Transformation of roxarsone by *Enterobacter* sp. CZ-1 isolated from an arsenic-contaminated paddy soil

K. Huang, F. Gao & F.-J. Zhao

College of Resources and Environmental Sciences,
Nanjing Agricultural University, Nanjing, P.R. China

ABSTRACT: Roxarsone [Rox(V)] is widely used as a feed additive in poultry industries. This arsenic-containing compound may be degraded by microbes to release toxic inorganic arsenic in the environment. To date, most of the studies of microbial mediated Rox(V) degradation have focused on anaerobic microorganisms. Here, we isolated a pure cultured aerobic Rox(V)-degrading bacterial strain, CZ-1, from an arsenic-contaminated paddy soil in China. On the basis of 16S rRNA gene sequence, strain CZ-1 was classified as a member of genus *Enterobacter*. This strain could efficiently degrade Rox(V), converting all of 10 μ M Rox(V) within 24 h in liquid culture. Four metabolites including n-acetyl-4-hydroxy-m-arsanilic acid (N-AHPAA[V]), 3-amino-4-hydroxyphenylarsonic acid (3-AHPAA[V]), arsenite (As[III]) and an unknown arsenic compound were detected and identified by HPLC-ICP-MS and HPLC-LC-MS. N-AHPAA(V) was the main product, likely to be formed from acetylation of 3-AHPAA(V). Based on these results, a novel degradation pathway of Rox(V) by *Enterobacter* sp CZ-1 is proposed.

1 INTRODUCTION

The extensive use of the organoarsenical 3-nitro-4-hydroxybenzene arsonic acid (roxarsone; Rox[V]) as a feed additive in the poultry industry can lead to increasing arsenic contamination of soil and water environments (Nachman *et al.*, 2005; O'Conner *et al.*, 2005). Previous studies using chicken feces and sewage sludge show that microbes play significant roles in degrading Rox(V) in the environment (Cortinas *et al.*, 2006; Stolz *et al.*, 2007). Stolz *et al.* (2007) reported that *Clostridium* species were mainly responsible for Rox(V) degradation under anaerobic conditions in their enrichment cultures obtained from chicken litter. They also isolated an anaerobic Rox(V)-degrading bacterium *Clostridium* OhILAs (Stolz *et al.*, 2007). Two other anaerobic bacteria, *Shewanella oneidensis* MR-1 and *Shewanella putrefaciens* CN32, have also been found to possess the Rox(V) degradation ability (Chen *et al.*, 2016; Han *et al.*, 2017). Roxarsone biodegradation activity of an aerobic microbial consortium has also been demonstrated (Guzman-Fierro *et al.*, 2015), but to date no strains of aerobic microbes capable of Rox(V) degradation have been isolated. The degradation pathway under aerobic conditions remains unknown. In this study, we isolated and characterized an aerobic Rox(V)-degrading strain of *Enterobacter*, named CZ-1, and propose a novel biodegradation pathway.

2 MATERIAL AND METHODS

2.1 Isolation and characterization of a Rox(V)-degrading bacterium

One strain with a Rox(V)-degrading ability was isolated from an As-contaminated paddy soil and named CZ-1. Its ability for Rox(V) degradation was determined quantitatively. The cell morphology was observed by SEM (S-3000N, Hitachi). The 16S rRNA gene was amplified by PCR and sequenced in GenScript Co. Ltd. The neighbor-joining phylogenetic tree was constructed by MEGA 5.0.

2.2 Chemical analysis

The metabolites produced in Rox(V) degradation were analyzed by HPLC (series 200; PerkinElmer)-ICP-MS (NexION 300D; PerkinElmer) using an anion-exchange column (Hamilton PRP-X100; 250 mm by 4.6 mm) eluted with the mobile phase [A: 50 mM (NH₄)₂HPO₄; B: water] in gradient elution and LC-MS consisting of an Agilent 1200 series HPLC coupled to an Agilent 6410B triple quadrupole mass spectrometer using an Agilent Eclipse Plus C18 column (2.1 $\times$ 150 mm, 3.5 μ m) with the mobile phase [95% solution A (0.1% formic acid) and 5% solution B (acetonitrile)] at a flow rate of 0.2 mL min⁻¹.

3 RESULTS AND DISCUSSION

3.1 Strain isolation and characterization

One strain, designated strain CZ-1, capable of degrading Rox(V) was isolated from an arsenic-contaminated paddy soil in Chenzhou city, Hunan province, China. After 24 h of inoculation, strain CZ-1 was able to degrade all of 10 μ M Rox(V) in ST10⁻¹ medium. Cells were Gram-negative, aerobic, and motile. A phylogenetic tree based on the 16S rRNA gene sequence of strain CZ-1 was constructed. Strain CZ-1 was closely related to *Enterobacter* sp. X72, with a sequence similarity score of 99%, suggesting that strain CZ-1 is an *Enterobacter* species.

3.2 Identification of the metabolites during Rox(V) degradation

The products of the degradation of Rox(V) by strain CZ-1 was first analyzed by HPLC-ICP-MS. The HPLC-ICP-MS analysis showed that nearly all of Rox(V) (10 μ M) in the culture medium was converted to four As-containing metabolites designated compounds 1, 2, 3 and 4. Compound 4 was found to be the main product. Compounds 1, 2 and 4 were preliminarily identified as As(III), 3-AHPAA(V) and N-AHPAA(V), respectively, while compound 3 was still unknown that did not correspond to several common phenylarsine compounds such as 4-hydroxyphenylarsonic acid (HPAA[V]), 4-aminophenylarsonic acid (4-APAA[V]), 2-aminophenylarsonic acid (2-APAA[V]) and carbarsone. When treated with 30% H₂O₂, compound 1 was transformed to As(V), providing further evidence that compound 1 was As(III). In addition, the LC-MS analysis demonstrated the presence of 3-AHPAA(V) and N-AHPAA(V), confirming the results from the HPLC-ICP-MS analysis.

On the basis of above results, we propose a novel transformation pathway of Rox(V) mediated by strain CZ-1, which is different from the putative degradation pathways in *Alkaliphilus oremlandii* strain OhILAs (Thomas *et al.*, 2014) and *Shewanella putrefaciens* strain CN32 (Han *et al.*, 2017). In the new pathway, the nitro group in Rox(V) is first reduced to amino group to form the compound 3-AHPAA(V), and the latter is then acetylated into N-AHPAA(V). N-AHPAA(V) was found to be stable.

4 CONCLUSIONS

To our best knowledge, strain CZ-1 is the first aerobic bacterium with a Rox(V)-degrading ability isolated

from an arsenic-contaminated paddy soil. A novel transformation pathway of Rox(V) by this strain is proposed.

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