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Biodegradation and detoxification of chlorimuron-ethyl by *Enterobacter ludwigii* sp. CE-1

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ABSTRACT

The application of the herbicide chlorimuron-ethyl has a lasting toxic effect on some succession crops. Here, a bacterium capable of utilizing chlorimuron-ethyl as the sole source of nitrogen was isolated from the contaminated soil and was identified as *Enterobacter ludwigii* sp. CE-1, and its detoxification and degradation of the herbicide were then examined. The biodegradation of chlorimuron-ethyl by the isolate CE-1 was significantly accelerated with increasing concentration (1–10 mg/l) and temperature (20–40 °C). The optimal pH for the degradation of chlorimuron-ethyl by the isolate CE-1 was pH 7.0. A pathway for the biodegradation of chlorimuron-ethyl by the isolate CE-1 was proposed, in which it could be first converted into 2-amino-4-chloro-6-methoxypyrimidine and an intermediate product by the cleavage of the sulfonylurea bridge and then transformed into saccharin via hydrolysis and amidation. The plant height and fresh weight of corn that had been incubated in nutrient solution containing 0.2 mg/l of chlorimuron-ethyl significantly recovered to 83.9% and 83.1% compared with those in the uninoculated control, although the root growth inhibition of chlorimuron-ethyl could not be alleviated after inoculation for 14 d. The results indicate that the isolate CE-1 is a promising bacterial resource for the biodegradation and detoxification of chlorimuron-ethyl.

1. Introduction

Chlorimuron-ethyl is a persistent sulfonylurea herbicide widely used in soybean field for the control of broadleaf weeds (Claus, 1987). The half-life of chlorimuron-ethyl in soil is approximately 7–70 days depending upon soil characteristics (Sharma et al., 2012). Like many other sulfonylurea herbicides, chlorimuron-ethyl inhibits the biosynthesis of essential branched-chain amino acids and stops plant cell division in the rapidly growing tips of roots and shoots (Zhang et al., 2009). Hence, seed germination and shoot and root elongation of after-reap crops, such as wheat and corn, have been inhibited by residual chlorimuron-ethyl in soil as a result of its extensive application (Wang et al., 2005; Zhang et al., 2011; Tan et al., 2013; Carvalho et al., 2015). DNA damage to aquatic organisms can also be caused through the runoff and leaching of chlorimuron-ethyl (Yin et al., 2008). Therefore, it is necessary to find an effective method to decrease or remove the toxicity of chlorimuron-ethyl-contaminated soil.

Bioremediation by microorganisms offers an efficient, economic, and safe way to remove herbicide residues in the environment (Singh and Singh, 2016). The degradation or detoxification of herbicide residues in agricultural soil has mainly relied on the direct spray of degrading microorganisms and plasmids (Ahmad et al., 2012; Zhang et al., 2012a, 2012b). A few strains capable of degrading chlorimuron-ethyl have been documented, including fungal strains (*Sporobolomyces* sp. LF1, *Serratia marcescens* N80, and *Aspergillus niger*) (Zhang et al., 2009; Sharma et al., 2012; Zhang et al., 2012a, 2012b) and bacterial strains (*Pseudomonas* sp. LW3, *Klebsiella jilinsis* 2N3, *Bacillus subtilis* YB1, *Hansschlegelia* sp. CHL1, *Rhodococcus* sp. D310-1, and *Stenotrophomonas maltophilia* D310-3) (Ma et al., 2009; Zhang et al., 2010; Lu et al., 2012; Yang et al., 2014; Li et al., 2016; Zang et al., 2016). The degradation rate of chlorimuron-ethyl at the concentration of 100 mg/l by *Klebsiella jilinsis* 2N3 were up to 92.5–96.6% in 12 h. *Pseudomonas* sp. LW3 could degrade more than 60% of a 50 mg/l chlorimuron-ethyl solution initially added to a medium provided as the sole source of nitrogen in 4 days. *Sporobolomyces* sp. LF1 could remove 46% of 50 mg/l chlorimuron-ethyl in 4 days. It was shown that chlorimuron-ethyl could be degraded almost completely by above microorganisms within 0.5–7 days. Although it has been revealed that sulfonylurea herbicides are degraded mainly by oxidation, de-esterification, and cleavage of the sulfonylurea bridge, few researchers have explicitly proposed a biodegradation pathway for a particular degrading microorganism (Yang et al., 2015).

In the present study, a highly efficient degrading strain that can utilize chlorimuron-ethyl as its sole nitrogen source was found. The

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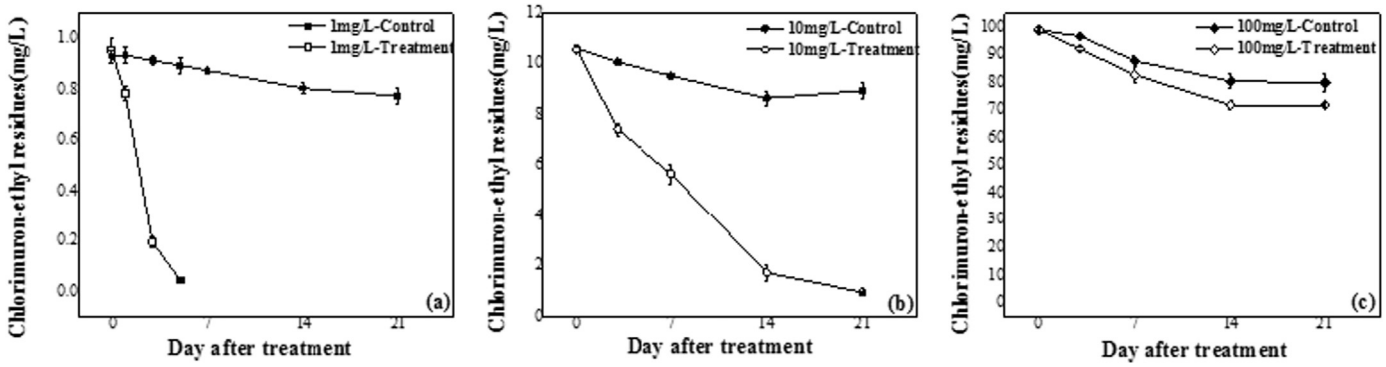

Fig. 1. Degradation of chlorimuron-ethyl at different concentrations by the isolate CE-1 in the mineral medium at 30 °C and pH 7.0.

Table 1
Degradation kinetics data for chlorimuron-ethyl degradation by the isolate CE-1.

Concentration (mg/l)	Temperature (°C)	pH	Kinetic function	Biodegradation rate (mg/l/d)	DT_{50}[a] (d)	r^2 (%)
1	30	7.0	$C = 1.1589e^{-0.6466*t}$	0.18	1.07	91.43
10	30	7.0	$C = 10.5743e^{-0.1136*t}$	0.38	6.10	99.92
100	30	7.0	$C = 96.3606e^{-0.0141*t}$	0.39	49.23	97.99
10	20	7.0	$C = 8.4085e^{-0.0178*t}$	0.11	38.94	86.22
10	30	7.0	$C = 10.5743e^{-0.1136*t}$	0.38	6.10	99.92
10	40	7.0	$C = 9.0529e^{-1.3701*t}$	1.86	0.51	99.34
10	30	5.0	$C = 10.5277e^{-0.0973*t}$	0	7.12	99.99
10	30	7.0	$C = 10.5743e^{-0.1136*t}$	0.38	6.10	99.92
10	30	9.0	$C = 11.1274e^{-0.0516*t}$	0.28	13.43	98.86

[a] DT_{50}: degradation half-life.

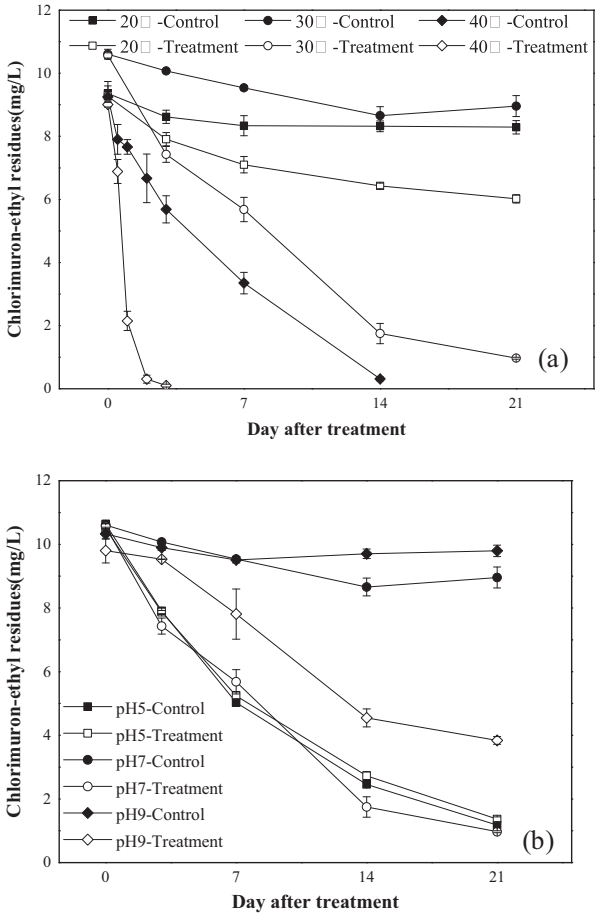

Fig. 2. Effect of temperature (a) and pH (b) on biodegradation of chlorimuron-ethyl at the concentration of 10 mg/l by the isolate CE-1 in mineral salts medium at 150 rpm.

objectives of this work with the isolated bacterial strain were to characterize its capability for degradation of chlorimuron-ethyl, to investigate its degradation pathway of chlorimuron-ethyl, and to estimate its bioremediation effect in water by phytotoxicity testing. The results will be useful for elucidating a possible application of the isolated bacterium for the remediation of chlorimuron-ethyl-contaminated soil.

2. Materials and methods

2.1. Chemicals

Chlorimuron-ethyl (purity ≥ 98.0%) was procured from Helishun Technology Co., Beijing, China. Saccharin (purity ≥ 98.0%), and 2-amino-4-chloro-6-methoxypyrimidine (purity ≥ 99.0%) were purchased from Aladdin Industrial Corporation, USA. Dichloromethane and anhydrous sodium sulfate of analytical grade were provided by Shuanglin Chemical Co., Hangzhou, China. HPLC-grade methanol was obtained from Tedia Co., USA.

2.2. Enrichment and isolation of chlorimuron-ethyl-degrading bacterium

The soil sample was collected from a soybean field in Xuzhou, Jiangsu, China and then periodically subjected to spraying with 100 mg/l chlorimuron-ethyl at an interval of 7 d. Approximately 10 g of the soil was sampled and placed in a 250-ml Erlenmeyer flask containing 100 ml of mineral salt medium (MSM, $MgSO_4{\cdot}7H_2O$ 0.2 g; KH_2PO_4 0.5 g; K_2HPO_4 0.5 g; NaCl 0.2 g; $CaCl_2$ 0.1 g; Glucose 5 g; H_2O, 1000 ml; pH 7.0) supplemented with 50 mg/l chlorimuron-ethyl. The mixture was incubated for 5 d in darkness at 30 °C and 150 rpm on a rotary shaker. Ceteris paribus, 1 ml of the incubated solution was then inoculated into a 150-ml Erlenmeyer flask containing 50 ml of the MSM supplemented with 50 mg/l of chlorimuron-ethyl as the sole nitrogen source. After three repetitions of the above process, a bacterial community was gradient diluted and then cultivated onto MSM-agar plates supplemented with 100 mg/l chlorimuron-ethyl and incubated for 24 h at 30 °C to isolate chlorimuron-ethyl-degrading bacteria.