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ScienceDirect
Ecotoxicology and Environmental Safety 72 (2009) 136–143
Ecotoxicology and Environmental Safety
www.elsevier.com/locate/ecoenv

Catalase and superoxide dismutase activities in a *Stenotrophomonas maltophilia* WZ2 resistant to herbicide pollution ☆

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Received 3 August 2007; received in revised form 7 January 2008; accepted 12 January 2008
Available online 4 March 2008

Abstract

Quinclorac bensulfuron-methyl is a mixed herbicide widely used on paddy rice field to effectively control barnyard grass and most broad-leaved grasses and sedges. We analyzed superoxide dismutase (SOD) and catalase activities in the quinclorac—highly degrading strain *Stenotrophomonas maltophilia* WZ2 and Gram-negative standard strain *Escherichia coli* K12 in an attempt to understand antioxidant enzymes in bacteria are produced in response to quinclorac or bensulfuron-methyl, which increases the virulence of the bacteria. MnSOD and two additional catalase isozymes were induced by quinclorac or bensulfuron-methyl in *S. maltophilia* WZ2, but not in *E. coli* K12. Quinclorac turned out to be a more sensitive inducer of SOD, whereas bensulfuron-methyl is a more sensitive one of catalase. A mixture of both has effects similar to quinclorac. Results indicate that catalase has a much weakly role in the defense against quinclorac or bensulfuron-methyl induced oxidative stress, whereas SOD could be critical.

Keywords: Superoxide dismutase; Catalase; *Stenotrophomonas maltophilia* WZ2; *Escherichia coli* K12; Quinclorac; Bensulfuron-methyl; Mixed herbicide

1. Introduction

Quinclorac (3, 7-dichloro-8-quinolinecarboxylic acid) belongs to a new class of highly selective, auxin-type herbicides used on paddy rice field to effectively control barnyard grass (*Echinochloa crusgalli*) (Grossmann, 1998). Bensulfuron-methyl (2-[[[[[(4, 6-dimethoxypyrimidin-2yl) amino] carbonyl] amino] sulfonyl]methyl]benzoate) is a highly active sulfonylurea herbicide that controls most broad-leaved grasses and sedges in paddy rice (Si et al., 2004). Mixtures of bensulfuron-methyl and quinclorac are also used widely as they have an improved weed-control effect and are effective on a wider spectrum of weeds, compared with bensulfuron-methyl or quinclorac individually, especially when the mixture ratio was 2% bensulfuron-methyl and 34% quinclorac (Lu et al., 2005). The widespread use of quinclorac and bensulfuron-methyl led to water pollution and problems for environmental health. Okamoto et al. (1998) reported that the concentration of bensulfuron-methyl in rivers and lakes of Japan was as high as 0.1–2.3 mg L^{-1}. The concentration of bensulfuron-methyl reached 0.02 mg L^{-1} in groundwater near paddy rice fields in Italy (Wei et al., 1998).

Previous studies have shown that several pollutants are redox active, and these redox-cycling drugs are able to enter microorganisms and affect aerobic metabolism, which generates univalent reduction of molecular oxygen including the superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical (•OH) (Barnes et al., 1999). These products of cell aerobic metabolism are toxic to many microorganisms, and can cause DNA damage (Huycke et al., 2002; Imlay and Linn, 1998), lipid peroxidative injury (Almagor et al., 1983) and enzyme inactivation (Flint et al., 1993; Liochev and Fridovich, 1993) by undergoing a series of redox reactions. Antioxidant enzymes, such as superoxide dismutases (SODs), catalase, and glutathione peroxidase (GSH-Px), are widely distributed in aerobic organisms. Among these enzymes, SODs, a family of four types of metalloenzymes dependent

☆ We confirm that in this study we did not use human or experimental animals.

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0147-6513/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2008.01.009

on a metal cofactor, Cu/Zn–SOD, Mn–SOD, Fe–SOD, and Ni–SOD (Lynch and Kuramitsu, 2000), detoxify oxygen radicals by conversion of O_2^-, the first reactive oxygen species formed in the metabolic reduction of oxygen, to H_2O_2 and O_2 (Fridovich, 1995). Subsequently, the catalases, which in bacteria can be divided into two groups, a bifunctional peroxidase/catalase and a monofunctional catalase (Loewen et al., 1985), decompose H_2O_2 to form oxygen and water (Barnes et al., 1999).

The oxidative toxicity induced by quinclorac to *Carassius auratus* (Song et al., 2002) and to *Echinochloa oryzicola* vasing (Sunohara and Matsumoto, 2004) has been investigated and the results indicate that quinclorac induces generation of active oxygen species (AOS) in animal cells and plant cells. The effects of the herbicide mefenacet (Ye et al., 2006) and the insecticide acetamiprid (Yao et al., 2006) on antioxidative enzyme and ATPase activities of bacteria have been studied in our laboratory.

Stenotrophomonas maltophilia WZ2, isolated from a pesticide contaminated soil, could utilize quinclorac as the sole carbon and energy source. Our results showed that pesticides had different effects on SOD and catalase activity of different bacteria. The enzyme activities of SOD and catalases could be induced as a way to against redox action from quinclorac and bensulfuron-methyl by *S. maltophilia*. To our knowledge, no previous studies have investigated the presence, type, and activity of SOD and catalase in bacteria treated with the herbicides quinclorac and bensulfuron-methyl. This study aimed to provide such information and determine the role of SOD and catalase in protection of the bacteria against oxidative stress. In addition, *S.maltophilia* is ubiquitous in environment although it is a condition pathogen. It is famous for its high resistance of antibiotics. As we known, most antibiotics have complex ringed structure. By comparison, structures of quinclorac and bensulfuron-methyl are much simple. Based on the partially similar structure of these compounds, our research could also provide reference on the mechanism of high antibiotics resistance in *S.maltophilia*, which is significant for clinical preventive medicine and health science.

2. Materials and methods

2.1. Chemicals and strains

Both quinclorac (98% purity) and bensulfuron-methyl (98% purity) were obtained from Zhejiang AMP Co Ltd. All other chemicals were purchased from the Shanghai Sangon Company. *S. maltophilia* WZ2, a Gram-negative strain that can efficiently degrade quinclorac was first isolated from activated sewage by our laboratory. Strain WZ2 decomposed 92% of quinclorac at original concentration of 1000 mg L^{-1} within 10 days. Another Gram-negative representative strain *Escherichia coli* K12 was used as a comparative strain in this study.

2.2. Media and cell growth

2.2.1. Exposition of cells to herbicides during growth

S. maltophilia WZ2 and *E. coli* K12 were inoculated into LB media (composed of: tryptone 10 g, yeast extract 5 g, NaCl 10 g, H_2O 1000 mL, adjust pH to 7.0 and autoclave to be sterilized) and incubated at 30 °C with shaking at 130 rpm for 24 h. Aliquots of above cultures were used to inoculate 30 mL LB media containing the herbicides quinclorac, bensulfuron-methyl or both (the mixture comprised 2% bensulfuron-methyl and 34% quinclorac in water). According to application concentration (500–1000 μg L^{-1}) to paddy rice fields, final concentrations of herbicides added ranged from 5 to 2000 μg L^{-1}. The cultures were incubated at 30 °C with shaking at 130 rpm for 18 h. Bacterial cells were then harvested by centrifugation at 6000 rpm for 15 min. The growth curve of *S. maltophilia* WZ2 at different herbicide concentrations were obtained with ultraviolet spectrophotometer at 600 nm.

2.2.2. Stationary-phase cells exposed to herbicides

S. maltophilia WZ2 and *E. coli* K12 were cultured in LB medium at 30 °C with shaking at 130 rpm for 24 h. Quinclorac, bensulfuron-methyl or both were added to final concentrations of 300, 150, and 300 μg L^{-1}, respectively (the concentrations were obtained according to the experiments mentioned above). These cultures were also incubated at 30 °C with shaking at 130 rpm, and were harvested by the same method outlined above after treatment with various herbicides for 0, 0.5, 1.5, 3.5, 4.5, 7, 12, and 18 h.

2.3. Preparation of crude extracts

Bacterial cells harvested were washed twice using 9 g L^{-1} NaCl and resuspended in 2 mL of the same solution. Suspensions were sonicated on ice for 800 s (interval of 3 s working with 5 s cooling between bursts) and centrifuged at 12 000 rpm for 20 min at 4 °C. The supernatant protein content was determined using the Bradford assay.

2.4. Enzyme activity assays

SOD and catalase activities of cell lysates were determined spectrophotometrically at 550 and 240 nm, respectively, with Kit A001 and A007. One unit of SOD activity was defined as the amount of lysate that inhibits the rate of xanthine/xanthine oxidase-dependent cytochrome c reduction at 25 °C by 50%. One unit of catalase activity was defined as the amount of enzyme that catalyzes the decomposition of 1.0 μmol of H_2O_2 per minute. All reagents were purchased from Nanjing Jiancheng Bioengineering Institute, Jiangsu Province, China.

2.5. Statistical analyses

All data were represented as means ± S.E. of three replicates. Statistical analyses of specific activities were performed using Duncan's honestly significant difference test. For all statistical analyses, the relationships were considered to be significant when $p<0.05$.

2.6. Electrophoresis and staining

The extracts of stationary-phase bacteria were separated by electrophoresis using 6% (for catalase) or 10% (for SOD) non-denaturing polyacrylamide gel electrophoresis (PAGE). Gel lanes were loaded with 17.8 and 27 μg total protein for SOD and catalase determinations, respectively. Bacterial extracts were then separated by electrophoresis in the presence of Tris (50 mmol L^{-1}), glycine (300 mmol L^{-1}), and EDTA (1.8 mmol L^{-1}) at a constant current (20 mA) for 2–3 h. SOD and catalase activities were visualized on gels according to the methods described by Beauchamp and Fridovich (1971) and Woodbury et al. (1971), respectively.

2.7. Determination of the type of SOD

The metal present in the active site of SOD molecule was determined using inhibition methods according to Dunlap and Steinman (1986).