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Research article

Cometabolic biodegradation of quizalofop-p-ethyl by *Methylobacterium populi* YC-XJ1 and identification of QPEH1 esterase

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ABSTRACT

Background: Quizalofop-p-ethyl (QPE), a unitary R configuration aromatic oxyphenoxypropionic acid ester (AOPP) herbicide, was widely used and had led to detrimental environmental effects. For finding the QPE-degrading bacteria and promoting the biodegradation of QPE, a series of studies were carried out.
Results: A QPE-degrading bacterial strain YC-XJ1 was isolated from desert soil and identified as *Methylobacterium populi*, which could degrade QPE with methanol by cometabolism. Ninety-seven percent of QPE (50 mg/L) could be degraded within 72 h under optimum biodegradation condition of 35°C and pH 8.0. The maximum degradation rate of QPE was 1.4 mg/L/h, and the strain YC-XJ1 exhibited some certain salinity tolerance. Two novel metabolites, 2-hydroxy-6-chloroquinoxaline and quinoxaline, were found by high-performance liquid chromatography/mass spectroscopy analysis. The metabolic pathway of QPE was predicted. The catalytic efficiency of strain YC-XJ1 toward different AOPPs herbicides in descending order was as follows: haloxyfop-p-methyl ≈ diclofop-methyl ≈ fluazifop-p-butyl > clodinafop-propargyl > cyhalofop-butyl > quizalofop-p-ethyl > fenoxaprop-p-ethyl > propaquizafop > quizalofop-p-tefuryl. The genome of strain YC-XJ1 was sequenced using a combination of PacBio RS II and Illumina platforms. According to the annotation result, one α/β hydrolase gene was selected and named *qpeh1*, for which QPE-degrading function has obtained validation. Based on the phylogenetic analysis and multiple sequence alignment with other QPE-degrading esterases reported previously, the QPEH1 was clustered with esterase family V.
Conclusion: M. populi YC-XJ1 could degrade QPE with a novel pathway, and the *qpeh1* gene was identified as one of QPE-degrading esterase gene.
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1. Introduction

Aromatic oxyphenoxypropionic acid esters (AOPPs) are selective postemergence herbicides for controlling annual and perennial grassy weeds (wild oat, barnyard grass, green bristlegrass, common crabgrass, wild millet, and goosegrass) containing homogeneous acetyl-coA carboxylase, and they show no activity for broadleaf weeds and dicotyledonous plants containing heterogeneous acetyl-coA carboxylase [1,2]. AOPPs belong to the internally absorbed conductive herbicides, absorbed from the leaf surface and translocated throughout the plant via the xylem and phloem, and accumulated in the meristematic tissue, inhibiting fatty acid biosynthesis, and thus causing weed necrosis [3]. In 2014, worldwide sales volume of AOPPs reached $12.17 billion, with 4.6% of the global herbicides market [4].

Quizalofop-p-ethyl (QPE), a unitary R configuration AOPP [5], which was developed by Nissan Chemical Industries in 1984, was declared as an active principle in commercial formulations of herbicides. QPE was predicted to rapidly increase in the coming years in an effort to overcome the widespread appearance of glyphosate-resistant weeds [6]. Because of the excellent performance, QPE was used in crops such as potatoes, soya beans, sugar beets, peanuts, oilseed rape, sunflowers, vegetables, cotton, flax, and other broad-leafed plants [7].

The widespread use of QPE has led to detrimental effects in following few aspects: a mixed cholestatic/hepatocellular liver injury [8]; alterations of gene expression in fatty acid degradation pathways [6]; reproductive toxicity in male rats [9,10]; endocrine disruption and acute toxicity to *Brachydanio rerio* [11]; genetic toxicity to *Misgurnus anguillicaudatus* and *Paramisgurnus dabryanus* [12]; phytotoxicity to *Scenedesmus obliquus* and *Lemna minor* [13,14]; microbial toxicity to

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Trichoderma harzianum and other soil microorganism [15,16]; QPE and its major metabolite quizalofop-p (QP) could be degraded enantioselectively, and QP (LC_{50}: 26.69 μg cm^{-2}) has much higher toxicity than the QPE (LC_{50}: 209.2 μg cm^{-2}) to *Eisenia fetida* [17]. According to the World Health Organization (WHO) definition of adverse drug reactions (2000) and U.S. Environmental Protection Agency (EPA) toxicity class III (2011), QPE is a toxic chemical, already prohibited by the European Union. Some countries set limits to its use: the maximum residue limit of QPE on vegetables is 0.06 mg/L in Canada, but QPE can be freely used without restrictions in China. Overused QPE has even been a raw water contaminant [14], so eliminating the residue of QPE was crucial to environmental remediation.

Remediation of polluted sites using a microbial process has proven effective and reliable due to its eco-friendly features [18]. Therefore, a series of microorganisms with degradation capacity for different AOPPs have been isolated in recent years. Some esterases from *Pseudomonas* [7,19], *Aquamicrobium* [20], *Acinetobacter* [21], *Ochrobactrum* [22], and *Rhodococcus* [23] were identified to degrade QPE extracellularly. Most of them belong to family V, VII, and VIII, and the similarity of amino acid sequences is quite different. However, research on degradation characteristics of bacteria was not adequate that they could be used in practice for bioremediation. The degradation of QPE only stayed on the preliminary stage, i.e., the transformation from QPE to QP, which was the main metabolite of QPE. To date, no studies have reported on the degradation of QP by a single strain. Its degradation mechanism remains unclear. Therefore, the superior degrading bacterial resources and complete mineralization pathways of QPE were needed emergently.

In this research, we report the isolation and QPE-degrading characteristics of *Methylobacterium populi* YC-XJ1, and we further explored the metabolites of QPE. The function of *qpeh1* was verified based on the genomic sequence and the annotation of Swiss-Prot.

2. Materials and methods

2.1. Chemicals and reagents

Standards of QPE (98.7% of purity), cyhalofop-butyl (96.5% of purity), clodinafop-propargyl (98.2% of purity), fenoxaprop-p-ethyl (99.2% of purity), haloxyfop-p-methyl (98.2% of purity), fluazifop-p-butyl (94.8% of purity), diclofop-methyl (99.6% of purity), quizalofop-p-tefuryl (97.9% of purity), propaquizafop (99% of purity), and metamifop (94.1% of purity) were purchased from Shenyang Research Institute of Chemical Industry Co., Ltd. Quizalofop-p (QP, >95% of purity), (4-(6-Chloroquinoxalin-2-yloxy) phenol (CYP, 95% of purity), and quinoxaline (95% of purity) were purchased from Sigma. Stock solutions (1×10^4 mg/L) of all standard substances were prepared by dissolving them in methanol (HPLC grade).

2.2. Medium

Mineral salts medium (MSM) composed of 1.5 g NH_4NO_3, 0.5 g $KH_2PO_4 \cdot 12\ H_2O$, 1.5 g K_2HPO_4, 0.2 g $MgSO_4 \cdot 7\ H_2O$, 0.5 g NaCl, and 1‰ (*v*/v) trace element solution (TES) in 1.0 L water [24]. TES contained $FeSO_4 \cdot 7\ H_2O$ (2.0 g/L), ZnSO4 (0.1 g/L), $CuSO_4 \cdot 5\ H_2O$ (0.03 g/L), $MnCl \cdot 4\ H_2O$ (0.03 g/L), $CoCl \cdot 7\ H_2O$ (0.3 g/L), $Na_2MoO_4 \cdot 2\ H_2O$ (0.03 g/L), and $Na_2WO_4 \cdot 2\ H_2O$ (0.02 g/L) [25]. NaCl solution (200 mg/mL) contained 20 g NaCl and 100 mL MSM. Luria-Bertani (LB) medium contained 10 g/L peptone, 5 g/L yeast, and 10 g/L NaCl. The pH values of all media were adjusted to 7.0 ± 0.2 with NaOH or HCl (2 mol/L). All media were sterilized by autoclaving at 121°C for 20 min. The solid medium of MSM and LB was prepared by adding 16 g agar per liter.

2.3. Analytical methods

Three biological replications of 10 mL aqueous samples were extracted with the equal volume of dichloromethane and 800 μL extracts were evaporated in fuming cupboard. Residues were dissolved in 800 μL methanol and the solution was filtered through 0.22 μm membrane (ANPEL, Shanghai, China) before being determined by high-performance liquid chromatography (HPLC) system (1200 series, Agilent Technologies Inc., California, USA) equipped with a C18 column (Agilent Eclipse XDB, 5 μm, 4.6 × 150 nm) and a diode array detector. Parameters of the detection method were appropriately modified based on the description as given [7,26]. See Table S1 for details.

2.4. Enrichment and isolation of QPE-degrading bacteria

The soil sample was collected from farmland near the desert region (latitude, 39°23′24″N and longitude, 99°26′33″E) in the Gansu province of China. A soil sample of 10 g was added to 100 mL MSM medium with 25 mg/L QPE and 2.5‰ (*v*/v) methanol (i.e., containing 250 μL QPE stock solutions). After incubating at 30°C and 180 rpm for 7 d, 10 mL enrichment culture was transferred into another fresh MSM medium containing double volume (500 μL) QPE stock solutions and incubated for 7 d. After four rounds of transfer, the enrichment culture was diluted and spread on MSM agar plates supplemented with 100 mg/L QPE and 10‰ methanol. After incubation for 3 d, a single colony was picked out and inoculated into 10 mL MSM medium containing 50 μL QPE stock solutions and incubated for 7 d to detect the degrading capabilities by HPLC. The target colonies will be purified by repetitive streaking on MSM agar plates and tested for their degrading capabilities.

2.5. Growth and degradation experiments

The isolate YC-XJ1 growing in 100 mL MSM medium containing 50 mg/L QPE and 5‰ methanol (i.e., supplemented with 500 μL QPE stock solutions) for about 28 h was harvested by centrifugation (5000 rpm, 10 min), then washed thrice with fresh MSM and adjusted to about OD_{600} 1.0. The bacterial suspension was inoculated (1%, *v*/v) into 10 mL MSM containing 50 mg/L QPE and 5‰ methanol (i.e., supplemented with 50 μL QPE stock solutions), and incubated at 30°C and 180 rpm. Bacterial growth was monitored by a UV–VIS spectrophotometer (Thermo Scientific, Massachusetts, USA) at every 12 h interval incubation, and residual concentrations of QPE were determined by HPLC. Each treatment was performed in three replicates, and the samples without inoculation were set as control.

2.6. Identification of QPE-degrading bacteria

The isolate YC-XJ1 was identified by morphological observation, physiological-biochemical characteristics, and 16S rRNA gene analysis. The strain YC-XJ1 was inoculated in the solid MSM medium plate for 3 d, and the morphology of the isolates was observed by using the gram-stain reaction under an Olympus microscope. Moreover, observation of cell morphology was performed by a transmission electron microscope (TEM) (H-7500, Hitachi, Tokyo, Japan). The physiological and biochemical characterizations were carried out according to instructions of BIOLOG Gen III microwell plate, for the detection of 71 kinds of sole carbon source and sensitivity of 23 kinds of chemical substances. Gene sequence, 16S rRNA, was sequenced by Invitrogen Biotechnology Co. Ltd., (Shanghai, China). The 16S rRNA gene sequences of other species were downloaded from NCBI. Phylogenetic tree analysis was constructed by the maximum likelihood method using MEGA 5.0 software, as described by Green and Ardley [27].

2.7. Effects of environmental factors on QPE degradation

Effects of various environmental conditions like temperature (10–50°C), pH (4–12), inoculums density (1–8%, v/v), maximum tolerance of QPE (50–300 mg/L), and salinity (0.5–80 g/L NaCl addition) were examined on the degradation of QPE. The initial concentration was set as 50 mg/L QPE and 5‰ methanol. Incubation time and time intervals would be adjusted when needed, and other settings were described earlier.

2.8. Analysis of QPE metabolic pathway

MSM (pH 8.0) culture solution of 10 mL was extracted with the equal volume of dichloromethane, and all extracts were evaporated under fuming cupboard. Residues were dissolved in 1000 μL methanol and the solution was filtered through 0.22 μm membrane before being determined by HPLC/mass spectroscopy (HPLC-MS) system as described by Ren et al. [28].

2.9. Substrate utilization tests

The initial concentration was set as 50 mg/L QPE and 5‰ methanol, i.e., 10 mL MSM (pH 8.0) containing 50 μL QPE stock solutions was incubated at 35°C and 180 rpm for 3 d. Each treatment was performed in three replicates, and the samples without inoculation were set as control. The residual concentration was detected by HPLC as described earlier, and standard curves of all substrates were shown in Fig. S1.

2.10. Genomic DNA extraction of strain YC-XJ1

Genomic DNA was extracted using Wizard® Genomic DNA Purification Kit (Promega) according to the manufacturer's protocol. Purified genomic DNA was quantified using a TBS-380 fluorometer (Turner BioSystems Inc., Sunnyvale, CA). High-quality DNA (OD260/280 = 1.8–2.0, >20 μg) was used to do further research.

2.11. Library construction and sequencing

The genome was sequenced using a combination of PacBio RS II Single Molecule Real Time and Illumina sequencing platforms. The Illumina data were used to evaluate the complexity of the genome.

2.12. Assembly and annotation

The data generated from PacBio and Illumina platform were used for bioinformatics analysis. All analyses were performed using I-Sanger Cloud Platform (www.i-sanger.com) from Shanghai Major bio. The glimmer 3.20 was used for prediction of coding DNA sequences (CDSs). The predicted CDSs were annotated from the NCBI nonredundant (NR) database, databases of Swiss-Prot and the Transporter Classification Database (TCDB) [29]. Signal peptides were predicted using signalP 4.0.

2.13. Sequence analysis of qpeh1

Nucleotides and amino acid sequence analyses of the *qpeh1* gene were performed using OMIGA 2.0. Blastn and Blastp tools (www.ncbi.nlm.nih.gov/Blast) were used for nucleotide and amino acid sequence identity searches, respectively. Phylogenetic analysis of protein sequences was performed using MEGA 5.0 software by the neighbor-joining method, bootstrapping of 1000 replicates, and Poisson model.

2.14. Cloning, expression, and purification of the recombinant QPEH1

The *qpeh1* gene was PCR-amplified from the genomic DNA of strain YC-XJ1 using the following primers: sense (5′- GCGC**GGATCC**GTGTTTCCCTTCGACCCAACC-3′), containing a *Bam*H I site (underlined) corresponding to positions 1–21; and antisense (5′- GCGC**AAGCTT**TCTGCGCGTCATCACCTCATCGAT-3′), containing a *Hin*d III site (underlined) after the stop codon and a 6 × His tag before the stop codon. The *qpeh1* PCR product and pET-29a (+) were digested with *Bam*H I and *Hin*d III, and the PCR product was inserted into the expression vector pET-29a (+) to generate the recombinant plasmid pET-*qpeh1*. The overexpression and purification followed the method described by Jia et al. [30].

2.15. Verification of QPE-hydrolysis activity

The initial concentration was set as10 mg/L QPE, and 10 mL MSM (pH 8.0) containing 500 μL purified QPEH1 solutions was incubated at 35°C and 180 rpm for 12 h. Each treatment was performed in three replicates, and the samples without purified QPEH1 solutions were set as control. All samples were extracted with the equal volume of dichloromethane, and 800 μL extracts were evaporated under fuming cupboard. The residues were dissolved in 800 μL methanol, and the residual concentration was detected by HPLC as described earlier.

2.16. Statistical analysis

All data were expressed as means ± standard deviation and were analyzed using SPSS software (version 25.0; IBM SPSS, Armonk, NY, USA). One-way analysis of variance was used to determine significant differences between values. Statistical significance was set at $P \le 0.05$, and $P \le 0.01$ was considered remarkably significant, as described by Wang et al. [31].

2.17. Accession numbers

The nucleotide sequences of the 16S rRNA, *qpeh1* gene, and the genomic sequences of *M. populi* YC-XJ1 have been deposited in the GenBank database under accession numbers **MK789859**, **MN179489**, and **CP039546**, respectively. The strain YC-XJ1 has been deposited in China General Microbiological Culture Collection Center (CGMCC) under the accession number CGMCC **18350**.

3. Results and discussion

3.1. Bacterial enrichment and their ability to degrade QPE

In this study, QPE (50 mg/L) was completely degraded within 5 d by the bacterial culture that resulted from continuous enrichment. An aerobic, pink, QPE-degradation bacterium was isolated and purified from the solid MSM plate, and the strain was named YC-XJ1. Fig. 1a shows the degradation of QPE and the growth of strain YC-XJ1. About 97% of QPE (50 mg/L) could be degraded in 96 h under the condition of pH 7.0 and 30°C. The degradation rate of QPE was positively correlated with the growth curve of strain YC-XJ1. The colony morphology of YC-XJ1 on MSM plate was pink, smooth, round, and eminent. The strain YC-XJ1 is gram-negative, rod-shaped bacteria and both its ends darkly stained under microscope. TEM photos showed a rod-shaped bacterium with the size of 2–8 μm × 0.5–2 μm, convex-bending structure on both ends and flagellum were long, thin, and hollow in Fig. 1b, as described by Fournier et al. [32].

The identification result of genus and species of BIOLOG Gen III microwell plate was *M. extorquens*, SIM value 0.682, >0.5, DIST value 4.533, <5, within the confidence interval (see Fig. S2 for details).

Physiological-biochemical characteristics revealed that strain YC-XJ1 had a rifamycin resistance, sensitivity to nalidixic acid, potassium tellurite, and tetrazolium purple. In carbon source detection experiments, positive results were found only in formic acid, α-ketoglutarate, and L-malic acid (Fig. S2). The results that YC-XJ1 could not utilize any saccharides may be the reason for not growing in LB.

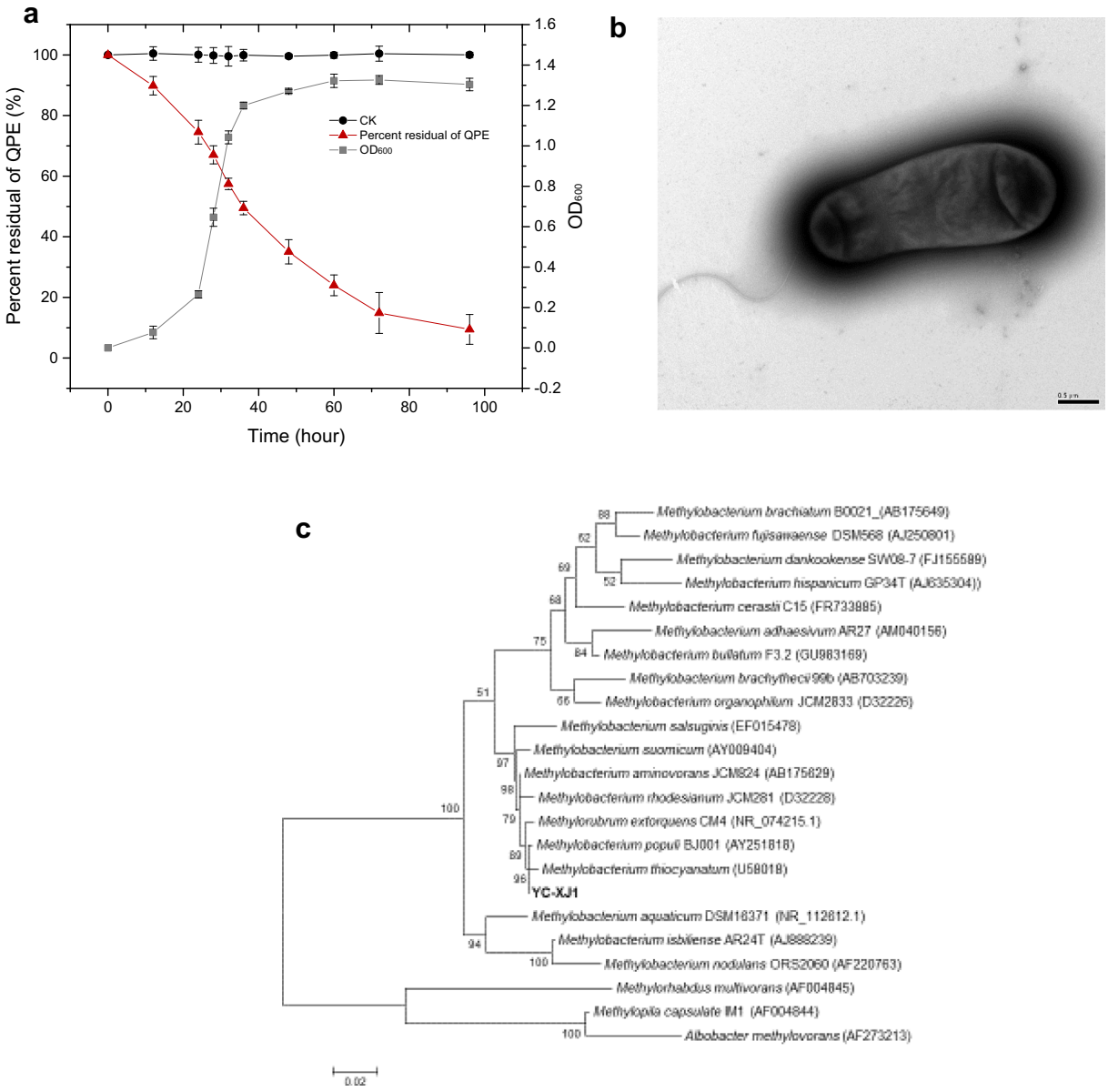

Fig. 1. The morphology and phylogenetic tree analysis of strain YC-XJ1. (a) The growing curve of strain YC-XJ1 and degrading curve of 50 mg/L QPE. (b) The pictures of strain YC-XJ1 under transmission electron microscope. (c) The phylogenetic tree of strain YC-XJ1 based on 16S rDNA sequence analysis. The maximum likelihood tree was constructed with Kimura two-parameter, G + I parameter model, and a bootstrap analysis with 1000 replicates.

The 16S rRNA gene sequence (1446 bp) alignment using BLAST software showed 99.7% identity to *M. populi* BJ001, 99.5% identity to *M. extorquens,* and 99.3% identity to *M. thiocyanatum*. A phylogenetic tree was constructed that included 23 type strains of *Methylobacterium* species. The results of maximum likelihood analysis showed the closest relatives of strain YC-XJ1 were *M. populi* and *M. thiocyanatum* in Fig. 1c.

The genome sequences of YC-XJ1 were 97.9% identical to the type genome of *M. populi* by average nucleotide identity, with 88.6% coverage of the genome. Therefore, the strain YC-XJ1 was finally identified as *M. populi*.

3.2. Effect of environmental factors on QPE degradation

The important factors in bacterial application process were pH and temperature. For the optimization of pH in this study, MSM buffers with a wide range of pH 5–11 were used at 30°C, as shown in Fig. 2a. The degradation rate was above 76.8% in pH 7–9 within 48 h, and the optimum pH was 8.0. For optimization of temperatures 15°C to 50°C were determined using the optimal pH 8.0. As shown in Fig. 2b, the optimum temperature was 35°C and the maximum degradation rate of QPE was 97% within 72 h.

Relatively few studies have reported on the optimal conditions of QPE-degrading bacteria. Zhou et al. [33] reported the optimum condition of *Bacillus pumilis* degrading QPE was 35°C and pH 7.0, the same as *Rhodococcus* sp. J-3 [34], and *B. subtilis* degrading QPE was 30–42°C and pH 7.0–9.0 [35]. In this study, the optimal degradation condition of QPE by *M. populi* YC-XJ1 was 35°C and pH 8.0, consistent with the above-reported data. Similar optimal degrading conditions of dichloromethane by *M. rhodesianum* H13 was 33.7°C and pH 7.55 [36].

Based on the reports, 99% of 100 mg/L QPE could be degraded by *Rhodococcus* sp. JT-3 and *Brevundimonas* sp. JT-9 within 60 h [7]. *Ochrobactrum* sp. QE-9 was able to degrade 78% of 100 mg/L QPE within 72 h, but it must be in the LB solution because *Ochrobactrum*

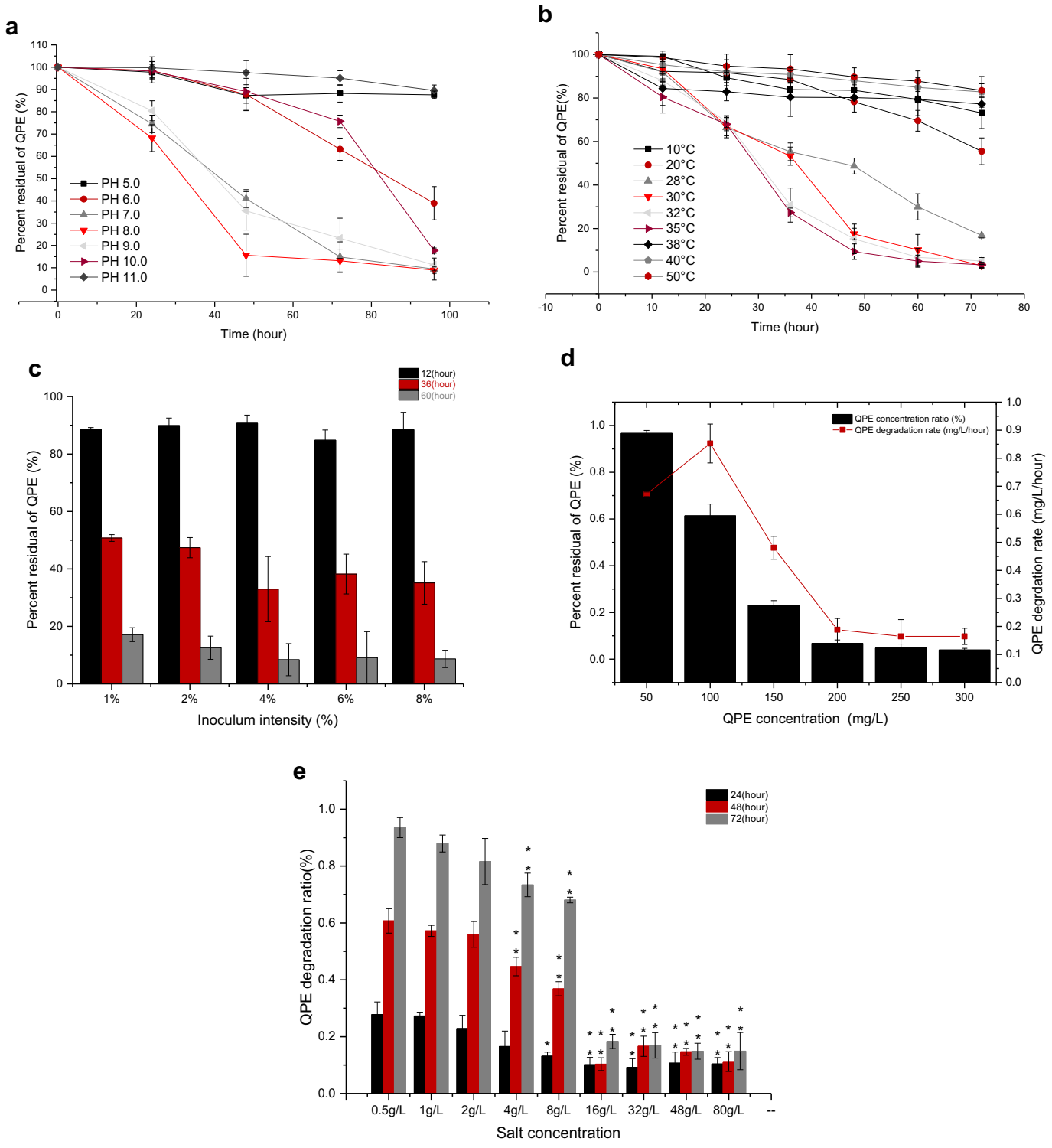

Fig. 2. Effects of environmental factors on QPE degradation by stain YC-XJ1. (a) Effects of pH. (b) Effects of temperature. (c) Effects of initial dose of strain YC-XJ1. (d) Effects of QPE concentration in MSM media. (e) Effects of NaCl concentration in MSM media. * represent significant ($P \leq 0.05$), ** represent remarkably significant ($P \leq 0.01$).

sp. QE-9 was not able to utilize QPE as the sole carbon source for growth [21]. Wang et al. [20] reported that the *Aquamicrobium* sp. FPB-1 was able to degrade 98.5% of 100 mg/L QE within 40 h. In comparison, the *M. populi* YC-XJ1 was able to degrade 97% of 50 mg/L QPE within 72 h.

Members of the *Methylobacterium* genus occupy different habitats due to their great phenotypic plasticity, including soil, water, leaf surfaces, nodules, grains, and air [37]. They potentially play an important role in mitigating ozone depletion resulting from methyl chloride and methyl bromide emissions [38]. Except methane, methanol, formaldehyde, and aminomethane C1 compounds [39,40,41,42], *Methylobacterium* genus also utilizes ethylamine [43], 1-Aminocyclopropane-1-carboxylate [44], isoprene [45], 2,4,6-trinitrotoluene, 4-nitro-2,4-diazabutanal [32], 2,2-bis(p-chlorophenyl)-1,1-dichloroethylene (DDE) [46], and polycyclic

aromatic hydrocarbon [47]. These capabilities suggest that bacteria from this genus may be used for the bioremediation of contaminated environments, such as soil and water [37]. In this study, it was reported for the first time that *Methylobacterium* could degrade AOPPs herbicides. This research provides a new source of degrading bacteria. The soil remediation experiments of *M. populi* YC-XJ1 would be carried out in the future, although none of the *Methylobacterium* have been reported for actual soil remediation.

The influence of inoculum density on QPE degradation is shown in Fig. 2c, and there was no effect on the degradation ratio when inoculum density was from 1% to 8%.

The substrate concentration was also an important factor affecting the degradation rate of degrading bacteria. Because of the lack of relevant information reported, the QPE concentration gradient experiment was performed. As shown in Fig. 2d, the degradation ratio first increased and then decreased with increasing concentration of QPE. The maximum degradation rate of QPE (1.4 mg/L/h) was observed at 100 mg/L QPE solution. When the concentration of QPE in MSM solution exceeded 100 mg/L, substrate inhibition effect occurred. When the QPE concentration reached 150 mg/L, the degradation rate was reduced by 46.3%, and the degradation rate was below 10% in 300 mg/L QPE solution.

The salinity tolerance of QPE degradation bacteria has not been found in previous reports; our studies showed that with the increase of salt concentration, the degradation rate decreased gradually based on three different detection times (24, 48, and 72 h). When the salt concentration reached 4 g/L, the degradation rate decreased significantly. The degradation rate maintained above 80% between 1 g/L and 8 g/L salt concentration, indicating that the strain YC-XJ1 had certain characteristics of salt tolerance. It might be that the saline-alkaline land environment where YC-XJ1 were isolated from affords them salt tolerance. When the salt concentration reached 16 g/L, the degradation rate decreased sharply to 20% in Fig. 2e. This research will provide guidance for the practical application of bioremediation.

3.3. Degrading pathway of QPE

The simultaneous detection method of QPE, QP, and CYP was established using the standard substance by HPLC, as shown in Fig. 3a. According to data that resulted from HPLC analysis shown in Fig. 3b, about 29.6 mg/L of QP and only 0.6 mg/L of CYP were generated after 48 h of incubation with strain YC-XJ1. Combined with the results of HPLC-MS, it can be concluded that the abundance of QP and the sparseness of CYP were generated in the QPE-degrading process. Apparently, QP was the main metabolite of QPE. The QP and CYP were used as sole substrates for continuous degradation, and samples were tested after every 2 d. The results showed a relatively weak degradation ability by strain YC-XJ1, as shown in Fig. 3c. Based on the result of HPLC-MS in Fig. 3d, a final degradation pathway was established, as shown in Fig. 3e.

Previous reports only revealed the degradation product of QP and CYP [7]. In this study, novel products of HCQ and quinoxaline were found. QP could be further degraded with the novel degradation pathway, and the process from HCQ to quinoxaline also demonstrated the dechlorination ability of strain YC-XJ1. The genus *Methylobacterium* showed dehalogenation with dehalogenase in previous reports [48,49].

Compared with QPE, the degradation rate of QP was relatively slow and so it was a rate-limiting step, as shown in Fig. 3c. The degradation of QP would become the key point for permineralization of QPE, so the microorganism that could utilize QP as the sole carbon source was screened and an efficient QP-degrading fungus was finally isolated. The relative degradation characteristics will be introduced in the future.

3.4. Substrate utilization experiments

To determine the biodegradability of the strain YC-XJ1 to other members of AOPPs, 10 of AOPPs herbicides were tested. As shown in Fig. 4a, AOPPs including haloxyfop-p-methyl, diclofop-methyl, and fluazifop-p-butyl were completely degraded (100%) after 72 h of incubation. The degradation rates of clodinafop-propargyl, cyhalofop-butyl, QPE, and fenoxaprop-P-ethyl were 99.5, 97, 96.2, and 95.5%, respectively. The degradation rate of propaquizafop and quizalofop-p-tefuryl were 79% and 77.6% but showed no degrading potential to metamifop.

Cyhalofop-butyl was widely used for the postemergence control of grasses in rice fields [50]. Based on the reports, 96% of 100 mg/L fluazifop-P-butyl was degraded by *Aquamicrobium* sp. FPB-1 within 40 h [20]. Cyhalofop-butyl (84.5% of 100 mg/L) could be degraded by *P. azotoformans* QDZ-1 within 5 d [26]. Compared to them, the *M. populi* YC-XJ1 was able to degrade 97% of 50 mg/L cyhalofop-butyl within 72 h. Fenoxaprop-p-ethyl was also widely used in China for controlling wild oats in wheat fields [51]. Based on the reports, 94.6% of 100 mg/L fenoxaprop-P-ethyl could be degraded by *R. ruber* JPL-2 within 54 h [24]; 94% of 100 mg/L fenoxaprop-P-ethyl could be degraded by *Rhodococcus* sp. T1 within 24 h [23]; 95.2% of 50 mg/L fenoxaprop-P-ethyl could be degraded by *Acinetobacter* sp. DL-2 within 5 d [22]; and 66% of 50 mg/L fenoxaprop-p-ethyl could be degraded by *Alcaligenes* sp. H within 5 d [52]. Compared to them, the *M. populi* YC-XJ1 was able to degrade 95.5% of 50 mg/L fenoxaprop-P-ethyl within 72 h. Besides QPE, cyhalofop-butyl and fenoxaprop-P-ethyl were the most used AOPPs in China. The excellent degradability of the above-mentioned three AOPPs was the basis for future application of YC-XJ1.

The catalytic efficiency of strain YC-XJ1 toward different AOPP herbicides has been demonstrated and the degrading rate was as follows in descending order: haloxyfop-p-methyl ≈ diclofop-methyl ≈ fluazifop-p-butyl > clodinafop-propargyl > cyhalofop-butyl > quizalofop-p-ethyl > fenoxaprop-p-ethyl > propaquizafop > quizalofop-p-tefuryl. The degrading characteristics of YC-XJ1 can be obtained from the above results based on the molecular structure of AOPPs. The more complex the side chain structure of ester, the slower the degradation rate. This may be closely related to the steric structure of esterase domain. Similar degradation characteristics have been reported in the biodegradation of chloroacetamide herbicides by *Paracoccus* sp. FLY-8 in vitro [53]. The longer the alkyl side chain, the slower the occurrence of degradation efficiencies.

The degradation rate of propaquizafop and quizalofop-p-tefuryl by YC-XJ1 was only 79% and 77.6%, because of the complex structure of the side chain, as shown in Fig. 4b. No degrading potential of metamifop indicated the strain YC-XJ1 was unable to break down the amide bond in Fig. 4b.

3.5. Assembly and annotation

The *M. populi* YC-XJ1 genome was assembled into one scaffold of 5,395,646 bp and 5375 CDSs were predicted. Genes, 3547 and 3174, were annotated by NR and Swiss-Prot database, respectively. Hydrolase genes (21 α/β) and 14 esterase genes (removed duplicate annotated genes) were obtained. Secreted protein genes (505) and 702 transporter genes were screened through TCDB database (Table S2).

3.6. Sequence analysis of qpeh1

According to the annotation results, one gene was selected and it was named as *qpeh1*. The cloned *qpeh1* gene was 1104 bp in length with a GC content of 72.7% and encoded a protein of 367 amino acids with a calculated molecular mass of 39,840 Da. No signal peptide was identified in corresponding protein sequence.

The QPEH1 was used as a query sequence in a homology search against nonreduntant protein sequences (NCBI). The search revealed

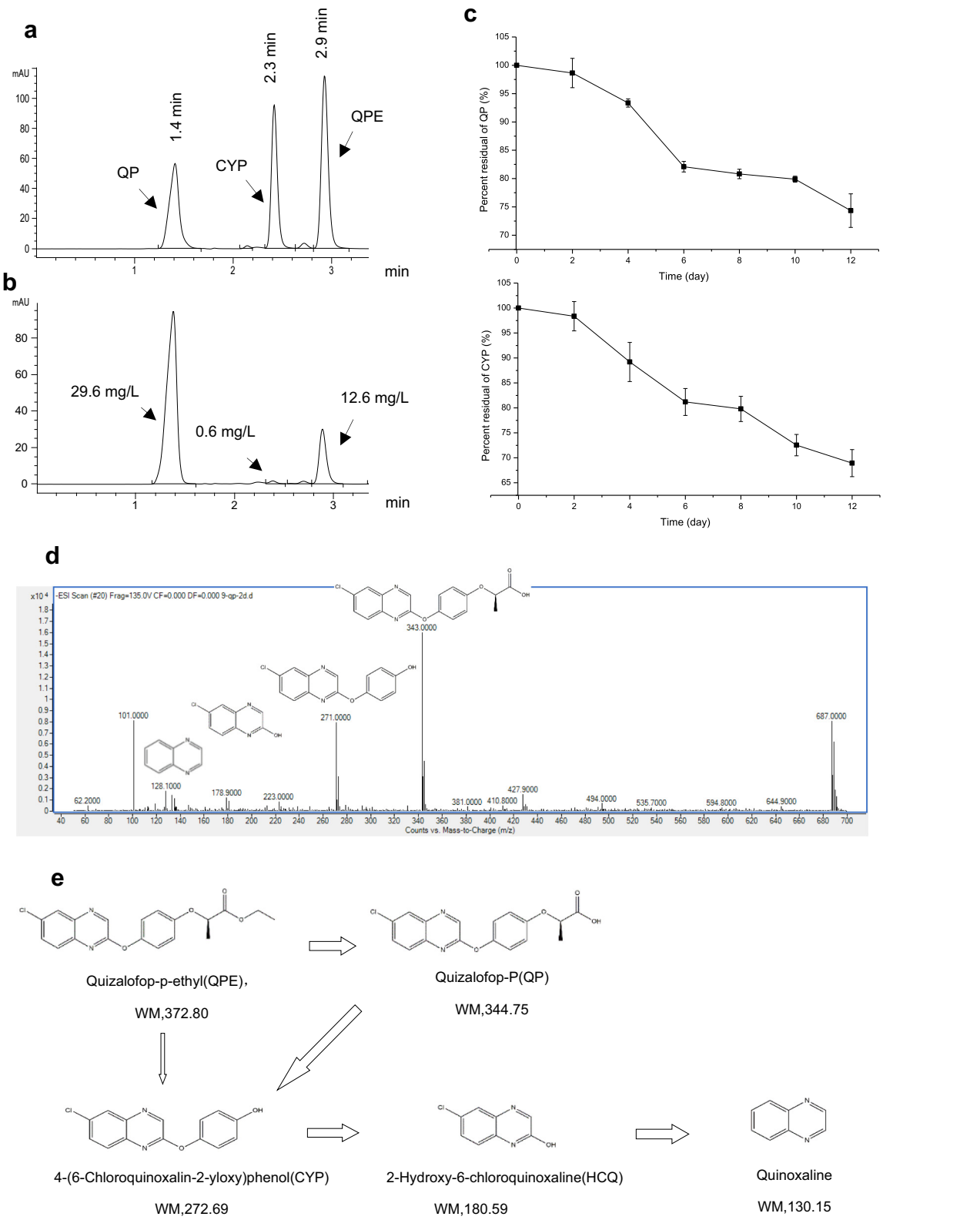

Fig. 3. The HPLC-MS analysis of intermediates and the QPE metabolic pathway. (a) The chromatogram of the standard solution of QPE, QP, and CYP (concentration was 20 mg/L and retention time was 2.9 min, 1.4 min, and 2.4 min, respectively). (b) The chromatogram of intermediates of QPE after the incubation of 48 h. (c) The degradation of QP and CYP by strain YC-XJ1. (d) The HPLC-MS analysis of intermediates of QPE degradation by strain YC-XJ1. (e) The QPE metabolic pathway.

that the most closely related proteins were α/β hydrolase. The A0A169RBE1 (α/β hydrolase of *Methylorubrum populi*, available in UniProt Knowledgebase), C5B0J6 (putative hydrolase of *M. extorquens* ATCC 14718), C7CGE7 (putative hydrolase of *M. extorquens* DSM 6343, available in the UniProt Knowledgebase), and EGF96305.1 (α/β hydrolase of *B. diminuta* ATCC 11568, available in the Protein Data

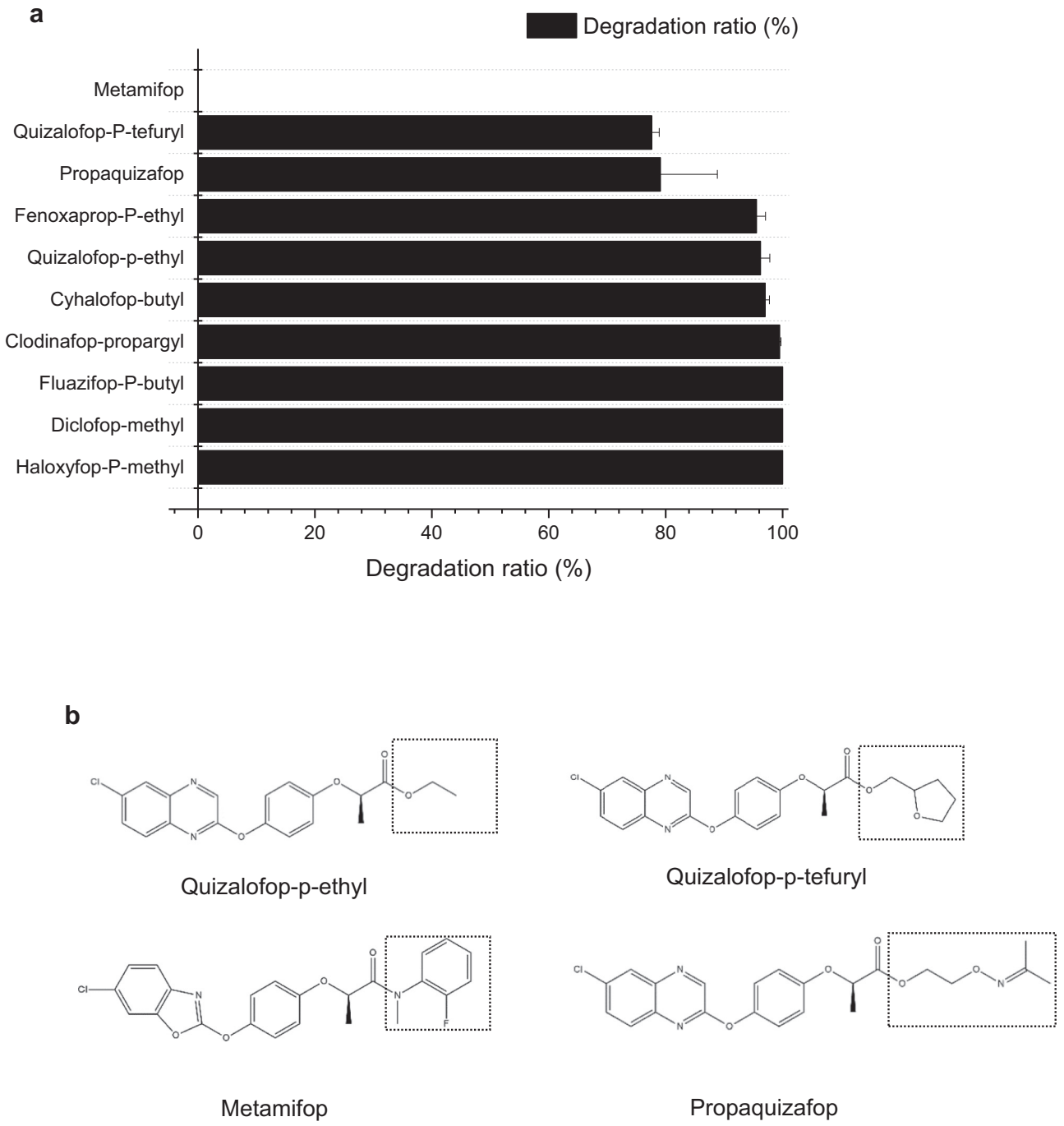

Fig. 4. The degrading ability of stain YC-XJ1. (a) The degradation of various substrates. (b) The structure of various substrates.

Bank) were selected for alignment analysis with QPEH1. The results showed QPEH1 contained the conserved esterase family sequence motif (G-X-S-X-G) and the catalytic triad (Ser-Asp-His) in Fig. 5. This suggested that QPEH1 was a member of esterase family.

A total of 27 members of subfamilies I–VIII and 8 esterases of QPE-degrading reported were used to construct a phylogenetic tree to verify the evolutionary relationship between QPEH1 and its closest relatives. As shown in Fig. 6, most of the 8 esterases of QPE-degrading reported were included in family V and VIII. The QPEH1 belonged to family V. Based on pairwise comparison with the most closely related esterase, the QPEH1 showed 30% and 29.1% identity to that of QpeH and CyHB from *Pseudomonas*, respectively. This result revealed significant differences of QPEH with other QPE-degrading hydrolases. Furthermore, there were more than 20 such similar α/β hydrolase genes in *M.* YC-XJ1, which was enough to demonstrate that the strain YC-XJ1 had abundant resources of hydrolase.

3.7. Verification of QPE-hydrolysis activity

In all, 47.1% of QPE (10 mg/L) was degraded after 12 h incubation with purified QPEH1, as shown in Table S4. The QPEH1 was identified as a novel QPE-degrading esterase from *M. populi* YC-XJ1.

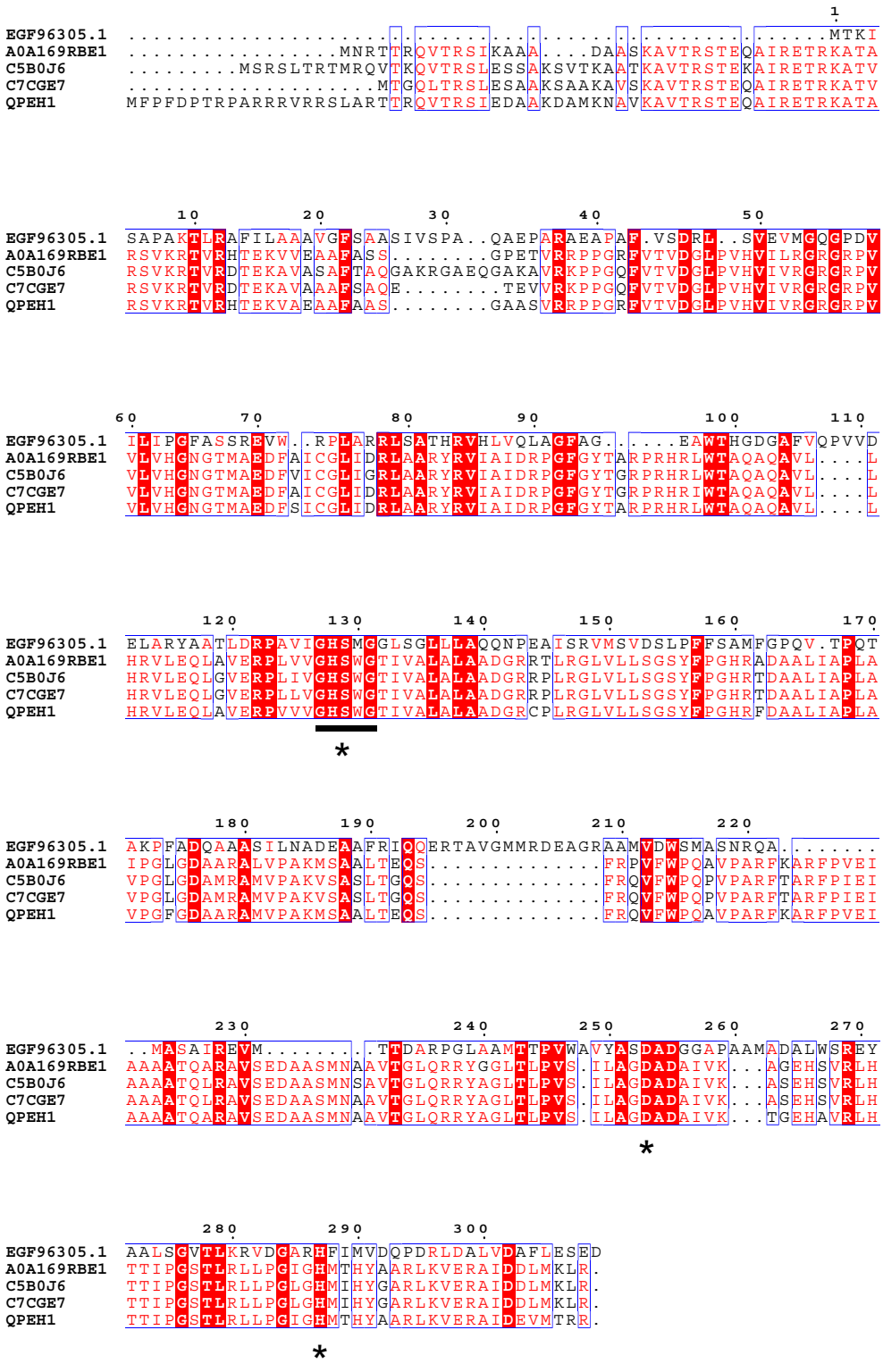

Fig. 5. The sequence alignment of QPEH1 with the most closely related proteins. EGF96305.1, α/β hydrolase fold family protein of *Brevundimonas diminuta* ATCC 11568, available in the Protein Data Bank (PDB); A0A169RBE1, α/β hydrolase of *Methylorubrum populi*, available in UniProt Knowledgebase; C5B0J6, Putative hydrolase of *M. extorquens* ATCC 14718; and C7CGE7, Putative hydrolase of *M. extorquens* DSM 6343, available in the UniProt Knowledgebase. The conserved hydrolase motif (G-X-S-X-G) was underlined, and the amino acids that form the catalytic triad (Ser-Asp-His) was indicated by asterisks. The identical amino acid residues are shown in red color.

3.8. Localization analysis of degradation reaction

Because of the lack of relevant reports, it was not clear whether the degradation processing occurs extracellularly or intracellularly. If extracellularly, it must involve the function of the secretory system to transport hydrolase out of the cell. If intracellularly, it was inevitable to need transporters on the cell membrane to assist QPE to enter the cell. Therefore, the

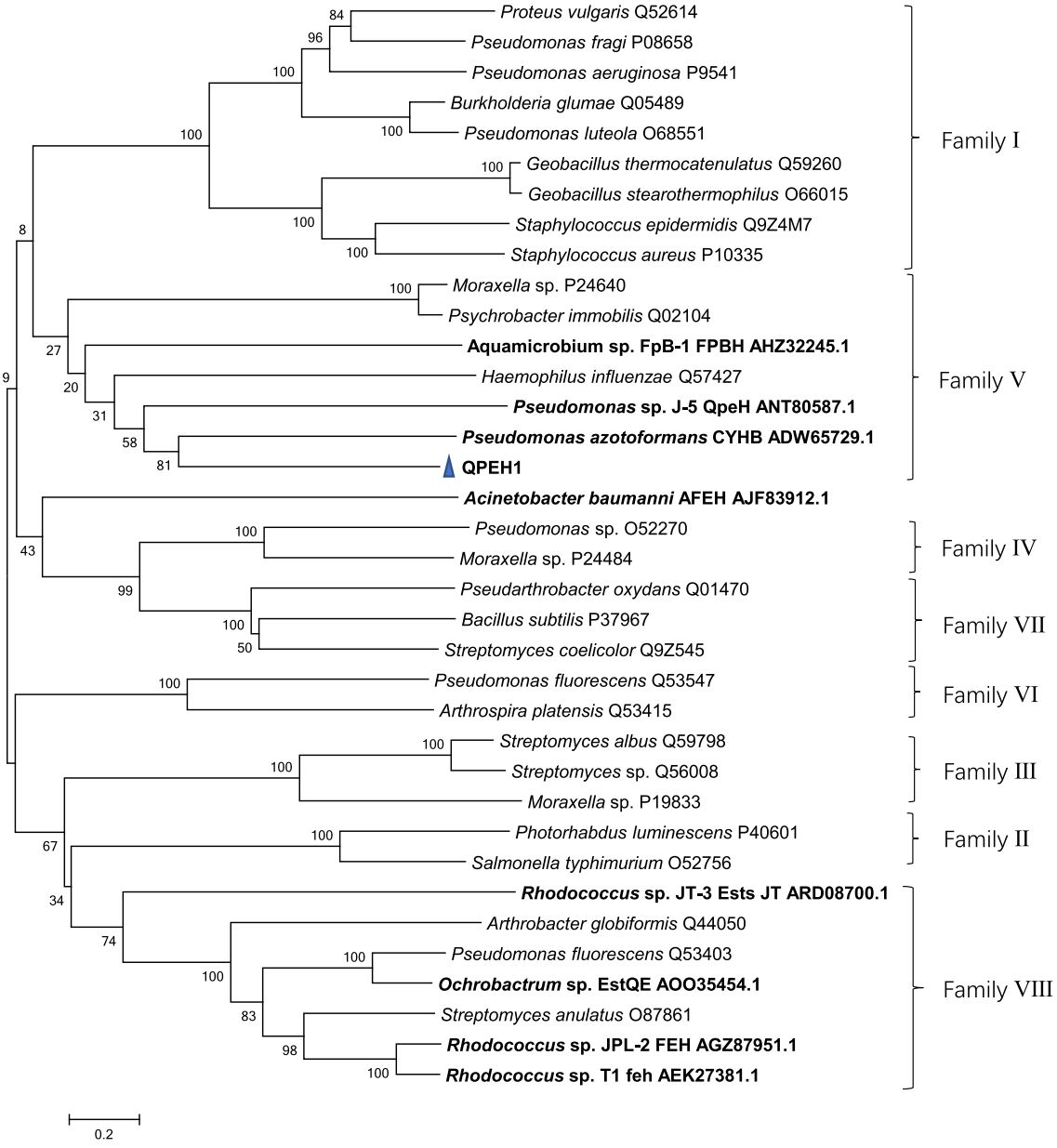

Fig. 6. The phylogenetic analysis of QPEH1. The phylogenetic tree was constructed using Mega 5.0 by the neighbor-joining method, bootstrapping of 1000 replicates and Poisson model, and details of sequences were shown in Table S3. The bold-type letters represent all QPE-degrading esterases reported.

secretory system, secreted proteins, and membrane transporters of YC-XJ1 were of particular concern. The complete proteome of YC-XJ1 was screened against the TCDB database.

Among 505 secreted protein genes predicted, no α/β hydrolase genes were included. Based on the annotation result of NR and Swiss-Prot database, a total of 21 α/β hydrolase genes, including QPEH1 gene, were screened as candidate QPE-degrading genes, which have no signal peptides. Presumably, the degradation processing was likely to occur intracellularly. However, it was true that cells used multiple protein transport systems in parallel. It was possible that cells use alternative secretory systems instead of recognizing a typical signal peptide [54]. After all, the intermediates of QPE-degrading pathway were identified extracellularly in this research.

A total of 702 transporter genes were obtained, and the possible related transporters are listed in Table S5. Transporters related to ATP binding accounted for the largest percentage, and the number was 166 accounting for 23.6%. The total number of transporter genes that may be related to substrate degradation were 123, accounting for 17.5%.

4. Conclusion

A strain YC-XJ1 capable of degrading QPE was isolated and identified. The associated degradation characteristics were determined. Two novel metabolites, HCQ and quinoxaline, were observed by HPLC-MS, and the metabolic pathway of QPE was predicted. It was first reported that *Methylobacterium* could degrade AOPPs, and its dechlorination ability was demonstrated. The *qpeh1* gene was identified as a new QPE-degrading esterase gene because of its low similarity of amino acid sequence with esterases reported previously. This study provided a microorganism source for AOPP biodegradation. The actual in situ remediation of *M.* YC-XJ1 in soil or

water need further research, and the molecular mechanism of enzymatic degradation of QPE by QPEH1 need to be elucidated.

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Conflict of interest

The authors declare that they have no conflict of interest.

Supplementary material

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