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Biodegradation of nicosulfuron by a novel *Alcaligenes faecalis* strain ZWS11

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ABSTRACT

A bacterial strain ZWS11 was isolated from sulfonylurea herbicide-contaminated farmland soil and identified as a potential nicosulfuron-degrading bacterium. Based on morphological and physicochemical characterization of the bacterium and phylogenetic analysis of the 16S rRNA sequence, strain ZWS11 was identified as *Alcaligenes faecalis*. The effects of the initial concentration of nicosulfuron, inoculation volume, and medium pH on degradation of nicosulfuron were investigated. Strain ZWS11 could degrade 80.56% of the initial nicosulfuron supplemented at 500.0 mg/L under the conditions of pH 7.0, 180 r/min and 30°C after incubation for 6 days. Strain ZWS11 was also capable of degrading rimsulfuron, tribenuron-methyl and thifensulfuron-methyl. Four metabolites from biodegradation of nicosulfuron were identified, which were 2-aminosulfonyl-N, N-dimethylnicotinamide (M1), 4, 6-dihydroxypyrimidine (M2), 2-amino-4, 6-dimethoxypyrimidine (M3) and 2-(1-(4,6-dimethoxy-pyrimidin-2-yl)-ureido)-N,N-dimethyl-nicotinamide (M4). Among the metabolites detected, M2 was reported for the first time. Possible biodegradation pathways of nicosulfuron by strain ZWS11 were proposed. The degradation proceeded mainly *via* cleavage of the sulfonylurea bridge, O-dealkylation, and contraction of the sulfonylurea bridge by elimination of a sulfur dioxide group. The results provide valuable information for degradation of nicosulfuron in contaminated environments.

Introduction

Sulfonylurea is one of the most important groups of herbicide being used worldwide for control of broadleaf weeds in various crops and vegetables, due to its low application amount, high herbicidal activity (2–75 g of active ingredient per hectare), broad spectrum of action, good selectivity and low mammalian toxicity (Brown et al., 1997; Sarmah and Sabadie, 2002).

Nicosulfuron, as a sulfonylurea herbicide, has been commercialized since the 1990s for weed control and is widely used around the world. However, with the widespread application of nicosulfuron, its residue has been reported in soil and surface waters (Battaglin et al., 2000; Brusa et al., 2001; Regitano and Koskinen, 2008). The potential risks of environmental contamination as well as the phytotoxicity problems of nicosulfuron under certain conditions have raised increasing concerns (Sabater et al., 2002; Seguin et al., 2001; Yue et al., 2007).

The fate and behavior of nicosulfuron in the environment have been investigated by some researchers (Benzi et al., 2011; Berger and Wolfe, 1996; Oliveira et al., 2001). The results showed that different chemical hydrolysis processes of nicosulfuron occurred under acidic, neutral, and alkaline

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conditions, and microbial degradation was prevalent in native sediments at neutral pH (Berger and Wolfe, 1996). The sorption coefficient of nicosulfuron was not significantly correlated with soil organic carbon content, and it could potentially leach into ground-water (Oliveira et al., 2001). A photodegradation study of nicosulfuron in aqueous solutions showed that some new metabolites formed during the degradation process. Additionally, nicosulfuron has a low degradation or hydrolysis rate in aqueous solution, and can persist in neutral and alkaline conditions for long times (Benzi et al., 2011). Rapid degradation of nicosulfuron has been considered an urgent issue for contaminated environments.

Microbial degradation is an important environmental biotechnology for elimination of organic pollutants (Harbottle et al., 2009; Valle et al., 2006). Sulfonylurea herbicides are mainly degraded or transformed by microorganisms or chemical hydrolysis in soil and water (Brusa et al., 2001; Joshi et al., 1985). To date, some studies on degradation of sulfonylurea herbicides by microorganisms have been published (Boschin et al., 2003; Brusa et al., 2001; Sondhia et al., 2013; Xu et al., 2009; Zanardini et al., 2002). Biodegradation of nicosulfuron by bacteria or fungi has also been reported, such as *Aspergillus niger* YF1 and *Bacillus subtilis* YB1 (Lu et al., 2012), *Serratia marcescens* N80 (Zhang et al., 2012), and *Talaromyces flavus* LZM1 (Song et al., 2013). Among the microorganisms mentioned above, the efficiency of *T. flavus* LZM1, which could degrade 100% of an initial nicosulfuron concentration of 100 mg/L within 5 days, was high (Song et al., 2013). *S. marcescens* N80 could degrade 93.6% of nicosulfuron at an initial concentration of 10 mg/L within 4 days (Zhang et al., 2012). The highest degradation rates of *B. subtilis* YB1 and *A. niger* YF1 could reach 87.9% and 98.8%, respectively, after incubation for 5 days; however, the initial concentration of nicosulfuron was only 2 mg/L (Lu et al., 2012). Studies regarding nicosulfuron-degrading bacteria are still limited.

In the present study, a novel bacterium *Alcaligenes faecalis* strain ZWS11, which could degrade nicosulfuron over a broad range of initial concentrations, was isolated from sulfonylurea herbicide-contaminated farmland soil, and the effects of different conditions on degradation of nicosulfuron were evaluated. Further experiments were carried out to determine the ability of strain ZWS11 to degrade other sulfonylurea herbicides. The metabolites of nicosulfuron were identified by HRLC-MS, and biodegradation pathways of nicosulfuron by strain ZWS11 were proposed. The results obtained from this investigation are expected to provide valuable information for the biodegradation of nicosulfuron.

1. Materials and methods

1.1. Chemicals

Technical grade nicosulfuron (purity ≥ 97.8%) was obtained from Zibo Nab Agrochemicals Limited, Shandong, China. Nicosulfuron (analytical standard 99.8%, 111991-09-4) was purchased from Sigma-Aldrich Co. (MO, USA). 2-Amino sulfonyl-N, N-dimethylnicotinamide (112006-75-4), 2-amino-4, 6-dimethoxypyrimidine (36315-01-2) and 4, 6-dihydroxy pyrimidine (1193-24-4) were purchased from J&K Scientific Ltd. (Beijing, China). HPLC acetonitrile and methanol were purchased from Mreda Technology Inc. (USA). All other reagents used in this study were of analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China).

1.2. Media

Mineral salt medium (MSM) was composed of (per liter) 1.5 g K_2HPO_4, 0.5 g KH_2PO_4, 1.0 g NaCl, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.025 g $FeSO_4$, 0.02 g $CaCl_2$, and 1.0 g NH_4NO_3, the glucose-mineral salt medium (GSM) was made with addition of 1.0 g glucose in MSM, and the sucrose-mineral salt medium (SSM) was made with addition of 1.0 g sucrose in MSM. The peptone-yeast extract-mineral salt medium (PYM) was supplemented with 1.0 g peptone and 0.5 g yeast extract in MSM. Lysogeny broth medium (LB) contained peptone 10.0 g, yeast extract 5.0 g, and NaCl 5.0 g. The media were dissolved in 1000 mL distilled water and adjusted to a pH value of 7.0 before autoclaving at 121°C for 30 min. The plates were prepared by adding 1.5% (W/V) agar into the liquid media.

1.3. Enrichment and isolation of nicosulfuron-degrading bacteria

Sulfonylurea herbicide-contaminated soil was collected from the surface layer (0–10 cm) of farmlands of Tangshan Academy of Agricultural Sciences, Hebei, China. The soil had been exposed to sulfonylurea herbicides every growing season for more than 10 years. Five grams of soil sample was added to an Erlenmeyer flask (250 mL) containing 100 mL MSM, supplemented with 10.0 mg/L nicosulfuron as the nutrient substance, and incubated on a rotary shaker (180 r/min) at 30°C for 7 days in the dark. About 5 mL enrichment culture was then transferred into fresh MSM every 7 days. Increasing with each successive transfer, the concentration of nicosulfuron was gradually increased to 200.0 mg/L. Seven subcultures were performed before the isolation of effective pure cultures. The final enrichment culture (microbial flora) was serially diluted with sterile distilled water and spread on MSM plates containing 200.0 mg/L nicosulfuron. Different colonies (single bacterium) were picked up by the streaking plate method on LB plates containing 200.0 mg/L nicosulfuron, in order to further purify the strain. After being incubated at 30°C for 3 days, the bacterium strain was isolated, and the pure culture was inoculated into liquid medium containing nicosulfuron to verify the degrading capability. Nicosulfuron was extracted from liquid cultures based on the methods of Anastassiades et al. (2003), Jiang et al. (2009) and Wu et al. (2013), with some modifications. Five milliliters of fermentation broth taken from 100 mL liquid culture (adjusted pH to 7.0 by HCl) was put into a 15 mL centrifuge tube (Corning Inc., Acton, Massachusettes, USA, USA), 5.0 mL acetonitrile (include 0.1% acetic acid, V/V) was added and the mixture was homogenized by a shaker (Vortex-Genie 2, Scientific Industries, Inc., New York, USA) at maximum speed for 1 min; then anhydrous sodium chloride (3.0 g) was added, and the tube was shaken at 220 r/min by a shaker for 35 min at room temperature, before centrifugation for 5 min at 4000 r/min. About 1.0 mL of the upper organic phase was transferred into vials after filtration through a 0.22 μm membrane filter for HPLC analysis. To ensure the accuracy and precision of experiments, triplicate analyses