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Characterization of a Strain Capable of Degrading a Herbicide Mixture of Quinclorac and Bensulfuronmethyl*[1]

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ABSTRACT

A bacterial strain, designated as LS, was isolated from a contaminated soil and was found to be capable of utilizing quinclorac, bensulfuronmethyl, and a mixture of the two as carbon and energy sources for growth. Strain LS was identified as *Ochrobactrum* sp. based on its physiological-biochemical properties, 16S rDNA sequences, and phylogenetic analysis. The extent of degradation of quinclorac and bensulfuronmethyl at initial concentrations of 1.5 and 0.1 g L^{-1} was 90% and 67%, respectively, as measured by high performance liquid chromatography (HPLC). When a herbicide mixture of 0.34 g L^{-1} quinclorac and 0.02 g L^{-1} bensulfuronmethyl was applied as carbon sources, quinclorac and bensulfuronmethyl were degraded at 95.7% and 67.5%, respectively. It appears that quinclorac is utilized more easily in a mixture than in a single state. The optimal temperature for growth of strain LS was 37 °C. Strain LS grew well at pH 6 to 9 and had the highest degradation level for quinclorac and bensulfuronmethyl at an initial pH of 7 and 8, respectively. Addition of 0.25 g L^{-1} yeast extract could promote the growth and extent of degradation of quinclorac and bensulfuronmethyl by strain LS. Strain LS also showed the capability to degrade other aromatic compounds such as catechol, propisochlor, 4-chloro-2-methylphenoxyacetic acid sodium (MCPA-Na) and imazethapy. The isolate LS shows a huge potential to be used in bioremediation for treating complex herbicide residues.

Key Words: bensulfuronmethyl, degradation, herbicide mixture, *Ochrobactrum* sp., quinclorac

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INTRODUCTION

Quinclorac, 3,7-dichloro-8-quinolinecarboxylic acid, belongs to a new class of highly selective, auxin-type herbicides used on paddy rice to effectively control barnyard grass (*Echinochloa crusgalli*) (Grossmann, 1998). Bensulfuronmethyl, methyl-2-[(4,6-dimethoxy-pyrimidin-2-yl)aminocarbonylamino]sulfonyl-methyl benzoate, is a highly active sulfonylurea herbicide used to control most broad-leaved grasses and sedges in paddy rice (Brown, 1990). Mixtures of bensulfuronmethyl and quinclorac have been widely applied for their improved weed-control effect and wider weed spectrum, compared with bensulfuronmethyl or quinclorac individually, especially in a mixture of 0.02 g L^{-1} bensulfuronmethyl and 0.34 g L^{-1} quinclorac in water (Lu *et al.*, 2005). Synthetic agrochemicals, such as quinclorac (Lü *et al.*, 2003b, 2004, 2006) and bensulfuronmethyl (Xie *et al.*, 2004), can affect the microbial activity and cause an overall toxic effect on the environment. Therefore, their residues in the soil may act as potential environmental hazards and disturb the natural ecological equilibrium (Alexandre and Claudio, 2000). The widespread use of quinclorac and bensulfuronmethyl has led to controversy with respect to water and soil pollution. Kyung (1997) reported that a very small fraction of quinclorac was distributed in different parts of rice plants, whereas about 95% of the originally applied quinclorac remained in the 30-cm

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surface layer of soil after harvest. Okamoto (1998) found that the concentration of bensulfuronmethyl in rivers and lakes of Japan was as high as 0.1–2.3 mg L^{-1}. The concentration of bensulfuronmethyl reached 0.02 mg L^{-1} in groundwater near paddy rice fields in Italy (Wei *et al.*, 1998).

Bioremediation, based on certain species of microorganisms, is a cheap and effective way to decontaminate pesticide in contaminated soils and water. There are many successful examples of *in-situ* bioremediation of pesticide residues by microorganisms. At present, only one quinclorac degradation strain has been reported (Lü *et al.*, 2004), and the degradation of bensulfuronmethyl is also rarely reported (Brusa *et al.*, 2001; Zhu *et al.*, 2005). However, no information on the biodegradation of the mixture of quinclorac and bensulfuronmethyl has been published in the literature. Therefore, understanding the degraders of herbicide mixtures, as well as the dynamics of biodegradation, is of great importance. The purpose of the present work was to study the phylogenetic and degradation characterization of a pure bacterium capable of high degradation of the herbicide mixture.

MATERIALS AND METHODS

Chemicals and culture medium

Quinclorac (98%) and bensulfuronmethyl (98%) were kindly offered by Zhejiang Xinanjiang Chemical Group Co., Ltd. and Zhejiang Tianyi Agrochemical Co., Ltd., respectively. The herbicide mixture (0.02 g L^{-1} bensulfuronmethyl and 0.34 g L^{-1} quinclorac) in our study is the same as the ratio widely and commonly used in China. All other chemicals used were of analytical grade purity or chromatographic purity.

The composition of mineral salt (MS) medium was as follows: $MgSO_4 \cdot 7H_2O$ 0.2, KH_2PO_4 1.0, K_2HPO_4 1.0, $(NH_4)_2SO_4$ 0.5, $CaCl_2$ 0.01, $FeSO_4$ trace, and $NaNO_3$ 0.5 g L^{-1}. The medium was adjusted to initial pH of 7.0–7.5 and then sterilized at 121 °C for 30 min.

Isolation of herbicide mixture-degrading bacterium

One gram soil sample, taken from Tianyi Agrochemical Co., Ltd. in Zhejiang Province, China, was inoculated into 100 mL MS medium in the presence of 20–50 mg herbicide mixture at 30 °C and shaken at 130 r min^{-1} for 7 d. The enrichment cultures were continuously transferred to a fresh liquid MS medium with herbicide mixture every 7 d. After enrichment, the liquid culture was spread onto solid MS medium using the dilution plate method and incubated at 30 °C for 48 h. The separated colonies were serially picked up and inoculated onto the new plates repeatedly until a pure isolate was obtained (Herigstad *et al.*, 2001). One purified isolate with higher capacity for degrading quinclorac and bensulfuronmethyl was named as strain LS.

Morphological characterization of strain LS

The strain LS cells were fixed by osmic acid, embedded in resin, and then sliced to 50–80 nm thick. After staining with lead salt, the cell morphology of strain LS was observed using a light microscope (Olympus BH-2, Japan) and a transmission electron microscope (TEM) (JEM-1200EX, Japan). In addition, punctured culturing of the strain in semisolid medium and a bacterial flagella staining test were used to test for bacterial movement.

Biochemical characteristics

Conventional physiological characteristics were determined by the procedures according to the 'Manual of Identification for General Bacteriology' (Dong and Cai, 2001). The Vitek system (BioMerieux, St. Louis, Mo.) was used for the identification of physiological characteristics of the isolate, whereas the Biolog microstation (GN) (Biolog Hayward, CA, USA) was used for the identification of its carbon source utilization.