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A key esterase required for the mineralization of quizalofop-*p*-ethyl by a natural consortium of *Rhodococcus* sp. JT-3 and *Brevundimonas* sp. JT-9

Hui Zhang, Mengya Li, Jie Li, Guangli Wang*, Feng Li, Dayong Xu, Yuan Liu, Minghua Xiong

College of Life Sciences, Huaibei Normal University, Huaibei 235000, China

HIGHLIGHTS

- This is the first report on the mineralization of QE by a -two-bacterial consortium.
- Synergistic degradation and growth of the consortium was investigated against QE.
- A metabolite during QE degradation by *Rhodococcus* sp. was identified by LC-MC.
- A key esterase for the mineralization of QE was obtained from *Rhodococcus* sp.
- The recombinant esterase was expressed, purified and characterized.

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ABSTRACT

A natural consortium, named L1, of *Rhodococcus* sp. JT-3 and *Brevundimonas* sp. JT-9 was obtained from quizalofop-*p*-ethyl (QE) polluted soil. The consortium was able to use QE as a sole carbon source for growth and degraded 100 mg L^{-1} of QE in 60 h. Strain JT-3 initiated the catabolism of QE to quizalofop acid (QA), which was used by strain JT-9 as carbon source for growth and to simultaneously feed strain JT-3. A novel esterase EstS-JT, which was responsible for the transformation of QE to QA and essential for the mineralization of QE by the consortium, was cloned from strain JT-3. EstS-JT showed low amino acid identity to other reported esterases from esterase family VIII and represents a new member of this family. The deduced amino acid sequence contained the esterase family VIII conserved motifs S-X-X-K, YSV and WAG. The purified recombinant EstS-JT displayed maximal esterase activity at 35 °C and pH 7.5. An inhibitor assay, site-directed mutagenesis and 3D modeling analysis revealed that S_{64}, K_{67} and Y_{175} were essential for catalysis and probably comprised the catalytic center of EstS-JT. Additionally, EstS-JT had broad substrate specificity and was capable of hydrolyzing *p*-nitrophenyl esters (C_2-C_8) and various AOPP herbicides.

1. Introduction

Quizalofop-*p*-ethyl {QE; ethyl(R)-2-[4-(6-chloroquinoxalin-2-yloxy) phenoxy] propionate} is a member of the aryloxyphenoxypropionate (AOPP) family of herbicides, which are widely used for post emergence control of annual and perennial grass weeds [1]. QE is a systemic herbicide that acts by inhibiting acetyl-CoA carboxylase (ACCase), an enzyme essential for fatty acid biosynthesis in broadleaved weeds [2]. Quizalofop acid (QA), the carboxyl ester hydrolysate of QE in soil, plants and animals [3–5], has been identified as the major active herbicide that inhibits the growth of weeds with broad leaves [6]. Although QE and its primary transformation product QA exhibit low toxicity and potential for harm, the widespread application of QE has caused environmental contamination and aquatic ecosystem destruction owning to high accumulation and persistence [7,8]. QE has been proven to result in reproductive toxicity [9] and genotoxicity [10], induce liver injury occupational exposure to QE [11]. Ma et al. investigated the toxicity of QE and QA to earthworms, which contribute to the breakdown of large pieces of organic matter and enhance microbial activity, and showed that QA had higher toxicity than the parent compound against *Eisenia foetida* [12]. Meanwhile, QA has been found

* Corresponding author.
E-mail address: guangliwang@aliyun.com (G. Wang).

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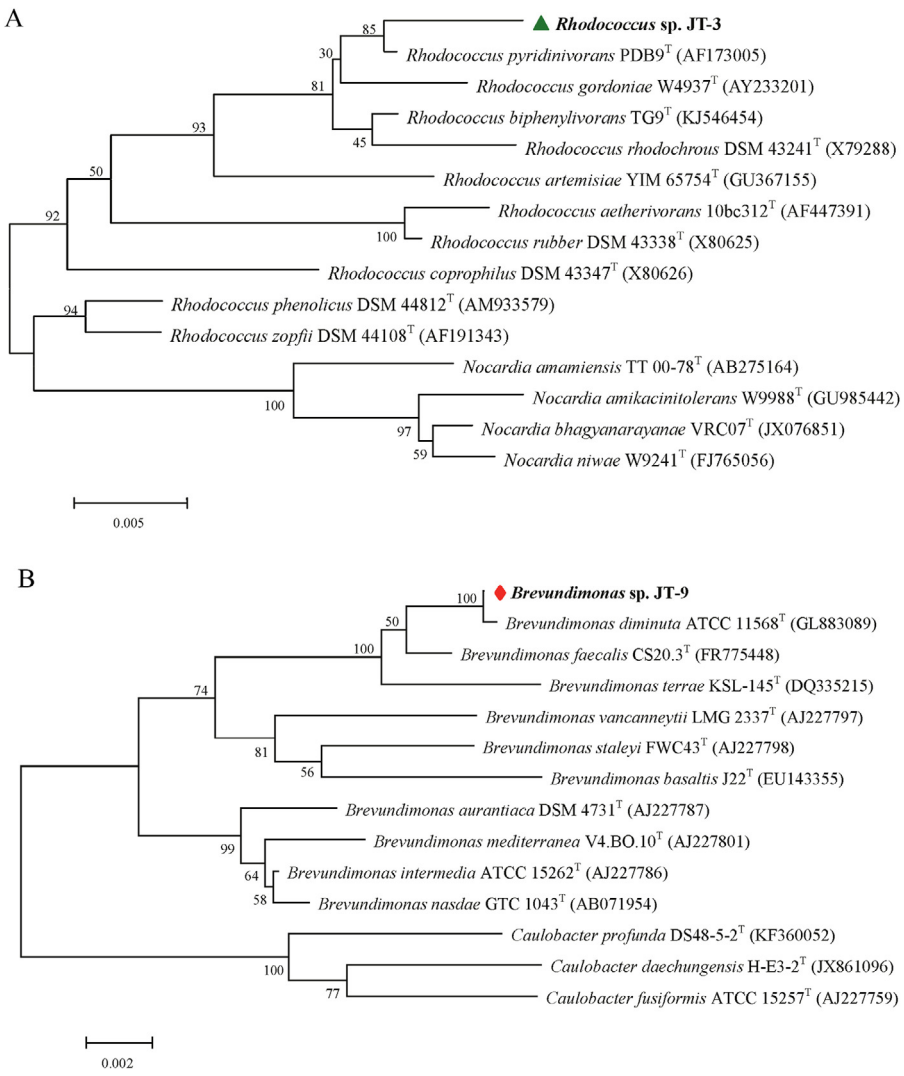

Fig. 1. Phylogenetic tree based on the 16S rRNA gene sequences from strain JT-3 (A) and JT-9 (B) as well as related species, respectively. Bootstrap values (%) obtained with 1000 replicates are indicated at the branch points.

in surface water ($2.5 \pm 0.9\ \mu g\ L^{-1}$) and muscle tissue of freshwater fish ($5 \pm 1\ \mu g\ g^{-1}$) [13], leading to an potential risk for ecosystems. Accordingly, it is extremely important to evaluate the degradation of these two compounds in the environment.

In general, QE used in agriculture can be transformed by physical and chemical processes, however, microorganism mediated degradation is the main environmental remediation mechanism without harmful transformation products. Until now, four bacterial strains have been reported to be capable of hydrolyzing another AOPP herbicide, fenoxaprop-ethyl (FE), including *Rhodococcus* sp. strain T1 [14], *Pseudomonas azotoformans* QDZ-1 [15], *Acinetobacter* sp. strain DL-2 [16], and *Rhodococcus ruber* JPL-2 [17]. Four esterase genes hydrolyzing the ester bond of AOPP herbicides to produce the corresponding acid have been identified from above mentioned four bacterial strains [14–17]. To the best of our knowledge, there have been no reports showing that pure microbial cultures can completely degrade QE. A microbial consortium was able to utilize FE as a sole carbon and nitrogen source for growth and transform FE to the corresponding fenoxaprop acid (FA), 6-chloro-2,3-dihydrobenzoxazol-2-one (CDHB) [18]. Recently, two enrichment cultures were identified that could completely degrade FE, and three intermediate metabolites (FA, CDHB and 2-(4-hydroxyphenoxy) propionic acid (HPP)) were identified by HPLC/MS [14,19]. Other studies have demonstrated that diclofop-methyl could be transformed to diclofop acid, 4-(2,4-dichlorophenoxy) phenol, 2,4-dichlorophenol and phenol by *Chryseomonas luteola and Sphingomonas paucimobilis* [20,21]. Nevertheless, QE mineralization by a microbial consortium has never been reported and QE hydrolases have not been extensively explored.

Here, we report a natural consortium, named L1, comprising of two bacterial species (*Rhodococcus* sp. strain JT-3 and *Brevundimonas* sp. strain JT-9) that was capable of using QE as its sole carbon source for growth, and completely degraded 100 mg L^{-1} QE within 60 h. Additionally, a novel QE-hydrolyzing esterase, EstS-JT, from *Rhodococcus* sp. strain JT-3 was characterized and was found to be involved in the transformation of QE to QA, which is the key step for the mineralization of QE by consortium L1.

2. Materials and methods

2.1. Chemicals and media

Quizalofop-*P*-ethyl (97.8%), quizalofop-*P*-tefuryl (97.5%), fenoxaprop-*P*-ethyl (97.0%), haloxyfop-*P*-methyl (97.5%), cyhalofop-butyl (98.1%) and clodinafop-propargyl (97.6%)