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Short communication

Transformation of methylthio-*s*-triazines via sulfur oxidation by strain JUN7, a *Bacillus cereus* species

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Abstract

It is known that methylthio-*s*-triazines can be transformed to the corresponding 2-hydroxy derivatives through sulfoxides and sulfones in aerobic and flooded soil; however, production of sulfoxides and/or sulfones from methylthio-*s*-triazines by isolated *s*-triazine-degrading bacteria has not been reported yet. In the present study, a new bacterial strain, JUN7, was obtained from Japanese soil; the bacterium is capable of transforming simetryn to 2-methylsulfinyl 4,6-bis(ethylamino)-*s*-triazine (sulfoxide simetryn) and 2-hydroxy-4,6-bis(ethylamino)-*s*-triazine (2-hydroxy simetryn) in a Luria-Bertani (LB) medium. This is the first isolation of the specific microorganism that mediates sulfur oxidation of methylthio-*s*-triazines, as far as we know. Strain JUN7 could decrease other methylthio-*s*-triazines such as dimethametryn and prometryn, but not chlorinated *s*-triazines (atrazine, simazine, and terbuthylazine) and methoxy-*s*-triazine (atraton) in 1/10 LB medium. Strain JUN7 did not possessed gene *atzA* or *trzN* encoding triazine-degrading enzymes, suggesting that the strain may have another metabolic system. Characterizations of strain JUN7 based on comparative morphology, physiological classification, and comparison of the partial 16S rRNA sequence indicated that it is assigned as a *Bacillus cereus* species.

Keywords: *Bacillus cereus*; Co-metabolic transformation; Isolation; Liquid chromatography-mass spectrometry; Methylthio-*s*-triazines; Simetryn; Sulfur oxidation

Methylthio-*s*-triazines are popular herbicides in Asia, since they are used in the control of broadleaf weeds in irrigated rice fields. In a metabolic study of methylthio-*s*-triazines in aerobic and flooded soil using ring and meththio-^{14}C-labeled prometryn (Kaufman and Kearney, 1976), the labeled sulfoxide, sulfone and the corresponding 2-hydroxy derivative were produced (Fig. 1). As microorganisms capable of degrading methylthio-*s*-triazines, Cook and Hütter (1982) isolated three Gram-negative bacterial strains that could utilize ametryn and/or prometryn as the sole sulfur source for growth. More recently, Aislabie et al. (2005) reported that atrazine-degrading *Arthrobacter nicotinovorans* strain HIM isolated from agricultural soil in New Zealand could degrade prometryn. Enzymatic transformation of methylthio-*s*-triazines to 2-hyroxy derivatives using bacterial species that can degrade atrazine, a chlorinated-*s*-triazine, was also reported. Topp et al. (2000) showed anaerobic transformation of methylthio-*s*-triazines by whole cells or cell extracts of *Nocardioides* sp. strain C190, and Seffernick et al. (2000) transformed ametryn to 2-hydoroxy ametryn using cell extracts obtained from *Clavibacter michiganensis* strain ATZ1. In addition, *Arthrobacter aurescens* strain TC1 could grow in the medium including ametryn or prometryn as the sole nitrogen source (Strong et al., 2002), and recombinant triazine hydrolase (*trzN*) derived from the strain could rapidly transform ametryn and ametryn sulfoxide to 2-hydroxy ametryn (Shapir et al., 2005). However, these available reports regarding the isolated degrading-bacteria did not demonstrate production of sulfoxide and/or sulfone during bacterial or enzymatic transformation of methylthio-*s*-triazines to 2-hydroxy derivatives.

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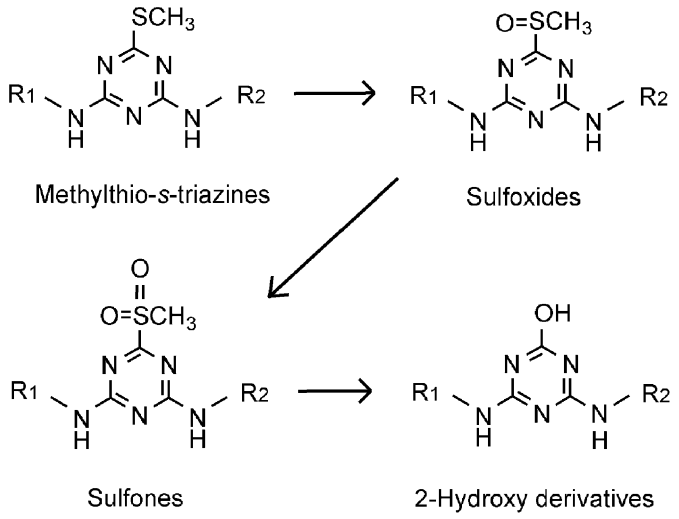

Fig. 1. Metabolic pathway of methylthio-*s*-triazines in aerobic and flooded soil proposed by Kaufman and Kearney (1976).

The present study was conducted to obtain microorganisms capable of degrading simetryn, which is the most popular methylthio-*s*-triazine herbicides in Japan, and a new bacterial strain was successfully isolated. The possible metabolic pathway was, then, investigated and sulfoxides were determined as the intermediate metabolites by liquid chromatography-mass spectrometry (LCMS) analyses, as mentioned below.

Analytical-grade *s*-triazines were purchased from Kanto Kagaku (Tokyo, Japan). Concentrations of *s*-triazines in culture media were determined by reverse-phase high-performance liquid chromatography (HPLC; Waters, Milford, Mass.) under the following conditions: separation, 40 °C with an ODS column (CapcellPaK C18 UG120 S5; 3 × 250 mm, Shiseido, Tokyo, Japan); mobile phase, water/acetonitrile (e.g., 3:2 for simetryn); and flow rate, 1 ml min^{-1}; detection, UV at 220 nm; injection volume, 10 μL; quantification, the external standard method.

Fourteen soil samples collected in Japan were examined as the bacterial sources. Each 0.1 g of the soil samples was suspended in 10 ml of sterile saline, and an aliquot (0.5 ml) of the suspension was added into L-shaped glass tubes (16 mmϕ; long leg, 120 mm; short leg, 70 mm) containing 6 ml of the minimum salt (MS) medium (0.5 g of $NaNO_3$, 0.5 g of K_2HPO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, and trace amount of $FeSO_4 \cdot 7H_2O$ per liter, adjusted to pH7) or the 1/10 Luria-Bertani (LB) medium (1 g of Bacto-tryptone, 0.5 g of yeast extract, and 1 g of NaCl per liter), supplemented with 5 mg of simetryn per liter. Cultivation was performed under dark conditions with shaking (26 °C). Concentrations of simetryn in the cultures were periodically measured by HPLC. Of the 14 soil samples, one showed a significant reduction in the simetryn concentration, when it was cultured in 1/10 LB medium. The culture was diluted in sterile saline, and an aliquot was spread on 1/10 LB agar plates. After a few days incubation at 30 °C, a number of colonies found on the plates were individually inoculated into L-shaped glass tubes containing 6 ml of 1/10 LB medium supplemented with simetryn and were shaken at 26 °C. Changes in the simetryn concentrations were examined by HPLC and positive cultures were selected. Colonization on 1/10 LB agar plates supplemented with simetryn and examination of simetryn-degrading ability in 1/10 LB medium were repeated several times. Thereby, a bacterial strain, named strain JUN7, was finally isolated as a bacterium that could significantly decrease simetryn concentration. Growth of strain JUN7 and its simetryn degradation in 1/10, 1/5 (2 g of Bacto-tryptone, 1 g of yeast extract, and 2 g of NaCl per liter), 1/2 (5, 2.5 and 5 g l^{-1}, respectively), and normal LB (10, 5 and 10 g l^{-1}, respectively) media supplemented with 5 mg L^{-1} simetryn (30 °C) is shown in Fig. 2. The presence of the higher nutrient levels led to higher cell numbers (Fig. 2a) and faster reduction in the simetryn concentrations (Fig. 2b). An unknown peak temporarily increased indicating that it may

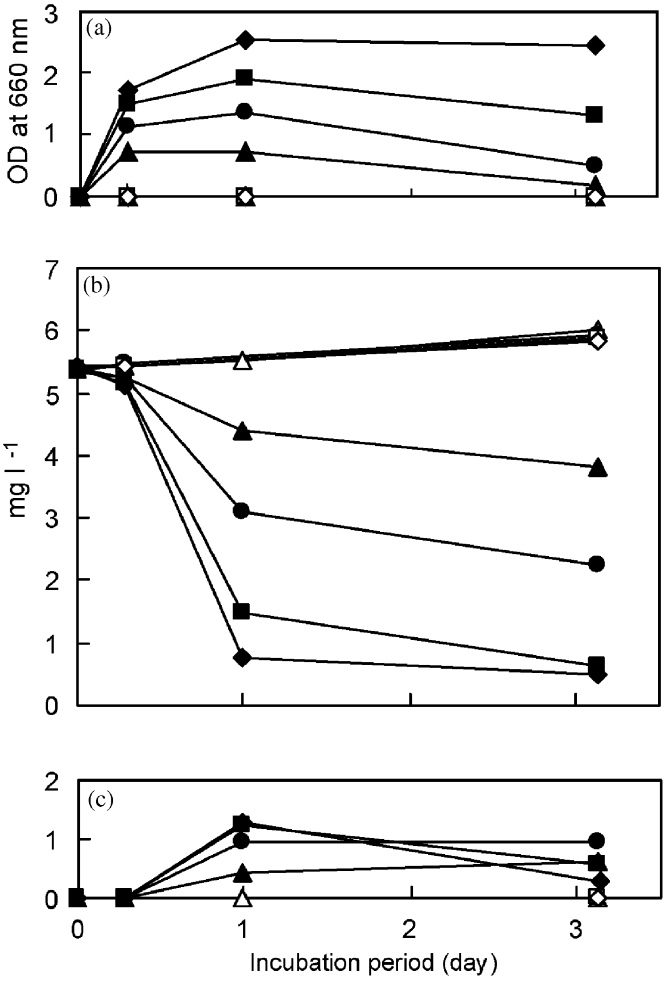

Fig. 2. Growth of strain JUN7 (a), simetryn degradation by the isolate (b), and the subsequent appearance of an unknown metabolite (c) in 1/10 (▲), 1/5 (●), 1/2 (■) and normal LB media (◆) supplemented with 5 mg l^{-1} of simetryn. White-colored symbols mean the respective uninoculated controls. Concentration of the unknown metabolite was temporarily calculated based on the peak areas of the simetryn standard, presuming that their molar extinction coefficients at 220 nm are similar.