Microbial Degradation of Chemical Pesticides and Bioremediation of Pesticide-Contaminated Sites in China

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Chemical pesticides as the major compounds to control pests and diseases have been popularly used worldwide in modern agricultural systems. However, the intensive use of chemical pesticides has also resulted in serious environmental problems because they are either recalcitrant or biodegraded very slowly. Owing to their highly toxic nature, there is significant concern regarding the large quantities of pesticide residues in the environment. Many methods were thought out for remediation of these pesticide residues including incineration, landfills, and bioremediation. Due to the limitation of physical-chemical remediation methods, bioremediation constitutes an attractive alternative to physicochemical methods of remediation and is considered as the most effective, less-expensive, and non-secondary pollution method by many biological and environmental scientists.

Bioremediation is an innovative technology that has the potential to alleviate numerous pesticide contamination problems and was often chosen for the cleanup of contaminated sites. Bioremediation processes can be broadly categorized into two groups: ex situ and in situ. Ex situ bioremediation technologies include bioreactors, biofilters, land farming, and some composting methods. In situ bioremediation technologies include bioventing, biosparging, biostimulation, liquid delivery systems, and some composting methods. In situ treatments tend to be more attractive because they require less equipment, generally have a lower cost, and generate less disturbance to the environment.

Microorganisms play key roles in the biodegradation of chemical pesticides. In recent years, there is an increasing interest in the use of microorganisms with versatile biodegradative properties to deal with the pollution of pesticides. This can be done by using indigenous microorganisms or by adding an enriched culture

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of microorganisms that have specific characteristics to degrade the desired pesticides at a quicker rate. Ideally, bioremediation results in the complete mineralization of chemical pesticides to H_2O and CO_2 without the buildup of intermediates.

The lab for environmental microbiology in Nanjing Agricultural University has been engaged in the pesticide degradation and remediation by microorganisms for more than 20 years and has made some progress in this field, including isolation and collection of high efficient pesticide-degrading bacteria, elucidation of the catabolic pathway of pesticides in microbes, cloning of the key gene(s) involved in pesticide degradation, the ecological process during biodegradation of pesticides, and removal of pesticide contamination by microbes.

1 Isolation and Collection of High Efficient Pesticide-Degrading Bacteria

More than 150 bacterial strains capable of degrading various herbicides have been isolated from pesticide-contaminated soil or water samples all over China. These isolates are able to degrade various kinds of pesticides including organochlorine, organophosphorus, and organic nitrogenous insecticides, fungicides, and herbicides (Tables 1 and 2). As regards the herbicides, sulfonylurea herbicides, phenylurea herbicides, chloroacetanilide herbicides, aryloxyphenoxy propanoate herbicides, benzonitrile, and diphenyl ether herbicides are all found to be degraded by these isolates. These isolates will provide us with abundant microbial resources for the study the mechanisms involved in the herbicide biodegradation and the application of bioremediation technology for the herbicide-contaminated sites.

It was found that 38 strains are the first time reported from that genus to be capable of degrading specific herbicides, and 20 species were found to be novel species (Table 2), showing the diverse of microbial strains for the degradation of chemical herbicides.

2 Elucidation of the Catabolic Pathway of Pesticides in Microbes

Through GC-MS, HPLC-MS, and MS/M, the metabolites produced during herbicide degradation were identified, and 17 metabolic pathways have been elucidated including the pathways for chlorimuron-ethyl, fomesafen, lactofen, propanil, butachlor, metsulfuron-methyl, cyhalofop-butyl, thifensulfuron-methyl, isoproturon, chlorpropham, acetochlor, bromoxynil, and bromoxynil octanoate (Table 3). Among these pathways, eight pathways are the first time reported, indicating the diversity of metabolism of herbicides.

Table 1 Microbial isolates capable of degrading various pesticides

Isolates	Pesticides degraded	References
Lysinibacillus sp. ZB-1	Diphenyl ethers herbicides (fomesafen, lactofen, fluoroglycofen)	Liang et al. (2009)
Pseudomonas sp. LW3	Sulfonylurea herbicides (chlorimuron-ethyl)	Ma et al. (2009)
Brevundimonas sp. LY-2	Diphenyl ethers herbicides (lactofen)	Liang et al. (2010b)
Pseudomonas aeruginosa L36	Sulfonylurea herbicides (metsulfuron-methyl)	Huang et al. (2010)
Sphingomonas sp. Y57	Propanil, isoproturon	Zhang et al. (2011b)
Ancylobacter sp. XJ-412-1	Sulfonylurea herbicides (metsulfuron-methyl, thifensulfuron-methyl, bensulfuron-methyl)	Lu et al. (2011)
Pseudomonas azotoformans QDZ-1	Aryloxyphenoxy propanoate herbicide (cyhalofop-butyl, quizalofop-P-ethyl, fenoxaprop-P-ethyl, diclofop-methyl, haloxyfop-P-methyl)	Nie et al. (2011)
Paracoccus sp. FLY-8	Chloroacetanilide herbicides (alachlor, acetochlor, propisochlor, butachlor, pretilachlor)	Zhang et al. (2011a)
Pseudomonas zeshuii BY-1	Diphenyl ethers herbicides (fomesafen, lactofen, acifluorfen, fluoroglycofen)	Feng et al. (2012a)
Rhodococcus sp. B1	Chloroacetanilide herbicides (butachlor, alachlor, acetochlor, pretilachlor)	Liu et al. (2012)
Sphingobium sp. YBL2	Phenylurea herbicides (Isoproturon)	Zhang et al. (2012c)
Catellibacterium caeni DCA-1	Chloroacetanilide herbicides (butachlor)	Zheng et al. (2012)
Paracoccus sp. FLN-7	Chlorpropham, propham, propanil	Zhang et al. (2012a)
Sphingopyxis sp. OB-3	Bromoxynil (bromoxynil octanoate)	Chen et al. (2013b)
Comamonas sp. 7D-2	Benzonitriles herbicides (bromoxynil)	Chen et al. (2013a)
Sphingobium quisquiliarum DC-2 and *Sphingobium baderi* DE-13	Chloroacetanilide herbicides (acetochlor)	Li et al. (2013b)

3 Cloning of the Key Gene(s) Involved in Pesticide Degradation

During the past two decades, over 20 key genes involved in the pesticide degradation have been cloned, including the genes for the catabolism of organochlorine, organophosphorus, and organic nitrogenous insecticides, fungicides, and herbicides. Among these genes, 15 genes were the first time reported novel genes and showed relatively low similarities to known genes (Table 4).

Table 2 Novel species isolated capable of degrading pesticides

Novel species isolated	Pesticide degraded	Reference
Rhodococcus qingshengii sp. nov.	Carbendazim	Xu et al. (2007)
Candida mengyuniae sp. nov.	Metsulfuron-methyl	Chen et al. (2009)
Flavobacterium haoranii sp. nov.	Cypermethrin	Zhang et al. (2010)
Rhodococcus jialingiae sp. nov.	Carbendazim	Wang et al. (2010d)
Sphingobium faniae sp. nov.	Pyrethroid	Guo et al. (2010)
Sphingobium qiguonii sp. nov.	Carbaryl	Yan et al. (2010)
Sphingobium wenxiniae sp. nov.	Pyrethroid	Wang et al. (2011a)
Lysobacter ruishenii sp. nov.	Chlorothalonil	Wang et al. (2011b)
Comamonas zonglianii sp. nov.	Phenol	Yu et al. (2011)
Methylopila jiangsuensis sp. nov.	Acetochlor	Li et al. (2011)
Catellibacterium caeni sp. nov.	Butachlor	Zheng et al. (2011)
Hansschlegelia zhihuaiae sp. nov.	Sulfonylurea herbicides	Wen et al. (2011)
Sphingobium jiangsuense sp. nov.	3-phenoxybenzoic acid	Zhang et al. (2012c)
Sphingobacterium wenxiniae sp. nov.	Cypermethrin	Zhang et al. (2012d)
Burkholderia zhejiangensis sp. nov.	Methyl parathion	Lu et al. (2012)
Catellibacterium nanjingense sp. nov.	Propanil	Zhang et al. (2012a)
Pseudomonas zeshuii sp. nov.	Fomesafen	Feng et al. (2012b)
Dokdonella kunshanensis sp. nov.	Butachlor	Li et al. (2013a)
Paracoccus huijuniae sp. nov	Chlorpropham, propham, propanil	Sun et al. (2013)

Table 3 The identified metabolic pathways of pesticides in microbes

Pesticides	Degrading strains	Metabolites identified	References
Cypermethrin	*Sphingobium wenxiniae* JZ-1	2, 2-dimethyl-3- (2, -2-dichlorovinyl) -cyclopropanecarboxylic acid, 3-phenoxybenzoic acid, catechol	Wang et al. (2009)
Triazophos	*Diaphorobacter* sp. TPD-1	1-phenyl-3-hydroxy-1, 2, -4-triazole, 2-phenylhydrazinecarboxylic acid	Yang et al. (2011)
Dimethoate	*Paracoccus* sp. lgjj-3	Dimethoate carboxylic acid, O, O, S-trimethyl thiophosphorothioate, phosphorothioic O, O, S-acid, O, O, O-trimethyl phosphoric ester	Li et al. (2010)
Isocarbophos	*Arthrobacter* sp. scl-2	Isopropyl salicylate, salicylic acid, gentisate	Li et al. (2009)
Methamidophos	*Hyphomicrobium* sp. MAP-1	O, S-dimethyl hydrogen thiophosphate, S-methyl dihydrogen thiophosphate, methyl dihydrogen phosphate, phosphoric acid	Wang et al. (2010b)

(continued)

Table 3 (continued)

Pesticides	Degrading strains	Metabolites identified	References
Chlorothalonil	*Pseudomonas* sp. CTN-3	4-hydroxyl-chlorothalonil	Liang et al. (2010a)
Buprofezin	*Pseudomonas* sp. DFS35-4	2-imino-5-phenyl-3- (propan-2-yl) -1, 3, 5-thiadiazinan-4-one, 2-imino-5-phenyl-1, 3, 5-thiadiazinan-4-one, methyl (phenyl) carbamic acid	Chen et al. (2011)
Carbofuran	*Novosphingobium* sp. FND-3	2-hydroxy-3- (3-methypropan-2-ol) phenol, 2-hydroxy-3- (3-methylpropan-2-ol) benzene-N-methylcarbamate, 5-hydroxycarbofuran	Yan et al. (2007)
Carbendazim	*Rhodococcus jialingiae* djl-6-2	2-aminobenzimidazole, 2-hydroxybenzimidazole	Wang et al. (2010a)
Chlorimuron-ethyl	*Pseudomonas* sp. LW3	N-formyl-benzosulfimide, 4-chloro chloromethoxy-pyrimidin-2-amine	Ma et al. (2009)
Fomesafen	*Lysinibacillus* sp. ZB-1	N-[4-{4- (trifluoromethyl) phenoxy}-2-methanamidephenyl] acetamide	Liang et al. (2009)
Lactofen	*Brevundimonas* sp. LY-2	1- (carboxy) ethyl-5- (2-chloro-4- (trifluoromethyl) phenoxy) -2-nitrobenzoate	Liang et al. (2010b)
Propanil	*Sphingomonas* sp. Y57	3, 4-dichloroaniline, 4, 5-dichlorocatechol	Zhang et al. (2011b)
Butachlor	*Paracoccus* sp. FLY-8	[2-chloro-N- (2, 6-dimethylphenyl) -N- (methoxymethyl) acetamide]; 2-chloro-N- (2, 6-dimethylphenyl) acetamide; 2, 6-diethylaniline	Zhang et al. (2011a)
Metsulfuron-methyl	*Ancylobacter* sp. XJ-412-1	2-[[[[(4-methoxy-6-methyl-1, 3, 5-triazin-2-yl) amino]carbonyl] amino]sulfonyl]benzoic acid (metsulfuron acid)	Lu et al. (2011)
Cyhalofop-butyl	*Pseudomonas azotoformans* QDZ-1	Butanol, cyhalofop acid	Nie et al. (2011)
Butachlor	*Rhodococcus* sp. B1	Butoxymethanol; 2-chloro-N- (2, -6-dimethylphenyl) -acetamide	Liu et al. (2012)
Thifensulfuron-methyl	*Hansschlegelia zhihuaiae* S113	Thifensulfuron acid	Hang et al. (2012)
Isoproturon	*Sphingobium* sp. YBL2	MDIPU; DDIPU; 4-IA	Zhang et al. (2012c)
Chlorpropham	*Paracoccus* sp. FLN-7	3-chloroaniline	Zhang et al. (2012a)
Fomesafen	*Pseudomonas zeshuii* BY-1	[5- (4-trifluoromethylphenoxy) -2-nitro-benzoyl]-sulfinamic acid; [5- (2-chloro-4-difluoromethyl-phenoxy) -2-nitro-benzoyl]-sulfinamic acid; N-[4- (difluoromethyl-phenoxyl) -2-formylamino-phenyl]-acetamide	Feng et al. (2012)

(continued)

Table 3 (continued)

Pesticides	Degrading strains	Metabolites identified	References
Butachlor	*Catellibacterium caeni* DCA-1	N-hydroxymethyl-2-chloro-N-(2, 6-diethyl-phenyl) -acetamide; 2-chloro-N- (2, 6-diethyl-phenyl) -acetamide; N- (2, 6-diethyl-pheny) -N-hydroxymethyl-form-amide; (2, 6-diethyl-phenyl) -ethoxymethyl-carbamic acid	Zheng et al. (2012)
Acetochlor	*Sphingobium quisquiliarum* DC-2 and *Sphingobium baderi* DE-13	2-chloro-N- (2-methyl-6-ethylphenyl) acetamide; 2-methyl-6-ethylaniline; 2-methyl-6-ethylaminophenol; 2-methyl-6-ethylbenzoquinoneimine	Li et al. (2013b)
Bromoxynil	*Comamonas* sp. 7D-2	3, 5-dibromo-4-hydroxybenzoate, 3-bromo-4-hydroxybenzoate, p-hydroxybenzoic acid, protocatechuic	Chen et al. (2013a)
Bromoxynil octanoate	*Sphingopyxis* sp. OB-3	3, 5-dibromo-4-hydroxybenzonitrile, octanoic acid	Chen et al. (2013b)
Isoproturon	*Sphingobium* sp. YBL2	(1- (4-isopropylphenyl) -3-methylurea), (1-(4-isopropylphenyl) urea)	Gu et al. (2013)

Table 4 The key genes cloned that are involved in the degradation of pesticides

Gene	Pesticides degraded	References
mpd	Organophosphorus insecticide	Cui et al. (2001)
pytH	Permethrin	Wang et al. (2009)
chd	Chlorothalonil	Wang et al. (2010c); Liang et al. (2011, 2012)
sulE	Sulfonylurea herbicides	Hang et al. (2012)
ampA	Dimethoate	Zhang et al. (2012a)
chbH	Cyhalofop	Nie et al. (2011)
feH	Fenoxaprop-P-ethyl	Liu et al. (2015)
pamh	Propanil	Shen et al. (2012)
cmeH	Diclofop-methyl	Li et al. (2013b)
broH	Bromoxynil octanoate	Chen et al. (2013b)
bhbAB	Bromoxynil	Chen et al. (2013a)
pdmAB	Isoproturon	Gu et al. (2013)
pbaAB	3-phenoxybenzoic acid	Wang et al. (2014)
cndA	Acetochlor	Chen et al. (2014)
ddhA	Isoproturon	Yan et al. (2016)

3.1 *A Novel Pyrethroid-Hydrolyzing Carboxylesterase Gene from* Sphingobium *sp. Strain JZ-1*

A novel esterase gene, *pytH*, encoding a pyrethroid-hydrolyzing carboxylesterase was cloned from *Sphingobium* sp. strain JZ-1. The gene contained an open reading frame of 840 bp. Sequence identity searches revealed that the deduced enzyme shared the highest similarity with many α/β-hydrolase fold proteins (20–24 % identities). *PytH* was expressed in *Escherichia coli* BL21 and purified using Ni-nitrilotriacetic acid affinity chromatography. It was a monomeric structure with a molecular mass of approximately 31 kDa and a pI of 4.85. *PytH* was able to transform p-nitrophenyl esters of short-chain fatty acids and a wide range of pyrethroid pesticides, and isomer selectivity was not observed. No cofactors were required for enzyme activity.

3.2 *A Novel Hydrolytic Dehalogenase Gene Involved in the Degradation of the Fungicide of Chlorothalonil*

Dehalogenases play key roles in the detoxification of halogenated aromatics. Interestingly, only one hydrolytic dehalogenase for halogenated aromatics, 4-chlorobenzoyl- CoA dehalogenase, has been reported. Here, we characterize another novel hydrolytic dehalogenase for halogenated aromatic compound from the 2, 4, 5, 6-tetrachloroisophthal-onitrile (chlorothalonil) degrading strain of *Pseudomonas* sp. CTN-3, which we have named Chd. Chd catalyzes a hydroxyl substitution at the 4-chlorine atom of chlorothalonil. The metabolite of the Chd dehalogenation, 4-hydroxy-trichloroisophth- alonitrile, was identified by reverse-phase high-performance liquid chromatography (HPLC), tandem mass spectrometry (MS/MS), and nuclear magnetic resonance (NMR). Chd dehalogenates chlorothalonil under anaerobic and aerobic conditions and does not require the presence of cofactors such as coenzyme A (CoA) and ATP. Chd contains a putative conserved domain of the metallo-β-lactamase superfamily and shows the highest identity with several metallohydrolases (24–29 %). Chd is a monomer (36 kilodaltons), and the isoelectric point (pI) of Chd is estimated to be 4.13. Chd has a dissociation constant (*K*m) of 0.112 mmol/L and an overall catalytic rate (*K*cat) of 207 s^{-1} for chlorothalonil. Chd is completely inhibited by 1, -10-phenanthroline, diethyl pyrocarbonate, and N-bromosuccinic acid. Site-directed mutagenesis of Chd revealed that histidine 128 and 157; serine 126; aspartate 45, 130, and 184; and tryptophan 241 were essential for the dehalogenase activity. Chd differs from other reported hydrolytic dehalogenases based on the analysis of amino acid sequences and catalytic mechanisms. This study provides an excellent dehalogenase candidate for mechanistic study of hydrolytic dehalogenation of halogenated aromatic compound.

3.3 Novel Gene Clusters Involved in the Catabolism of the Herbicide Bromoxynil

Dehalogenation is the key step in the degradation of halogenated aromatics, while reductive dehalogenation is originally thought to rarely occur in aerobes. An aerobic strain of *Comamonas* sp. 7D-2 was shown to degrade the brominated aromatic herbicide bromoxynil completely and release two equivalents of bromides under aerobic conditions. The enzymes involved in the degradation of bromoxynil to 4-carboxy-2-hydroxymuconate-6-semialdehyde, including nitrilase, reductive dehalogenase (*BhbA*), 4-hydroxybenzoate 3-monooxygenase, and protocatechuate 4, 5-dioxygenase, were molecularly characterized. The novel dehalogenase *BhbA* was shown to be a complex of a respiration-linked reductive dehalogenase (RdhA) domain and a NAD (P) H-dependent oxidoreductase domain and to have key features of anaerobic respiratory RdhAs, including two predicted binding motifs for [4Fe-4S] clusters and a close association with a hydrophobic membrane protein (*BhbB*). *BhbB* was confirmed to anchor *BhbA* to the membrane.

BhbA was partially purified and found to use NAD (P) H as electron donors. Full-length *bhbA* homologues were found almost exclusively in marine aerobic *Proteobacteria*, suggesting that reductive dehalogenation occurs extensively in aerobes and that *bhbA* is horizontally transferred from marine microorganisms. The discovery of a functional reductive dehalogenase and ring-cleavage oxygenases in an aerobe opens up possibilities for basic research as well as the potential application for bioremediation.

3.4 Sulfonylurea Herbicide De-esterification Esterase Gene *from* Hansschlegelia zhihuaiae *S113*

De-esterification is an important degradation or detoxification mechanism of sulfonylurea herbicide in microbes and plants. However, the biochemical and molecular mechanisms of sulfonylurea herbicide de-esterification are still unknown. A novel esterase gene, *sulE*, responsible for sulfonylurea herbicide de-esterification, was cloned from *Hansschlegelia zhihuaiae* S113. The gene contained an open reading frame of 1,194 bp, and a putative signal peptide at the N terminal was identified with a predicted cleavage site between Ala37 and Glu38, resulting in a 361-residue mature protein. *SulE* minus the signal peptide was synthesized in *Escherichia coli* BL21 and purified to homogeneity. *SulE* catalyzed the de-esterification of a variety of sulfonylurea herbicides that gave rise to the corresponding herbicidally inactive parent acid and exhibited the highest catalytic efficiency toward thifensulfuron-methyl. *SulE* was a dimer without the requirement of a cofactor. The activity of the enzyme was completely inhibited by Ag^{+}, Cd^{2+}, Zn^{2+}, methamidophos, and sodium dodecyl sulfate. A *sulE*-disrupted mutant strain, $sulE^{-}$, was constructed by insertion mutation. $sulE^{-}$ lost the de-esterification ability

and was more sensitive to the herbicides than the wild type of strain S113, suggesting that *sulE* played a vital role in the sulfonylurea herbicide resistance of the strain. The transfer of *sulE* into *Saccharomyces cerevisiae* BY4741 conferred on it the ability to de-esterify sulfonylurea herbicides and increased its resistance to the herbicides. This study has provided an excellent candidate for the mechanistic study of sulfonylurea herbicide metabolism and detoxification through de-esterification, construction of sulfonylurea herbicide-resistant transgenic crops, and bioremediation of sulfonylurea herbicide-contaminated environments.

3.5 A Novel Arylamidase Gene from Paracoccus *sp. Strain FLN-7 That Is Involved in the Hydrolysis of Amide Pesticides*

The bacterial isolate *Paracoccus* sp. strain FLN-7 hydrolyzes amide pesticides such as diflubenzuron, propanil, chlorpropham, and dimethoate through amide bond cleavage. A gene, *ampA*, encoding a novel arylamidase that catalyzes the amide bond cleavage in the amide pesticides was cloned from the strain. *ampA* contains a 1,395-bp open reading frame that encodes a 465-aminoacid protein. *AmpA* was expressed in *Escherichia coli* BL21 and homogenously purified using Ni-nitrilotriacetic acid affinity chromatography. *AmpA* is a homodimer with an isoelectric point of 5.4. *AmpA* displays maximum enzymatic activity at 40 °C and a pH of between 7.5 and 8.0, and it is very stable at pHs ranging from 5.5 to 10.0 and at temperatures up to 50 °C. *AmpA* efficiently hydrolyzes a variety of secondary amine compounds such as propanil, 4-acetaminophenol, propham, chlorpropham, dimethoate, and omethoate. The most suitable substrate is propanil, with *K*m and *K*cat values of 29.5 μmol/L and 49.2/s, respectively. The benzoylurea insecticides (diflubenzuron and hexaflumuron) are also hydrolyzed but at low efficiencies. No cofactor is needed for the hydrolysis activity. *AmpA* shares low identities with reported arylamidases (less than 23 %), forms a distinct lineage from closely related arylamidases in the phylogenetic tree, and has different biochemical characteristics and catalytic kinetics with related arylamidases. The results in the present study suggest that *AmpA* is a good candidate for the study of the mechanism for amide pesticide hydrolysis, genetic engineering of amide herbicide-resistant crops, and bioremediation of amide pesticide-contaminated environments.

3.6 Novel Bacterial N-*demethylase Genes* PdmAB *Involved in the Initial Step of* N, N-*dimethyl-Substituted Phenylurea Herbicide Degradation*

The environmental fate of phenylurea herbicides has received considerable attention in recent decades. The microbial metabolism of *N*, *N*-dimethyl-substituted

phenylurea herbicides can generally be initiated by mono-*N*-demethylation. In this study, the molecular basis for this process was revealed. The *pdmAB* genes in *Sphingobium* sp. strain YBL2 were shown to be responsible for the initial mono-*N*-demethylation of commonly used *N*, *N*-dimethyl-substituted phenylurea herbicides. *PdmAB* is the oxygenase component of a bacterial Rieske nonheme iron oxygenase (RO) system. The genes *pdmAB*, encoding the α subunit *PdmA* and the β subunit PdmB, are organized in a transposable element flanked by two direct repeats of an insertion element resembling ISRh1. Furthermore, this transposable element is highly conserved among phenylurea herbicide-degrading sphingomonads originating from different areas of the world. However, there was no evidence of a gene for an electron carrier (a ferredoxin or a reductase) located in the immediate vicinity of *pdmAB*. Without its cognate electron transport components, expression of *PdmAB* in *Escherichia coli*, *Pseudomonas putida*, and other sphingomonads resulted in a functional enzyme. Moreover, coexpression of a putative [3Fe-4S]-type ferredoxin from *Sphingomonas* sp. strain RW1 greatly enhanced the catalytic activity of *PdmAB* in *E. coli*. These data suggested that *PdmAB* has a low specificity for electron transport components and that its optimal ferredoxin may be the [3Fe-4S] type. PdmA exhibited low homology to the α subunits of previously characterized ROs (less than 37 % identity) and did not cluster with the RO group involved in *O*-or *N*-demethylation reactions, indicating that *PdmAB* is a distinct bacterial RO *N*-demethylase.

3.7 Comparative Genomic Analysis of Isoproturon-Mineralizing Sphingomonads Reveals the Isoproturon Catabolic Genes

The worldwide use of the phenylurea herbicide, isoproturon (IPU), has resulted in considerable concern about its environmental fate. Though many microbial metabolites of IPU are known and IPU-mineralizing bacteria have been isolated, the molecular mechanism of IPU catabolism has not been elucidated yet. In this study, complete genes that encode the conserved IPU catabolic pathway were revealed, based on comparative analysis of the genomes of three IPU-mineralizing sphingomonads and subsequent experimental validation. The complete genes included a novel hydrolase gene *ddhA*, which is responsible for the cleavage of the urea side chain of the IPU demethylated products; a distinct aniline dioxygenase gene cluster *adoQTA*1*A*2*BR*, which has a broad substrate range; and an inducible catechol *meta*-cleavage pathway gene cluster *adoXEGKLIJC*. Furthermore, the initial mono-*N*-demethylation genes *pdmAB* were further confirmed to be involved in the successive *N*-demethylation of the IPU mono-*N*-demethylated product. These IPU-catabolic genes were organized into four transcription units and distributed on three plasmids. They were flanked by multiple mobile genetic elements and highly conserved among IPU-mineralizing sphingomonads. The elucidation of the

molecular mechanism of IPU catabolism will enhance our understanding of the microbial mineralization of IPU and provide insights into the evolutionary scenario of the conserved IPU-catabolic pathway.

4 Horizontal Transfer of the Chlorothalonil Hydrolytic Dehalogenase Gene Facilitates Bacterial Adaptation to Chlorothalonil-Contaminated Sites

Microorganisms have evolved multiple mechanisms to adapt to environmental stresses, such as mutation, DNA rearrangement, and horizontal gene transfer. The horizontal transfer of genes plays a key role in the evolution of catabolic genes, thereby facilitating bacterial adaptation to pollutant-contaminated sites. Bacterial dehalogenases catalyze the cleavage of carbon-halogen bonds of many man-made chlorinated compounds, which is a key step in the detoxification of these priority organic pollutants. Notably, many dehalogenase genes are associated with transmissible elements. In our laboratory, many diverse TPN-dechlorinating strains have been isolated, and the novel chlorothalonil hydrolytic dehalogenase (Chd), which catalyzes a hydroxyl substitution at the 4-chlorine atom of TPN to form 2, 4, 5-trichloro-6- hydroxybenzene-1, 3-dicarbonitrile (4-TPN-OH), was also identified. In this study, we discovered a close association between the highly conserved *chd* gene and a novel insertion sequence (IS), IS*Ocsp1*, in diverse TPN-dechlorinating strains. We suggest that the ecological role of horizontal gene transfer is to facilitate bacterial adaptation to TPN-contaminated sites, by allowing these bacteria to rapidly transform toxic TPN to less toxic 4-TPN-OH.

5 Removal of Pesticide Contamination by Microbes

5.1 Degradation of Fomesafen in Soil by Strain Lysinibacillus *sp. ZB-1*

The soil sample was collected from the top 0 to 20 cm from agricultural field in the campus of Nanjing Agricultural University, Nanjing, China. The soil with a pH value of 6.82 has never been treated with fomesafen. Soil samples were sterilized by autoclaving at 121 °C for 60 min. Subsamples (200 g) of fresh soil and sterile soil were weighed, and the solution of fomesafen was added to obtain a final concentration of 10 mg/kg soil and mixed well. One set of fresh soil and of sterile soil were inoculated with strain ZB-1 (10^8 cells/g). Another set of uninoculated soil was kept as a control. The inoculum was thoroughly mixed into the soils under sterile conditions, and the moisture was adjusted to 35 % (w/w of dry weight of soil). Each soil microcosm was incubated at 30 °C in the dark. At intervals of 7 day,

10 g of the soil samples were collected and the concentration of fomesafen was detected. It was found that only 11.1 % of the initially added 10 mg/L fomesafen was degraded in uninoculated sterilized soil after 5 weeks. When strain ZB-1 was inoculated to the sterilized soil, the degradation of fomesafen increased to 67.1 % during the same period, which showed obviously strain ZB-1 enhanced the degradation of fomesafen. In fresh soil samples with and without inoculation, the degradation of fomesafen was 77.9 % and 14.8 % after 5 weeks, respectively. The degradation rate of fomesafen in fresh soil inoculated with strain ZB-1 was clearly more rapid than that of without inoculation, which confirmed that strain ZB-1 could cooperate well with the indigenous microorganisms to degrade fomesafen in soil. The results of fomesafen degradation in soil indicated that strain ZB-1 could be used potentially for the bioremediation of fomesafen contaminated soils.

5.2 *Degradation of the Chloroacetamide Herbicide Butachlor by* Catellibacterium caeni *sp. nov DCA-1^T*

Three different soil samples (red soil, fluvo-aquic soil, and high sandy soil) were collected from Yingtan (Jiangxi Province), Suqian (Jiangsu Province), and Nantong (Jiangsu Province), China, respectively. Soil samples were treated as described previously. Two sets of non-sterile and sterile soils were inoculated with 5 % (v/w) cells of strain DCA-1^T, respectively. Soil samples inoculated with heat-killed DCA-1^T cells were kept as the control. Butachlor was added to soil at a concentration of 50 mg/kg dry soil and then adsorbed in the dark overnight. Sterile water was employed to adjust the soil moisture to 40 %. Soil samples were collected for butachlor analysis at 1, 3, 5, 10, 20, 30, and 45 day, respectively.

The recovery efficiencies of butachlor from the three soils ranged from 89.8 % to 101.5 %, indicating that butachlor could be recovered from these soils efficiently. In all three sterile soils inoculated with heat-killed cells, less than 20 % of butachlor was degraded in 45 days. However, in the non-sterile soils about 60–80 % of butachlor was degraded over 45 days, indicating that indigenous microorganisms could also degrade butachlor. The inoculation of DCA-1^T cells significantly accelerated the butachlor degradation in both sterile and non-sterile soils, with 57.2–90.4 % of 50 mg/kg butachlor removed in 5 days compared to 5.4–36 % in the controls (inoculated with heat-killed cells), showing that strain DCA-1^T played major roles in the removal of butachlor. In the sterile soils, the inoculation of strain DCA-1^T removed 68.6–86.0 % of 50 mg/k butachlor in 30–45 days. However, in the non-sterile soils, the inoculation of strain DCA-1^T removed nearly the whole 50 mg/kg butachlor in the same time span, indicating that the inoculated exogenous strain DCA-1^T was in good cooperation with indigenous microorganisms. In the sterile soils, the degradation rate of butachlor in the red soil (pH 4.8) by the inoculated strain DCA-1^T cells was 68.6 % in 45 days, which was relatively

lower than that in the fluvo-aquic soil (82.8 %, pH 6.3) and high sandy soil (86 %, pH 8.2), showing that soil type significantly affected the butachlor degradation by strain DCA-1^T.

5.3 Elimination of Chlorothalonil Inhibition by Chlorothalonil Hydrolytic Dehalogenase in Alcoholic Fermentation

The effect of different kinds of pesticide residues in grapes on alcoholic fermentation by *Saccharomyces cerevisiae* was evaluated. Among four types of pesticides added into the grape slurry, omethoate, triadimefon, and cyhalothrin did not inhibit the alcoholic fermentation at their proposed spraying concentration of 0.21, 0.10, and 0.10 g/L, respectively, whereas chlorothalonil concentration above 0.03 g/L behaved significantly negative influence on both *S. cerevisiae* growth and alcoholic fermentation efficiency. When the chlorothalonil concentration was lower than 0.01 g/L, the fermentation proceeded smoothly without any degradation of chlorothalonil. Considering the cumulative toxicity and adverse effect of chlorothalonil on fermentation, chlorothalonil hydrolytic dehalogenase (Chd) extracellularly expressed from the recombinant *Bacillus subtilis* WB800 was used to pretreat the chlorothalonil-contaminated grape slurry. After treatment by the Chd enzyme in an activity of 7.25 mU/L slurry for 60 min, the inhibition effect could be substantially eliminated even at an initial concentration of 0.10 g/L chlorothalonil. This study provides a potential approach for solving the conflict in fermentation industry with pesticides inhibition.

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