



Whole-Genome Sequencing of a Chlorimuron-Ethyl-Degrading Strain: *Chenggangzhangella methanolivorans* CHL1 and Its Degrading Enzymes

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ABSTRACT Chlorimuron-ethyl is a commonly used sulfonylurea herbicide, and its long-term residues cause serious environmental problems. Biodegradation of chlorimuron-ethyl is effective and feasible, and many degrading strains have been obtained, but still, the genes and enzymes involved in this degradation are often unclear. In this study, whole-genome sequencing was performed on chlorimuron-ethyl-degrading strain, *Chenggangzhangella methanolivorans* CHL1. The complete genome of strain CHL1 contains one circular chromosome of 5,542,510 bp and a G+C content of 68.17 mol%. Three genes, *sulE*, *pnbA*, and *gst*, were predicted to be involved in the degradation of chlorimuron-ethyl, and this was confirmed by gene knockout and gene complementation experiments. The three genes were cloned and expressed in *Escherichia coli* BL21 (DE3) to allow for the evaluation of the catalytic activities of the respective enzymes. The glutathione-S-transferase (GST) catalyzes the cleavage of the sulfonylurea bridge of chlorimuron-ethyl, and the esterases, PnbA and SulE, both de-esterify it. This study identifies three key functional genes of strain CHL1 that are involved in the degradation of chlorimuron-ethyl and also provides new approaches by which to construct engineered bacteria for the bioremediation of environments polluted with sulfonylurea herbicides.

IMPORTANCE Chlorimuron-ethyl is a commonly used sulfonylurea herbicide, worldwide. However, its residues in soil and water have a potent toxicity toward sensitive crops and other organisms, such as microbes and aquatic algae, and this causes serious problems for the environment. Microbial degradation has been demonstrated to be a feasible and promising strategy by which to eliminate xenobiotics from the environment. Many chlorimuron-ethyl-degrading microorganisms have been reported, but few studies have investigated the genes and enzymes that are involved in the degradation. In this work, two esterase-encoding genes (*sulE*, *pnbA*) and a glutathione-S-transferase-encoding gene (*gst*) responsible for the detoxification of chlorimuron-ethyl by strain *Chenggangzhangella methanolivorans* CHL1 were identified, then cloned and expressed in *Escherichia coli* BL21 (DE3). These key chlorimuron-ethyl-degrading enzymes are candidates for the construction of engineered bacteria to degrade this pesticide and enrich the resources for bioremediating environments polluted with sulfonylurea herbicides.

KEYWORDS gene knockout, gene complementation, microbial degradation, pollutant, sulfonylurea herbicide, whole-genome sequencing

Chlorimuron-ethyl, which is a sulfonylurea herbicide, is widely used to prevent the growth of broad-leaved weeds and sedge in soybean (1). However, the excessive application of chlorimuron-ethyl leads to long-term residue in soils. This can cause many ecological problems (2), such as soil degradation, toxic impact on susceptible

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crops, and effects on the soil microbial community (3–5). It is therefore necessary to eliminate chlorimuron-ethyl residues in soil. The degradation of chlorimuron-ethyl occurs mainly by biodegradation and chemical hydrolysis. Biodegradation strategies, which have high efficiency and are environmentally friendly, have been extensively studied (6–8).

In recent years, many chlorimuron-ethyl degrading microorganisms have been isolated, but their mechanisms of degradation have not been fully studied (9–11). Some studies have investigated the degrading enzymes and their coding genes (12). For example, two cytochrome P450 monooxygenases of *Streptomyces griseolus* and flavin-containing monooxygenases of *Talaromyces flavus* LZM1 broke the sulfonylurea bridge of sulfonylurea herbicides by oxidation (13–15). A hydrolase was obtained from *Oceanisphaera psychrotolerans*, which was expressed in *Escherichia coli* and hydrolyzed the sulfonylurea bridge (16). Esterase (SulE) from *Hansschlegelia zhihuaiae* S113 hydrolyzed the ester bond to degrade sulfonylurea herbicides (17). Carboxylesterase (CarE) of *Rhodococcus erythropolis* D310-1 de-esterified chlorimuron-ethyl (18). Glutathione-S-transferase (GST) from *Klebsiella jilinsis* 2N3 catalyzed the degradation of chlorimuron-ethyl (19). Two other key proteins (Kj-CysJ and Kj-SsuD) of *K. jilinsis* 2N3 were also involved in the degradation of chlorimuron-ethyl (20).

Strain *Chenggangzhangella methylocysteaceae* CHL1 is a novel sulfonylurea herbicide-degrading microorganism that was recently isolated and identified by our laboratory (8, 21). In the present study, the whole-genome of strain CHL1 was sequenced using Pacific Bioscience sequencing technology. On the basis of sequence and database annotations, genes involved in the degradation of chlorimuron-ethyl were predicted and subsequently confirmed by gene knockout and gene complementation experiments. The recombinant enzymes encoded by the key degradation genes were expressed in *E. coli*, and their catalytic activities were evaluated. The enzymatic degradation mechanisms of chlorimuron-ethyl were analyzed via ultra-high-performance liquid chromatography with Q Exactive high-resolution accurate mass spectrometry (UPLC-Q Exactive HRMS). This study lays a foundation for understanding the chlorimuron-ethyl degradation mechanism of strain CHL1 and also provides new approaches for the bioremediation of environments polluted with sulfonylurea herbicides.

RESULTS

Genome sequencing of strain CHL1. The complete genome of strain CHL1 contained one circular chromosome that was 5,542,510 bp long, with a G+C content of 68.17 mol%. The percentage of the protein coding genes was 87.32%, with a G+C content of 68.60 mol%. The mean coding sequence was 710.61 bp long. A total of 55 tRNAs and 6 rRNAs were predicted.

Key genes for chlorimuron-ethyl degradation and phylogenetic analyses. Glutathione-S-transferase from *K. jilinsis* 2N3, esterase SulE from *H. zhihuaiae* S113, and carboxylesterase CarE from *R. erythropolis* D310-1, previously reported to degrade chlorimuron-ethyl, were used as baits for a Basic Local Alignment Search Tool (BLAST) against the strain CHL1 genome (17–19). A SulE sequence identical to the SulE from *H. zhihuaiae* strain S113 was identified by BLAST (Table S1) (17). SulE is an esterase that de-esterifies sulfonylurea herbicides to their corresponding parent acid forms (Table S1) (17). In addition, a carboxylesterase of strain CHL1, PnbA, with high homology to the carboxylic ester hydrolase of *H. zhihuaiae* (68.27% amino acid similarity), was also identified (Fig. S1a; Table S1). *H. zhihuaiae* was isolated from polluted farmland soil and is capable of degrading the sulfonylurea herbicide metsulfuron-methyl via its carboxylic ester hydrolase activity. Thus, the PnbA of strain CHL1 might be involved in the de-esterification of chlorimuron-ethyl (22, 23). In addition to SulE and PnbA, a GST homologue was annotated in the categories “Drug metabolism-cytochrome P450” and “Metabolism of xenobiotics by cytochrome P450” by the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Table S1). The GST of strain CHL1 has high homology to the GSTs from *Methylobacterium dankookense* and *M. gnaphalii* (Fig. S1b). The GST from *K. jilinsis* 2N3 can break the sulfonylurea bridge of chlorimuron-ethyl (19). Therefore, the GST of strain CHL1 was predicted to participate in the cleavage of the

sulfonylurea bridge. We hypothesize that the genes *sulE*, *pnbA*, and *gst* are encoding chlorimuron-ethyl degradation enzymes (Table S1).

Gene knockout, gene complementation, and degradation of chlorimuron-ethyl.

To provide experimental evidence to verify the function of chlorimuron-ethyl degradation enzymes, the coding genes of *SulE*, *PnbA*, and *GST* were knocked out by the Lambda-Red system. The kanamycin resistance gene cassette was inserted into the position of the target gene by homologous recombination technology, and the expression of the flanking genes around the target gene was not affected (24, 25). Construction of the mutant strains of strain CHL1 with *sulE*, *pnbA*, and *gst* knockout and complementation was confirmed by agarose gel electrophoresis (Fig. 1a–c). The growth and chlorimuron-ethyl degradation abilities of the knockout strains, the complemented strains, and the wild-type strain are shown in Fig. 1d–f. The biomasses of the knockout strains (CHL1 Δ *sulE*, CHL1 Δ *pnbA*, CHL1 Δ *gst*) and the complemented strains (CHL1 Δ *sulE*[pEG-*sulE*], CHL1 Δ *pnbA*[pEG-*pnbA*], CHL1 Δ *gst*[pEG-*gst*]) were similar to that of the wild-type on the seventh day. Compared to the wild-type strain, on the seventh day, the ability of the knockout strains, CHL1 Δ *sulE*, CHL1 Δ *pnbA*, and CHL1 Δ *gst*, to degrade chlorimuron-ethyl decreased by 19.5%, 10.4%, and 10.8%, respectively ($P < 0.05$). The degradation ability of the complemented strains, CHL1 Δ *sulE*[pEG-*sulE*], CHL1 Δ *pnbA*[pEG-*pnbA*], and CHL1 Δ *gst*[pEG-*gst*], was not significantly different from that of the wild-type ($P > 0.05$). These results show that *sulE*, *pnbA*, and *gst* directly or indirectly participate in the degradation of chlorimuron-ethyl by strain CHL1.

Sequence analysis. The genes *sulE*, *pnbA*, and *gst* are located in different gene clusters (Fig. S2). *SulE*, *PnbA*, and *GST* were composed of 360 amino acids (1,083 bp), 594 amino acids (1,785 bp), and 203 amino acids (612 bp), respectively. *SulE*, *PnbA*, and *GST* were predicted to contain the structural domains, Esterase_713_like-3 (abhydrolase family), abhydrolase superfamily, and GST_C_7 (GST_C_7 family), respectively. The predicted binding sites for substrate chlorimuron-ethyl and *SulE*, *PnbA*, and *GST* were residues Ser-86 and His-198 (Fig. 2a), Ser-48 and Arg-94 (Fig. 2b), and Asp405 and Gln-428 (Fig. 2c), respectively.

Heterologous expression. Through heterologous expression assay in vitro, the mechanisms of chlorimuron-ethyl degradation enzymes will be further revealed. *GST*, *PnbA*, and *SulE* of strain CHL1 were successfully expressed in *E. coli* BL21 (DE3) and purified by Ni-NTA agarose chromatography. The concentrations of the purified *GST* and *PnbA* enzymes were 207.93 $\mu\text{g} \cdot \text{mL}^{-1}$ and 256.98 $\mu\text{g} \cdot \text{mL}^{-1}$, respectively, and their activities in the degradation of chlorimuron-ethyl were 1.62 $\text{U} \cdot \text{mL}^{-1}$ and 0.98 $\text{U} \cdot \text{mL}^{-1}$, respectively. The optimal pH values for chlorimuron-ethyl degradation by *GST* and *PnbA* were 5.0 and 7.0, respectively, and 30°C was the optimum reaction temperature for both *GST* and *PnbA* (Fig. 3). The activity of purified *SulE* in our study (554.44 $\mu\text{g} \cdot \text{mL}^{-1}$) was consistent with that documented in previous reports (17, 26, 27).

Enzymatic degradation mechanisms of chlorimuron-ethyl. The mass spectral ion peaks (m/z) of the chlorimuron-ethyl degradation products produced by *GST*, *PnbA*, and *SulE* from strain CHL1 were at 160.08 [M+H]⁺ and 230.14 [M+H]⁺, 387.13 [M+H]⁺, and 387.18 [M+H]⁺, respectively (Fig. 4a). On the basis of these results and the literature, the degradation products of chlorimuron-ethyl by *GST* were determined to be 4-chloro-6-methoxypyrimidin-2-amine and 2-sulfamoylbenzoate, and the degradation product of chlorimuron-ethyl by *PnbA* and *SulE* was 2-[[[(4-chloro-6-methoxy-2-pyrimidinyl) carbamoyl] sulfamoyl] benzoic acid (17–19). Considering these products, the degradation mechanisms of chlorimuron-ethyl by the enzymes were analyzed. *SulE* and *PnbA* could de-esterify chlorimuron-ethyl to produce 2-[[[(4-chloro-6-methoxy-2-pyrimidinyl) carbamoyl] sulfamoyl] benzoic acid, as reported previously for *SulE* (Fig. 4b) (17). *GST* catalyzed the cleavage of the sulfonylurea bridge of chlorimuron-ethyl to produce 2-sulfamoylbenzoate and 4-chloro-6-methoxypyrimidin-2-amine (Fig. 4b).

DISCUSSION

High-efficiency, sulfonylurea herbicide-degrading strain CHL1 represents a novel genus and species of the family Methylocystaceae (28). Here, the whole-genome of strain CHL1 was sequenced and analyzed. The results have important theoretical and

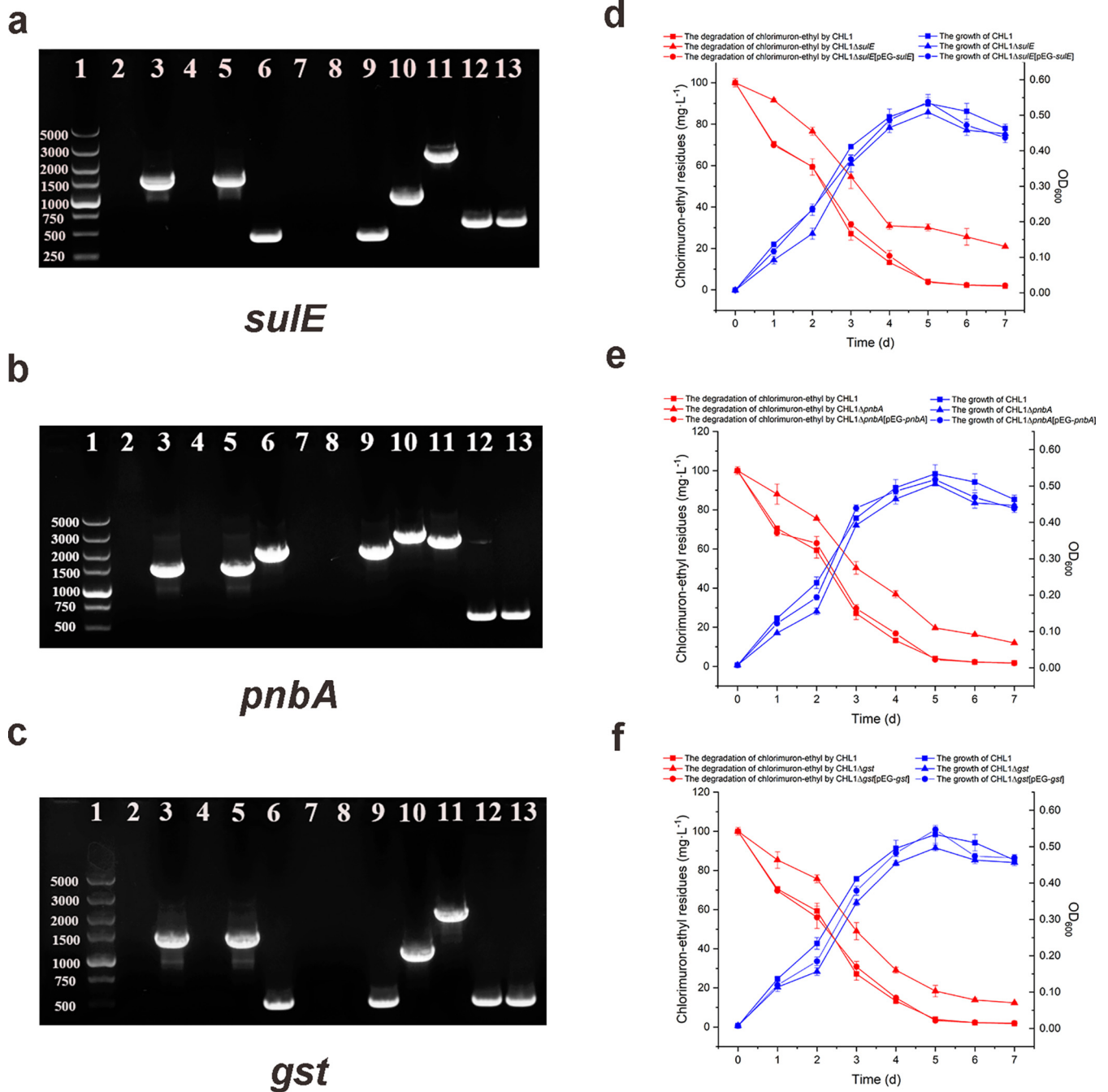


FIG 1 PCR verification and the degradation of chlorimuron-ethyl by mutant strains of strain CHL1 with *sulE* (a, d), *pnbA* (b, e), and *gst* (c, f) knockout and complementation. (a–c) Lane 1: DL₅₀₀₀ DNA marker. Lanes 2, 6, 10: wild-type strain CHL1. Lanes 3, 7, 11: knockout strain with kanamycin resistance gene. Lanes 4, 8, 12: knockout strain without kanamycin resistance gene. Lanes 5, 9, 13: complementation strain. Lanes 2, 3, 4, 5: amplification of the kanamycin resistance gene. Lanes 6, 7, 8, 9: amplification of the target gene using internal primers. Lanes 10, 11, 12, 13: amplification of the target gene, using external primers with 300 bp each, upstream and downstream. (d–f) Growth curves of strains (blue) and the degradation of chlorimuron-ethyl (red).

practical significance. The genes *sulE*, *pnbA*, and *gst* were predicted to be related to the degradation of chlorimuron-ethyl by strain CHL1, which was verified by gene knockout and gene complementation experiments (17–19, 22, 23). *H. zihuaiae* strain S113 almost completely lost its ability to degrade chlorimuron-ethyl after *sulE* was knocked out (17). In comparison, strain CHL1Δ*sulE* retained the ability to degrade 79.1% of the chlorimuron-ethyl. Moreover, the chlorimuron-ethyl degradation rates of strains CHL1Δ*pnbA* and CHL1Δ*gst* were >80%. Some other genes or pathways

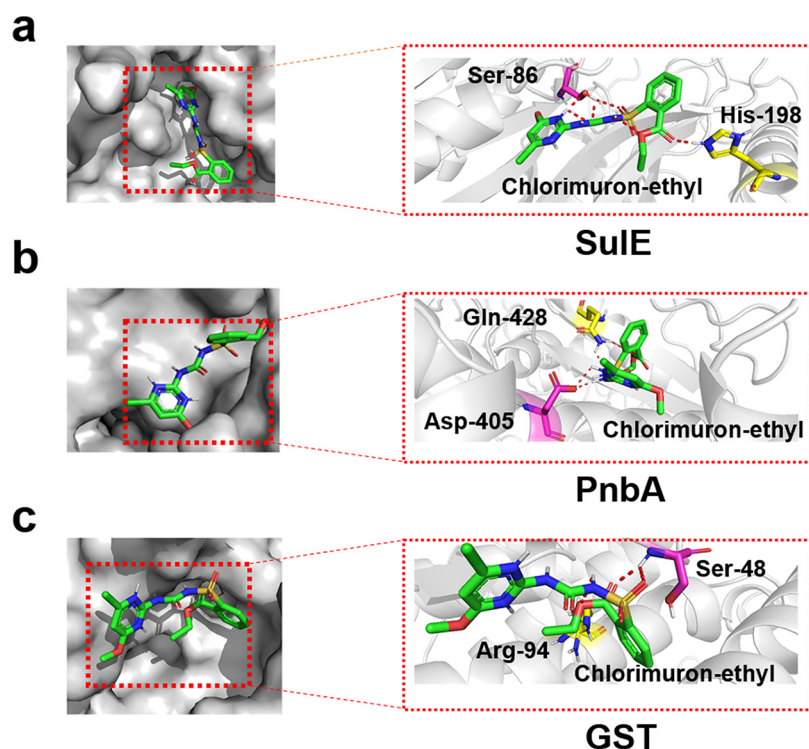


FIG 2 Binding site of SuIE (a), PnbA (b), and GST (c) with chlorimuron-ethyl. (a) Ser-86 and His-198. (b) Asp405 and Gln-428. (c) Ser-48 and Arg-94.

may be a response for chlorimuron-ethyl degradation in strain CHL1, or this strain may contain multiple isoenzymes with the same or similar degradation functions (19).

The predicted substrate binding sites for chlorimuron-ethyl were residues Ser-86 and His-198 of SuIE, Asp405, and Gln-428 of PnbA as well as Ser-48 and Arg-94 of GST. In the future, these key amino acid sites will be verified by site-directed mutagenesis and modified aiming to improve the enzyme activities (29). SuIE, PnbA, and GST from strain CHL1 were successfully recombinantly expressed in *E. coli* and purified. The optimum temperature for the degradation of chlorimuron-ethyl by SuIE, GST, and PnbA was 30°C in each case, and the optimal pH values for the reactions were 8.0, 5.0, and 7.0, respectively. Considering the mechanisms of degradation of chlorimuron-ethyl, GST cleaved the sulfo-

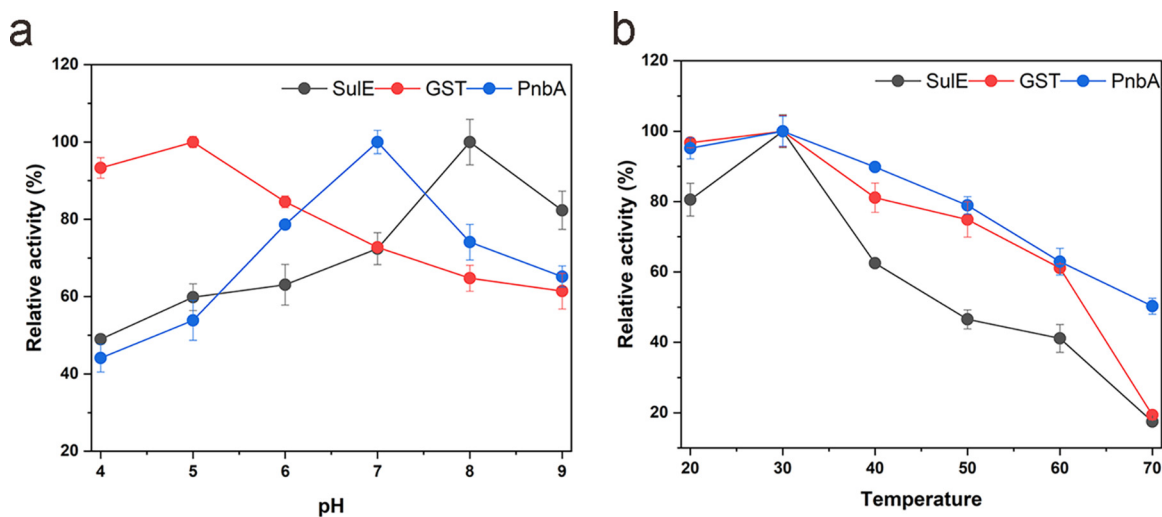


FIG 3 Effect of pH (a) and temperature (b) on the activity of SuIE, PnbA, and GST toward chlorimuron-ethyl.

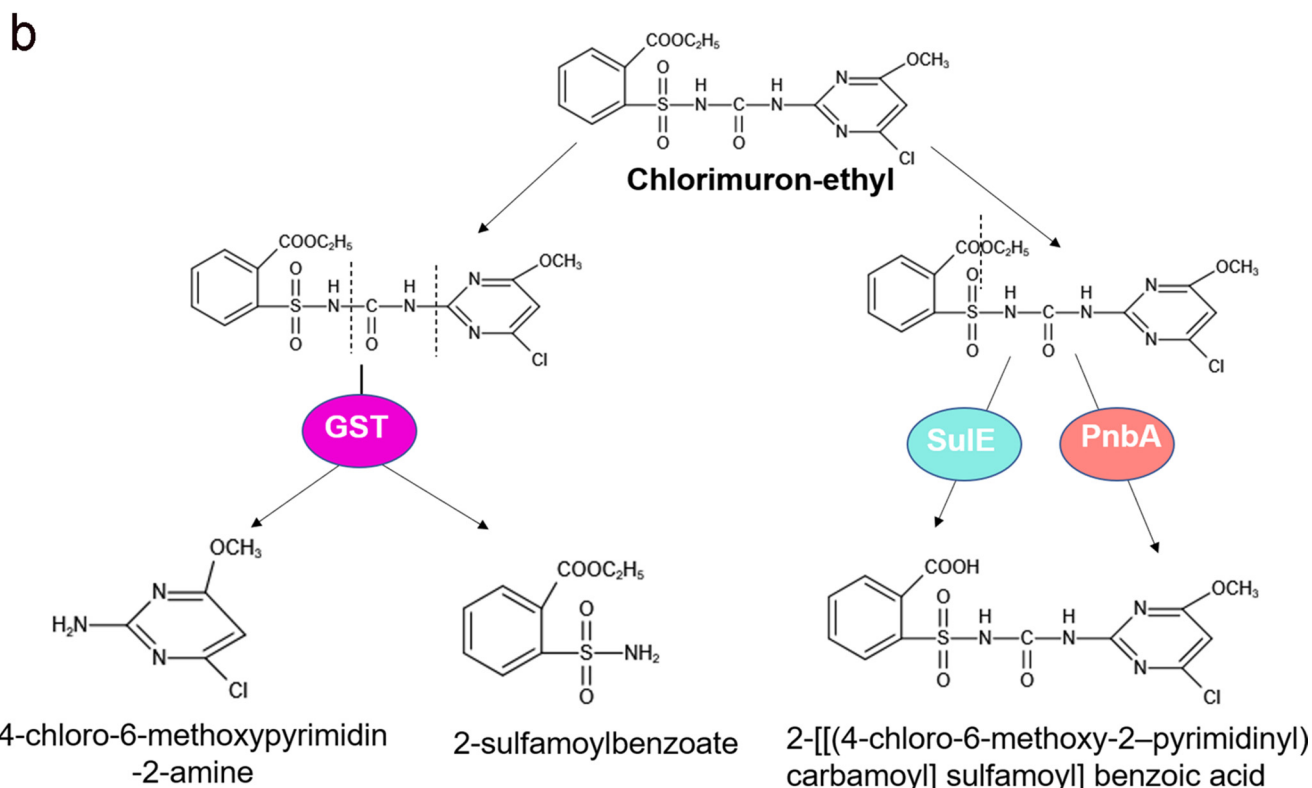
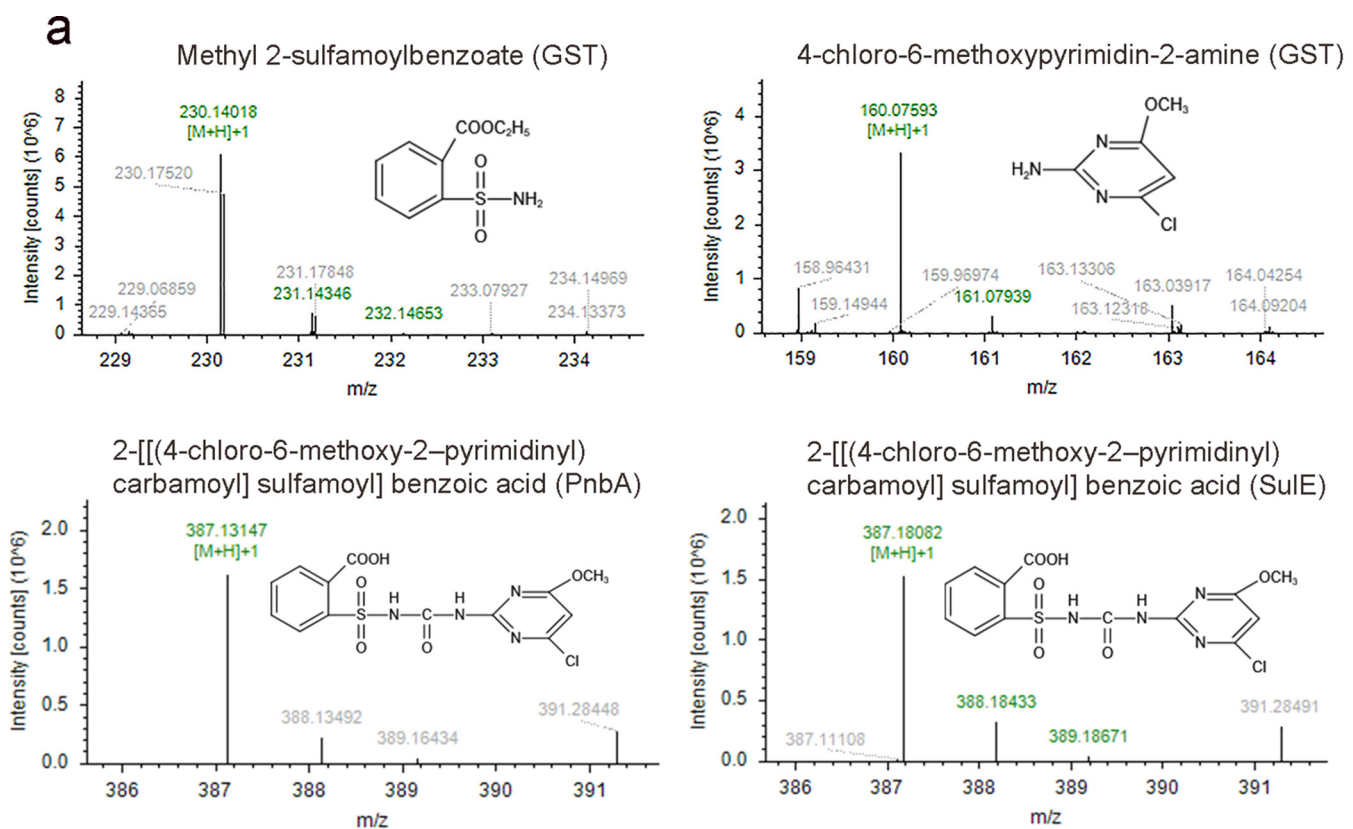


FIG 4 Degradation products (a) and degradation pathways (b) of chlorimuron-ethyl by SuIE, PnbA, and GST.

TABLE 1 The plasmids and bacterial strains used in this study

Strains and plasmids	Relevant genotype or characteristic	Reference or source	
Plasmid pKD4	Knockout vector, Kan ^R	This study	
Plasmid pKD46	Knockout vector, Amp ^R	This study	
Plasmid pCP20	Knockout vector, Amp ^R	This study	
Plasmid pEarleyGate100	Complementation vector, Kan ^R	Huiyuanyuan Biotechnology	
Plasmid pET-28a (+)	Expression vector, Kan ^R	This study	
Plasmid pEG- <i>gst</i>	<i>Gst</i> , <i>pnbA</i> , and <i>sulE</i> complementation vectors by pEarleyGate100, respectively	This study	
Plasmid pEG- <i>pnbA</i>			
Plasmid pEG- <i>sulE</i>			
Strain CHL1	Chlorimuron-ethyl utilizer, wild-type	This study	
Strain CHL1Δ <i>gst</i>	Strain CHL1 with <i>gst</i> , <i>pnbA</i> , and <i>sulE</i> deleted, respectively		
Strain CHL1Δ <i>pnbA</i>			
Strain CHL1Δ <i>sulE</i>			
Strain CHL1Δ <i>gst</i> [pEG- <i>gst</i>]	<i>Gst</i> , <i>pnbA</i> , and <i>sulE</i> were complemented by pEG- <i>gst</i> , pEG- <i>pnbA</i> , and pEG- <i>sulE</i> plasmids in CHL1Δ <i>gst</i> , CHL1Δ <i>pnbA</i> , and CHL1Δ <i>sulE</i> strains, respectively		
Strain CHL1Δ <i>pnbA</i> [pEG- <i>pnbA</i>]			
Strain CHL1Δ <i>sulE</i> [pEG- <i>sulE</i>]			
Strain CHL1[pKD46]	Strain CHL1 with plasmid pKD46		This study
<i>E. coli</i> BL21 (DE3)	F_ompT hsdS (rB_mB_) dcm gal (DE3)		Solarbio Biotechnology

nylurea bridge, and SulE and PnbA broke the ester bond. The results were consistent with the previous functional prediction for GST and PnbA in this experiment. GST degraded chlorimuron-ethyl to 2-sulfamoylbenzoate and 4-chloro-6-methoxypyrimidin-2-amine. The product 4-chloro-6-methoxypyrimidin-2-amine was previously identified in the degradation of chlorimuron-ethyl by *Pseudomonas* sp. LW3, *R. erythropolis* D310-1, and *K. jilinsis* 2N3 (6, 18, 19). Furthermore, 2-sulfamoylbenzoate was an intermediate of the degradation product *o*-sulfonate benzoic imide in chlorimuron-ethyl degradation by GST of *K. jilinsis* 2N3 (19). SulE and PnbA both degraded chlorimuron-ethyl to generate 2-[(4-chloro-6-methoxy-2-pyrimidinyl) carbamoyl] sulfamoyl benzoic acid, which was previously identified in the degradation of chlorimuron-ethyl by *R. erythropolis* D310-1 and *H. zihuaiae* S113 (17, 18).

It is necessary to study the genes and enzymes involved in herbicide degradation to remediate herbicide-contaminated environments (30–32). The known genes and enzymes involved in the degradation of sulfonylurea herbicides are still limited, and they need to be explored further. Our results reveal the genome sequence of strain CHL1 and its key genes and enzymes for degrading chlorimuron-ethyl, which could provide candidates for the construction of engineered bacteria for degradation and could enrich the resources of sulfonylurea herbicide-degrading enzymes for environmental bioremediation.

MATERIALS AND METHODS

Plasmids, bacterial strains, chemicals, and media. *Chenggangzhangella methanolivorans* strain CHL1 was isolated from sludge samples collected from sewage treatment tanks at an agricultural chemical factory (Jiangsu Province, China) through enrichment, and had been identified and stored in our laboratory (8). All of the plasmids and strains used in this study are displayed in Table 1.

The chlorimuron-ethyl and the reagents for the spectroscopic and chromatographic analyses were purchased from Sigma-Aldrich (Shanghai, China). All chemicals and analytical reagents were provided by TaKaRa Biotechnology (Dalian, China). The components of the mineral salt medium (MSM) were as follows: 2.0 g NaNO₃, 0.5 g NaCl, 2.0 g KH₂PO₄, 0.02 g FeSO₄·7H₂O, 0.125 g MgSO₄·7H₂O, 0.2 g yeast extract, and 20 mL methanol per 1,000 mL of distilled water, pH 7.0.

Genome sequencing, annotation, and analysis of strain CHL1. Strain CHL1 was cultured on MSM solid from a freeze-dried stock and incubated at 28°C. A single colony was selected and cultured in MSM to the exponential phase (160 rpm, 28°C). The culture medium was centrifuged (8,000 rpm, 5 min), and the harvested cells were washed repeatedly with PBS buffer (0.2 mol·L⁻¹, pH 7.8) and suspended in MSM (8). The bacterial precipitations were collected by centrifugation (8,000 rpm, 5 min) to extract the genomic DNA of strain CHL1 using a bacterial genomic DNA extraction kit (Tiangen, Beijing).

Sequencing of the genomic DNA of strain CHL1 was performed by Personal Biotechnology Co. (Guangzhou, China), using Pacific Bioscience Sequel technology (33–35). The complete genome sequence of strain CHL1 was assembled using Mecat2 and Unicycle and was annotated using the Non-Redundant Protein Sequence (NR), Swiss-Prot Protein Sequence (Swiss-Prot), Pfam protein domain (Pfam), Clusters of Orthologous Groups of Proteins (COG), Gene Ontology (GO), and Kyoto Encyclopedia

of Genes and Genomes (KEGG) databases. Key genes involved in the degradation of chlorimuron-ethyl by strain CHL1 were predicted based on the genome sequence combined with literature about degradation enzymes for sulfonylurea herbicides.

Phylogenetic analyses. On the basis of the protein database UniProt and sequence alignment, the sequences with the top 10 similarities were selected for construction of phylogenetic trees in MEGA 7.0 software (36), using the neighbor-joining method and 1,000 bootstrap replicates.

Gene knockout and complementation. Lambda-Red recombination technology was used as the gene replacement method, based on the recombination of functional genes, *exo*, *bet*, and *gam*, of the Lambda phage (37). The Lambda-Red system is widely used to verify gene function by replacing a target gene with an antibiotic resistance gene to achieve mutation (20, 38). *sulE*, *gst*, and *pnbA* were knocked out from strain CHL1 using the Lambda-Red system to test the roles of the respective gene products in degrading chlorimuron-ethyl (39). The primers used in the experiments are listed in Table S2. Strain CHL1 was cultured in 100 mL of MSM at 28°C with shaking at 160 rpm until the logarithmic growth phase ($OD_{600\text{ nm}} = 0.8$). The bacterial suspension was centrifuged at 9,500 rpm for 10 min and washed with pre-cooled 10% glycerol (4°C) four times to obtain competent cells.

The plasmid pKD46 was transformed into competent cells of strain CHL1 using a Gene Pulser Xcell (Bio-Rad) with parameters, capacitance 25 μF , voltage 1,800 V, resistance 200 Ω , and pulse time 5 ms. Competent cells of strain CHL1[pKD46] were prepared as described above, and the expression of the recombinases was induced by the addition of 100 mM arabinose.

Plasmid pKD4 contained the kanamycin resistance gene, and the 5'-end of each of the two primers contained a sequence of 60 bp, homologous to either end of *gst*, *pnbA*, or *sulE*, respectively. The gene-targeting linear box was amplified by one-step PCR, using the primers with homologous arms, pKD4, and PrimeSTAR HS DNA polymerase (TaKaRa) (Fig. S3a). Then, to obtain the knockout strains, it was transformed into competent cells of strain CHL1[pKD46] using the Gene Pulser Xcell with the same parameters as described above.

Plasmid pCP20, containing two FLP recombination target sites, was transformed into competent cells of the knockout strains using the Gene Pulser Xcell with the same parameters as described above to remove the kanamycin resistance gene (Fig. S3b).

Plasmid pEarleyGate100 was used to construct the corresponding complementation strains. *sulE* and *gst* amplified by PCR were ligated into pEarleyGate100, using the XbaI and HindIII restriction sites, respectively (Fig. S4). *pnbA* amplified by PCR was ligated into pEarleyGate100 using the XbaI and SpeI restriction sites. The resulting plasmids, pEG-*gst*, pEG-*pnbA*, and pEG-*sulE*, were transformed into the corresponding knockout strain to obtain the complemented strain.

The construction of mutants was confirmed by PCR of (i) the kanamycin resistance gene, (ii) the target gene, and (iii) the full length of the target gene (with 300 bp each, upstream and downstream).

Ability to degrade chlorimuron-ethyl. The abilities of the knockout strains and the complementary strains to degrade chlorimuron-ethyl were determined by monitoring cell growth and substrate concentration. These strains (inoculum size of 1%) were inoculated into 100 mL of MSM with 100 $\text{mg} \cdot \text{L}^{-1}$ chlorimuron-ethyl and were cultured at 28°C for 7 days. All assays had three replicates. The value of bacterial growth (OD_{600}) and the concentration of chlorimuron-ethyl were detected every day.

The collected samples were centrifuged (8,000 rpm, 10 min) to remove the bacterial precipitations. Supernatants were collected and extracted by dichloromethane in the same volume 3 times. The organic phase was combined and dried with N_2 . The samples were dissolved with 10 mL of methanol and filtered through 0.22 μm nylon filters. The concentration of chlorimuron-ethyl was determined by HPLC equipped with a Zorbax C-18 ODS Spherex column (4.6 \times 250 mm, 5 μm , Agilent Technologies, Palo Alto, CA, USA). The mobile phase was 0.5% methanoic acid:methanol (30:70, vol/vol) at a flow rate of 1 $\text{mL} \cdot \text{min}^{-1}$, and chlorimuron-ethyl was detected at 254 nm (6, 9).

Protein structure. The nucleotide sequences of genes *gst*, *pnbA*, and *sulE* of strain CHL1 have been submitted to the GenBank database with accession numbers [OK050582](#), [OK050584](#), and [OK050587](#), respectively. The primary, secondary, and tertiary structures of the enzymes encoded by the predicted functional genes *sulE* (encoding an esterase [SulE]), *pnbA* (encoding a carboxylic ester hydrolase [PnbA]), and *gst* (encoding a GST) were analyzed using DNAMAN, NCBI, and SWISS-MODEL software, respectively (36, 40, 41). The binding site of the enzymes and chlorimuron-ethyl were analyzed by AutoDock Vina software and were visualized by PyMOL (42).

Gene expression in *E. coli* and enzyme purification. The GST-encoding gene fragment was ligated into expression vector pET-28a (+) using the *Nde*I and *Hind*III restriction sites, and the constructed vector was transduced into *E. coli* BL21 (DE3). The expression of GST was induced with 1 mM isopropyl β -D-1-thiogalactopyranoside for 24 h at 30°C, and GST was purified by Ni-NTA agarose chromatography (43). The purity and concentration of GST were analyzed by 12% SDS-PAGE and the Bradford method (44, 45), respectively. The expression and purification of SulE and PnbA were the same as for GST, except the *Xho*I restriction site was used instead of the *Hind*III site for the expression of PnbA. The primers used in these experiments are listed in Table S3.

Enzyme assay and biochemical characterization. The initial concentrations of GST and chlorimuron-ethyl in 10 mL of PBS were 0.5 $\mu\text{g} \cdot \text{mL}^{-1}$ and 20 $\mu\text{g} \cdot \text{mL}^{-1}$, respectively. The enzymatic reactions were performed at 25°C for 10 min and were terminated by the addition of dichloromethane. One unit of enzyme activity of GST to chlorimuron-ethyl was defined as the amount of enzyme that converted 1 μmol of chlorimuron-ethyl into its parent acid form in 1 min under the detected condition. The optimal pH and optimal temperature of GST were determined by assaying enzyme activity in PBS buffer at ranges of pH values (4.0 to 9.0) and temperatures (20°C to 70°C), respectively. All assays were done in

triplicate, and the highest enzyme activity was set to 100%. The enzyme assay and the biochemical characterization of SulE and PnbA followed the same protocol as the above method.

Enzymatic degradation mechanisms of chlorimuron-ethyl. Purified SulE, GST, and PnbA (10 U) were added to 10 mL of phosphate-buffered saline containing 20 mg · L⁻¹ chlorimuron-ethyl and were incubated in their optimum reaction pH and temperature conditions for 24 h. The samples were measured by UPLC-Q Exactive HRMS and analyzed using the Compound Discoverer 3.2 software (46).

Statistical analysis. Differences were tested for statistical significance via independent-sample *t*-tests that were performed using SPSS 23.0. A *P* value of <0.05 was considered to be indicative of a statistically significant difference.

Data availability. The whole-genome sequence of *Chenggangzhangella methanolivorans* strain CHL1 was deposited into NCBI under the accession number CP081869.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

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