive mycelial, rapid colonization of selective substrates, etc.) make them attractive organisms for bioremediation. The aim of the present study was to investigate the diuron biodegradation potential of streptomycete strains isolated from soil.

2. Material and methods

2.1. Microbiology

2.1.1. Soils

Actinomycete strains were isolated from seven different soils: two uncultivated soils and five soils from citrus orchards regularly treated with diuron. The uncultivated soils were clay loam, with pH 8, high contents of calcium carbonate (41%) and organic matter (5–6%). The cultivated soils were silty loam to sandy loam, with pH 7.8–8.1, high content of calcium carbonate (38%) and low level of organic matter (1.5–2%). The samples were collected from the first 10 cm below the surface, aseptically transferred to sterile vials and stored at 4 °C until used.

2.1.2. Microorganisms

A total of 53 actinomycete strains were isolated from soil using standard isolation methods. Seventeen randomly chosen strains were tested for diuron degrading ability. Twelve out of them were isolated from the agricultural soils, and five strains from two uncultivated soils. The isolates for biodegradation assay are filed at the Microbiology Laboratory of Biotechnology Department, Polytechnic University of Valencia, as shown in Table 1. The strains were maintained at 4 °C on ISP-2 agar medium (yeast extract, 4 g; malt extract, 10 g; dextrose, 4 g; agar, 20 g; distilled water, 1000 mL). Biomass for biodegradation study was obtained after 7 days of cultivation in Petri dishes at 25 °C on ISP-2 agar medium, without any adaptation to herbicide. Five mL of ISP-2 liquid medium was added to the Petri dishes to obtain conidial suspensions. The final conidia concentration was estimated using a haemocytometer (improved Neubauer chamber).

2.1.3. Phenotypic characterization of actinomycete strains

The actinomycete strains were maintained on ISP-2 slants at 4 °C and nutrient broth with 20% glycerol at −20 °C. In order to observe pigment production and macroscopic morphology, the isolates were cultured on International Streptomyces Project (ISP) media (Shirling and Gottlieb, 1966). Phenotypic characteristics were determined by standard procedures, described previously by Williams et al. (1989).

2.1.4. DNA extraction and purification

Cells were cultured on ISP-2 broth for 3–5 days, washed and collected by centrifugation and frozen for 24 h. The thawed pellet was resuspended in 1 mL of TE buffer (1 mM EDTA, 10 mM Tris/HCl) with glass beads (3 mm) and vortexed to disaggregate the clumps of mycelium. Four hundred μL of this suspension was incubated with 100 μL lysozyme (50 mg mL^{-1}) and 15 μL of mutanolysine (1330 U mL^{-1}) at 37 °C for 30 min (Lanoot et al., 2005). After addition of 30 μL SDS (10%), 3 μL proteinase K (20 mg mL^{-1}) (Sigma) and glass beads (400 μm), cells were vortexed and incubated at 37 °C for 1 h. Cell debris and polysaccharides were selectively precipitated with CTAB (cetyl trimethyl ammonium bromide) at 65 °C for 10 min. DNA was purified with equal volumes of phenol:chloroform:isoamylalcohol (25:24:1). The suspension was shaken, centrifuged and the upper layer was removed to a clean tube. A final purification step with chloroform:isoamylalcohol (24:1) removed the residual phenol from the suspension. The upper layer was removed to a clean tube and DNA was precipitated with isopropanol. The solution was centrifuged to pellet the DNA, the supernatant was discarded and a wash step with 70% ice-cold (−20 °C) ethanol was made and the pellet was dried in vacuum. TE buffer was added to suspend the dried DNA.

2.1.5. PCR amplification of actinomycetales/streptomyces strains

PCR primers were designed previously and synthesized by TIB MOLBIOL (Berlin, Germany). Primers 243F and A3R amplified a

Table 1
Streptomycete isolates for biodegradation assays

Origin	Strain code[a]
Non-agricultural soil	F1-3, F1-5, F1-11, F1-12, F3-8
Agricultural soil	A1-1, A1-2, A1-5, A2-2, A2-4, A2-6, A2-11, A3-2, A3-14, A4-2, A5-3, A7-9

[a]All isolates are maintained in the Microbiology Laboratory of Biotechnology Department, Polytechnic University of Valencia, Spain.

Download English Version:

https://daneshyari.com/en/article/4366049

Download Persian Version:

https://daneshyari.com/article/4366049

Daneshyari.com