Supporting Information

Comparative analysis of DNA-SIP and Magnetic-nanoparticle Mediated Isolation (MMI) on unraveling dimethoate degraders

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Text S1. MNPs synthesis and functionalization of soil

1 mL of $FeCl_2$ (1.0 M) was mixed with 2 mL of $FeCl_3$ (2.0 M). Then, 25 mL of NaOH (2.0 M) was added dropwise to the mixture. After 30 min of continuous shock, the black Fe_3O_4 composite MNPs were retrieved using a magnet and then washed several times with a total of 30 mL of deionized water, until pH was 7.0. The concentration of synthetic MNPs was 9.1 g/L. The activated soil was functionalized with MNPs, by mixing 500 mg soil (dry weight) and 0.91 mg (0.1 mL) MNPs. MMI and MMI-R treatments referred to the MNP-functionalized soils without or with dimethoate (100 mg/kg) addition.

Text S2. DNA extraction, high-throughput sequencing and quantification

To determine microbial community structure, DNA was extracted from each treatment. To assess bacterial community composition and diversity, the extracted DNA was amplified and sequenced targeting the V3-V4 regions of 16S rRNA genes (Sangon Biotech Co., Ltd, Beijing, China). PCR was performed using the universal primer set of 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), and sequenced using 2×250 bp PE technology on an Illumina MiSeq sequencer, and analyzed using the MOTHUR software package. All the effective reads were clustered into operational taxonomic units (OTUs) at 97% similarity using an UPARSE pipeline. Ribosomal Database Project (RDP) classifier was used to annotate and blast the sequence of each OTU at a confidence threshold of 0.8. Bacterial alpha-diversity indices (Chao1, Shannon and Simpson) were analyzed by QIIME (v1.80) to assess bacterial species richness and diversity. The distance matrices from samples were generated by the Bray-Curtis metric and visualized by principal coordinates analysis (PCoA) by QIIME (Quantitative Insights Into Microbial Ecology) software.

DNA content was quantified using the UV-VIS Spectrophotometer (NanoDrop Technologies, Wilmington, DE). Approximately 5 μg DNA was added to Quick-Seal polyallomer tubes (13×51 mm, 5.1 mL; Beckman Coulter, Pasadena, CA) and mixed with Tris-EDTA (pH 8.0)-$CsCl_2$ solution at a final buoyant density (BD) of 1.748 g mL^{-1}. The BD was determined using a digital refractometer (model AR200; Leica Microsystems Inc., Buffalo Grove, IL). After balancing, the tubes were heat sealed and transferred to an ultracentrifuge (Optima L-100XP, Beckman Coulter) at 45000×g for 48 h. Subsequently, DNA was fractionated and collected using a fraction recovery system (Beckman Coulter). A total of 15 layers were obtained for each sample. The BD of each DNA fraction was determined by an AR200 refractometer and DNA was purified.

The isolated strains capable of degrading OPs were identified by 16S rRNA sequencing. The 16S rRNA genes were amplified by PCR with a pair of primers (27F: 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R: 5'-TACCTTGTTACGACTT-3') (Weisburg et al. 1991). The PCR conditions were as follows: initial denaturation at 94°C for 5 min; 30 amplification cycles of denaturation at 94°C for 1 min; primer annealing at 55°C for 1 min, and extension at 72°C for 1.5 min; final extension at 72°C for 5 min. The purified PCR products were cloned into a pGEM-T Easy Vector and transformed into *Escherichia coli* JM109 competent cells. The plasmids of positive clones were extracted using a plasmid extraction kit (Qiagen Ltd, Germany), and the 16S rRNA inserts were sequenced by Majorbio Technology Company (Shanghai, China). Sequence similarity search and alignments were performed by Basic Local Alignment Search Tool (BLAST) algorithm in NCBI. Neighbor-joining method with MEGA 7.0 software was used to construct the phylogenetic trees of the 16S rRNA and OPs-degrading genes.

The fastq files of *Rhodococcus* L-1:

GGCCTCGCGATGCAGCTTGCATGCCTGCAGGTCGACGATTCGTCGTCGGCTGGGCAGG

GTTACGAGTACTACCACCTCGTGCACGAGGGCACCACGATTTCGAGCAGCGTGTACGG

CTCGGTGTTCTACATGACCACCGGCTTCCACGGTCTGCACGTCATCGGCGGTCTCATCG

CCTTCGTCTTCTTGATCGCACGTACCCGGGTCAGCAAGTTCACGCCCGCCCAGGCCAC

CGCCGCGATCGTTGTTTCGTACTACTGGCACTTTGTCGACATCGTGTGGATCGCGCTGT

TTGCCACGATTTATTTCATCCGTTAATCAGCTGTACGCCCCAGGCAGCCCCTGACGTCC

AGTTCCGTCCCGACATACCAAAGGGATACAGATGAGTTCATCCCCCCCTCCCGCATCCG

ATAACTCAGCGAATGCCGCGAAGTCGCGTCGCCAGCGGAAAATCCGCCGGCGCGTCA

CTGGCGCACTCGTATTGATGATGGGATTGATCAGCGCAGGTTTCCTCGCTTCGGCGTTG

ACACCAGCTCCGCAGGTTGCGACCGCCAGTGACGATTCAGCAGCCCTGATTCGTGAAG

GCAAGCAGCTGTACGACACGTCATGCATCACGTGCCACGGCGCGAACTTGCAGGGCG

TGCAGGACCGCGGTCCCAGCCTCATCGGCGTCGACGAAGCAGCCGTGTACTTCCAGGT

CTCCTCTGGTCGTATGCCGGCAGCTCGCAACGAGGTAGGCCGCACGCAATCTCTAGAG

GATCCCCGGGTACCGAGCTCGAATTCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAT

GTTTTACTCCCCCCGCTTAAAAAAA

Text S3 Enumeration of *oph* gene

One standard curves was obtained by producing a 10-fold serial dilution of plasmid pGEM-T Easy Vector sequences (10^2-10^8 copies; Promega) containing the OPH genes. The amplification reactions were conducted using a three-step method in a 96-well optical plate on an ABI 7500 real-time PCR system (Applied Biosciences, USA) as follows: denaturation for 10 min at 95°C,

followed by 40 cycles of 10s at 95°C, 30s at 56°C, and 20s at 72°C. The SYBR green signal intensities were measured in each cycle at 72°C after extension. At the end of qPCR, a melting-curve analysis was performed by increasing the temperature from 55°C to 95°C. For each sample, the average of three replicates was determined as the copy number per fraction.

Text S4 Dimethoate analysis

Briefly, during each extraction procedure, 0.5 g NaCl, 2 mL acetone, and a certain volume of dimethoate (3 mL, 2 mL, and 1 mL for the 3 extractions, respectively) were added into the supernatant and sonicated for 10 min. Supernatants from the 3 successive extractions were mixed, and subsequently dried at 35°C by a rotary evaporator. The volume was then adjusted to 5 mL with dimethoate, followed by a membrane filtration (pore size, 0.22μm; nylon) into brown vials for analysis. Samples were analyzed for dimethoate and its metabolic with a gas chromatography mass spectrometry (GC-MS, Shimadzu, QP2010SE), with a DB-5MS capillary column (30 m length, 0.25 mm diameter, 0.25 μm thickness). A total of 2.0 μL sample was injected in the splitless mode with a 5-min solvent delay time. The carrier gas was helium (99%) at a rate of 1.0 mL/min and the injector temperature was 280ºC. The GC oven temperature was set at 60ºC for 2 min, raised to 180ºC at a rate of 30ºC/min and maintained at 180 ºC for 5 min, and finally raised to 300ºC at a rate of 10ºC/min and maintained at 300ºC for 2 min. Electron impact source and selected ion monitoring (SIM) mode were used to identify individual metabolite. The ion source temperature was 240°C and scanning range was from 45 to 450 atomic mass units (amu). The dimethoate standard concentrations (10-800 μg mL^{-1}) were used to derive the calibration curve for dimethoate. Mean recoveries of surrogate standards in the present study ranged from 85-98% and the final concentrations of dimethoate were corrected by surrogate recovery.

For the determination of dimethoate degradation metabolites, the molecular mass of each metabolite was searched against previous literatures and the database of dimethoate metabolites (National Institute of Standards and Technology, NIST). The chemical structure of each possible metabolite was confirmed by the pattern of fragment ions in the mass spectrum.

Table S1 The geochemical properties data of the soil samples

Parameters	**Value**
pH	7.5
H_2O (%)	16.4
Humus（g/kg）	10.3
SOM（g/kg）	17.7
TOC（g/kg）	10.3
TN（g/kg）	1.12
Available N（mg/kg）	79.3
TP（g/kg）	1.10
Available P（mg/kg）	52.5
Available K（mg/kg）	125

Table S2 The components of minimal medium

Components	Concentration
KH_2PO_4	0.5g/L
NaCl	0.5 g/L
$CaCl_2$	0.1g/L
$MgSO_4 \cdot 7H_2O$	0.2 g/L
$FeCl_3 \cdot 6H_2O$	0.5g/L
$MnSO_4 \cdot H_2O$	0.5g/L
$ZnSO_4 \cdot 7H_2O$	0.2g/L

Table S3 PCR primers used for the PCR of 16SrRNA and dimethoate-OPH genes

Target	Primer	Sequence (5'-3')
16S rRNA	27 F	AGAGTTTGATCCTGGCTCAG
	1492 R	GGTTACCTTGTTACGACTT
OPs-degrading genes	*ophB*-F	5'-CGTCGTCGGCTGGGCAGGGT-3'
	ophB-R	5'-GCGTGCGGCCTACCTCGTTG-3'
	ophC2-F	5-'ATGCGTCTTTTCTCGCTGAGC-3'
	ophC2-R	5'-TCAGCGGTCGCTACGGATCGG-3'

Table S4 The relative abundances of OTUs in the MMI-R, ^{12}C-R-SIP and ^{13}C-R-SIP treatments .

OTU ID	Phylum	Class	Order	Family	Genus	OS-0d	MFC-R-7	MFC-R-21	MFC-R-35	13C-R-SIP-7	13C-R-SIP-21	13C-R-SIP-35	MMI-R-7	MMI-R-21	MMI-R-35	12C-R-SIP-7	12C-R-SIP-21	12C-R-SIP-35
Otu4	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	2.02	46.84	36.82	2.6	11.72	2.57	1.83	1.96	2.84	2.8	1.29	0.72	6.32
Otu0	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	5.83	8.62	16.03	10.78	11.34	8.56	11.4	17.32	12.22	11.63	6.95	7.27	8.06
Otu21	Firmicutes	Bacilli	Bacillales	Bacillaceae 1	Bacillus	0.07	0.29	0.76	40.83	0.29	0.33	0.08	0.3	0.33	0.27	0.11	0.76	0.21
Otu11	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Ramlibacter	0.28	2.93	2.39	9.28	5.6	0.05	6.7	1.07	3.58	3.89	0.58	0.5	0.36
Otu84	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Arthrobacter	5.1	3.33	9.82	4.84	1.46	5.58	10.28	0.31	0.04	0.04	1.12	3.45	4.84
Otu62	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae	Rhodococcus	0.7	1.29	3.12	6.32	11.72	2.67	1.93	0.08	0.05	0.05	4.12	2.15	1.11

Table S5 Dimethoate and its metabolites identified by GC/MS

Metabolite	Chemical name	Rt(min)	Characteristic ions (m/z)
D	Dimethoate	10.89	229,143,125,93,87
D1	Omethoate	9.61	213,156,141,110,80
D2	O,O,S-trimethyl thiophosphorothioate	5.55	156,141,125,110,95
D3	N-methyl-2-sulfanylacetamide	5.205	105,73,58
D4	O,O-diethyl S-hydrogen phosphorodithioate	5.349	186,158,142,121,97
D5	O,O,O-trimethyl thiophosphate	4.31	156,125,95,79
D6	O,O,S-trimethylphosphorothiate	3.95	156,141,125,110,93,79
D7	O,O,O-trimethyl phosphoric ester	3.109	109,95,80,78,65

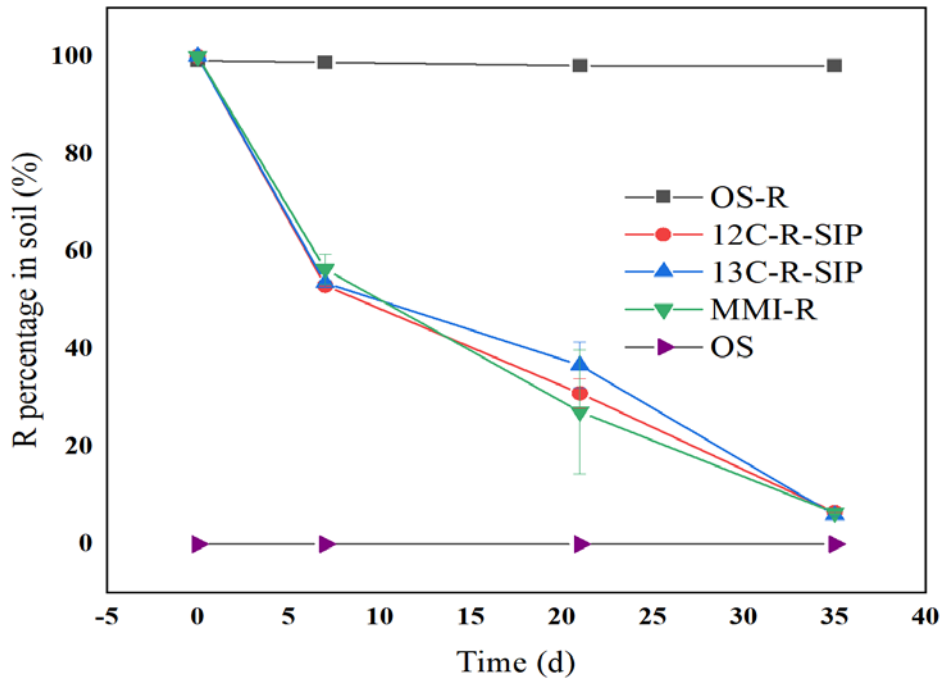


Figure S1 Residual dimethoate percentage in different treatments.

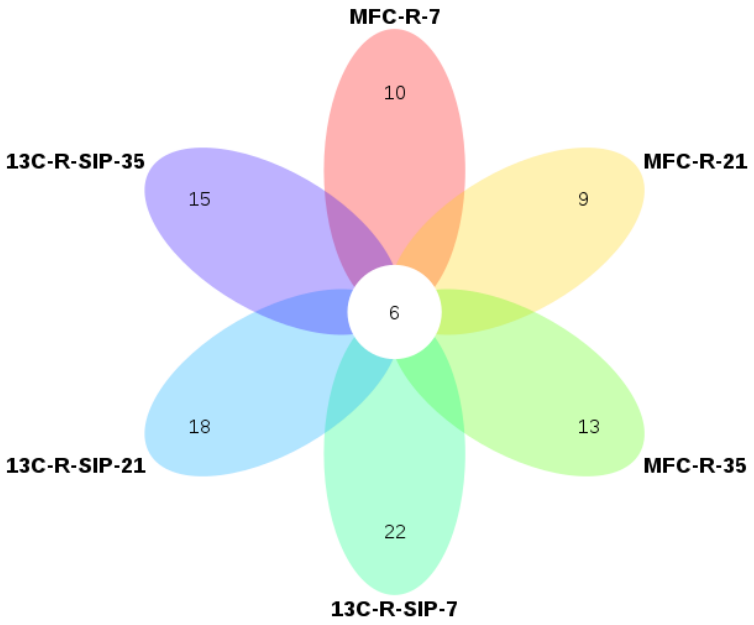
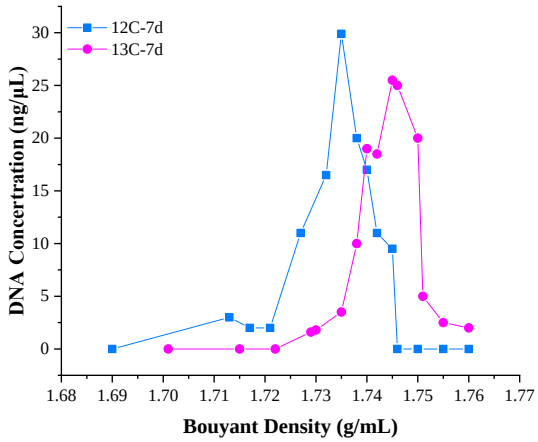
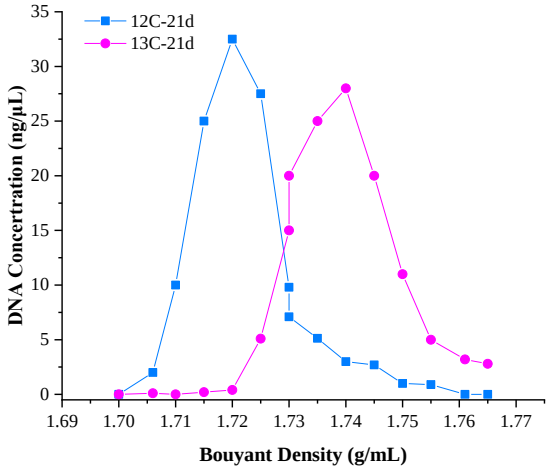


Figure S2 Venn diagram showing the shared and unique archaeal OTUs at 5% distance threshold between the MMI and DNA-SIP treatments.





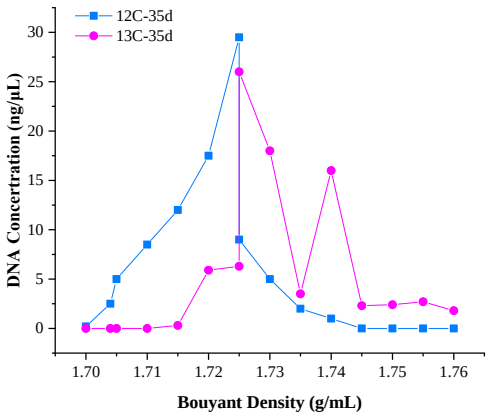
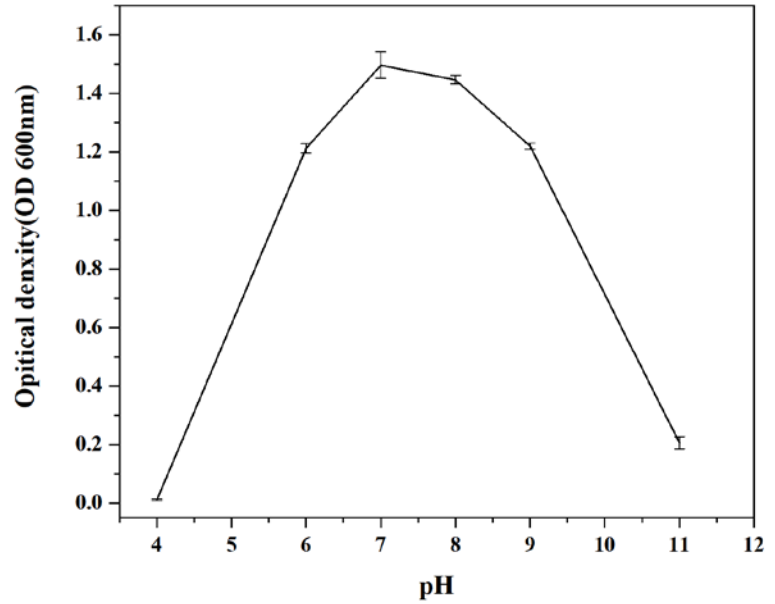


Figure S3 DNA concentration in each fraction relative to the BD on day 7, 21 and 35.



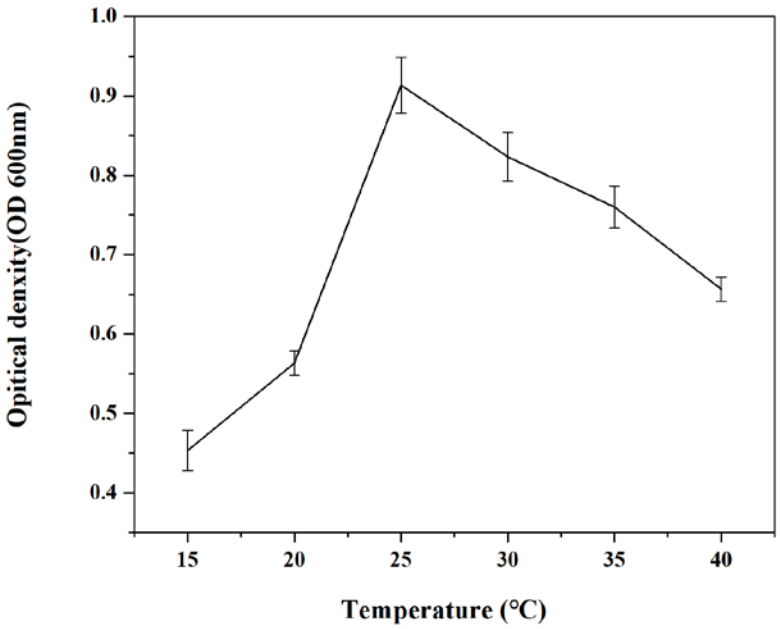
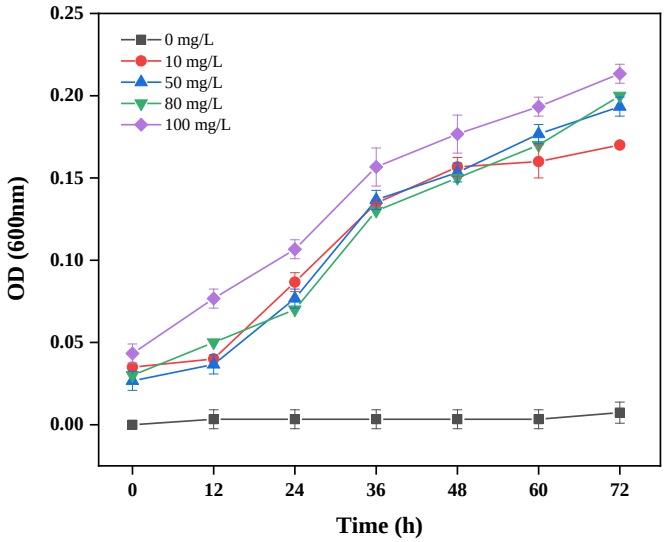
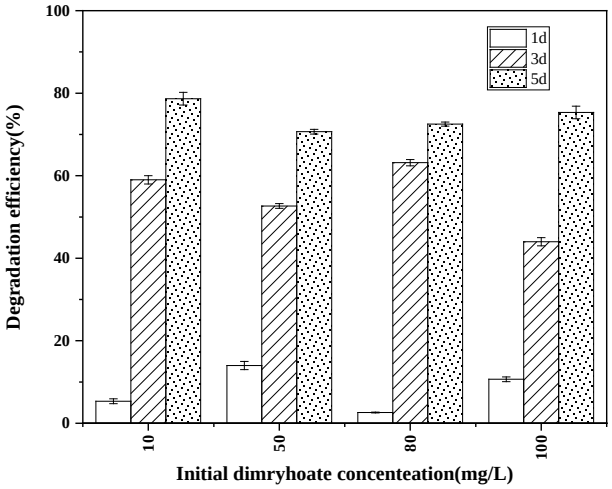


Figure S4. Growth curve of *Rhodococcus sp.* L-1 under different pH and temperature . Cell growth was evaluated by measuring the optical density of the culture at 600 nm after incubation for 72 h.



(a)



(b)

Figure S5. Growth curve (a) and dimethoate degrading efficiency (b) of Rhodococcus sp. L-1 in mineral medium supplied with different concentrations of dimethoate as carbon source. The initial dimethoate ranged from 10 to 100 mg/L.

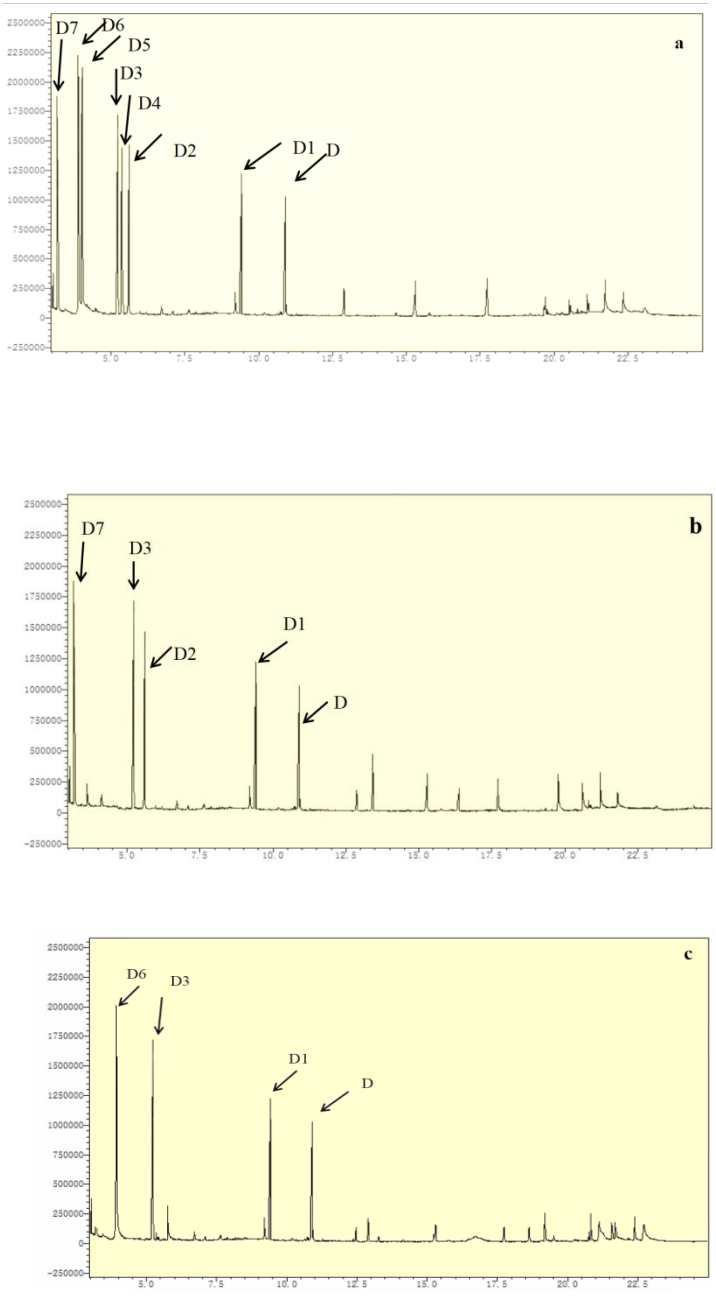
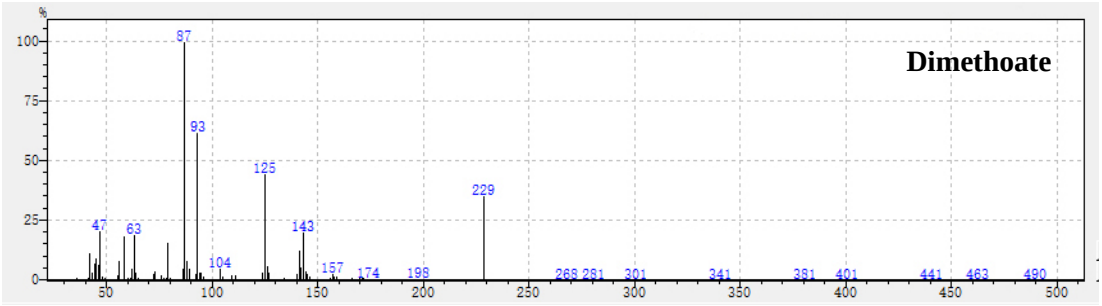
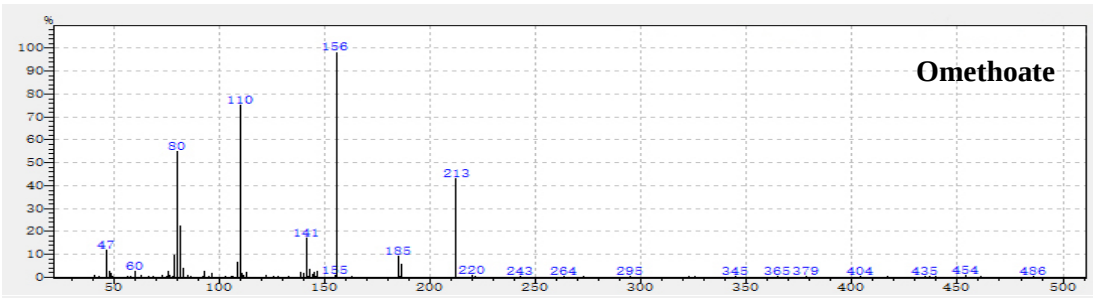
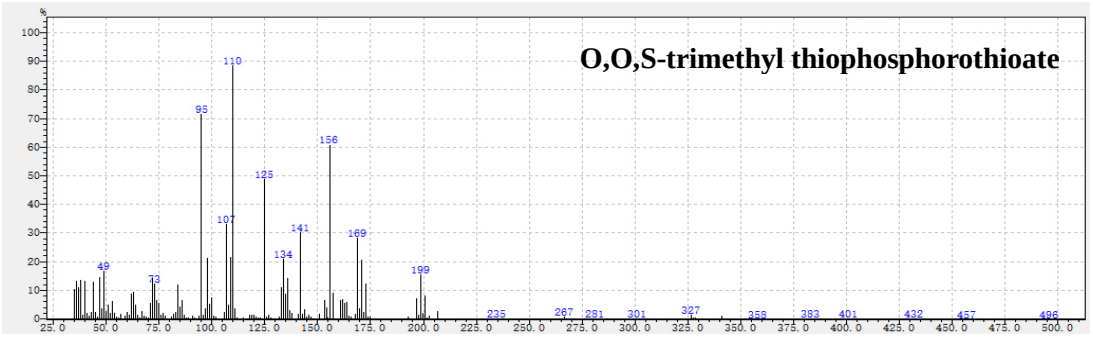
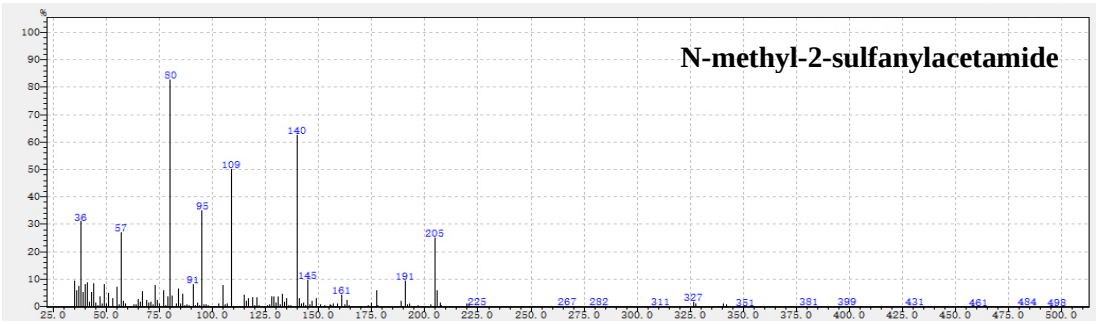


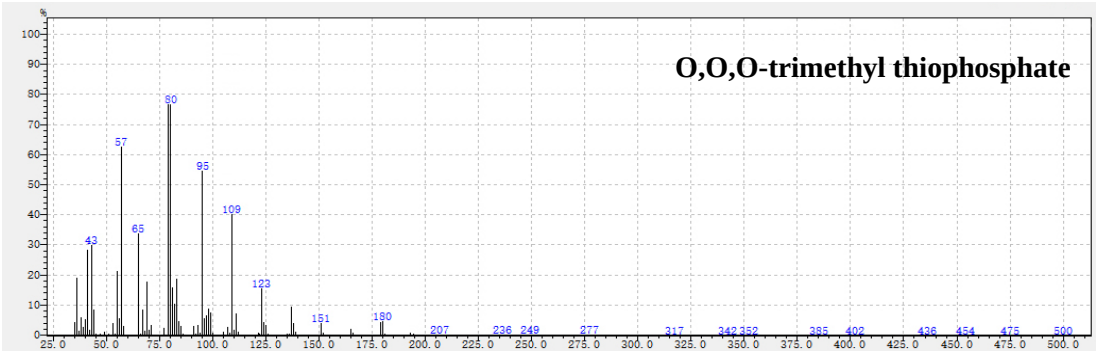
Figure S6 The GC profiles for dimethoate and its metabolites in different treatment at 35 days (a, SIP, b, MMI and c, Rhodococcus sp.L-1)


Dimethoate


Omethoate


O,O,S-trimethyl thiophosphorothioate


N-methyl-2-sulfanylacetamide


O,O,O-trimethyl thiophosphate

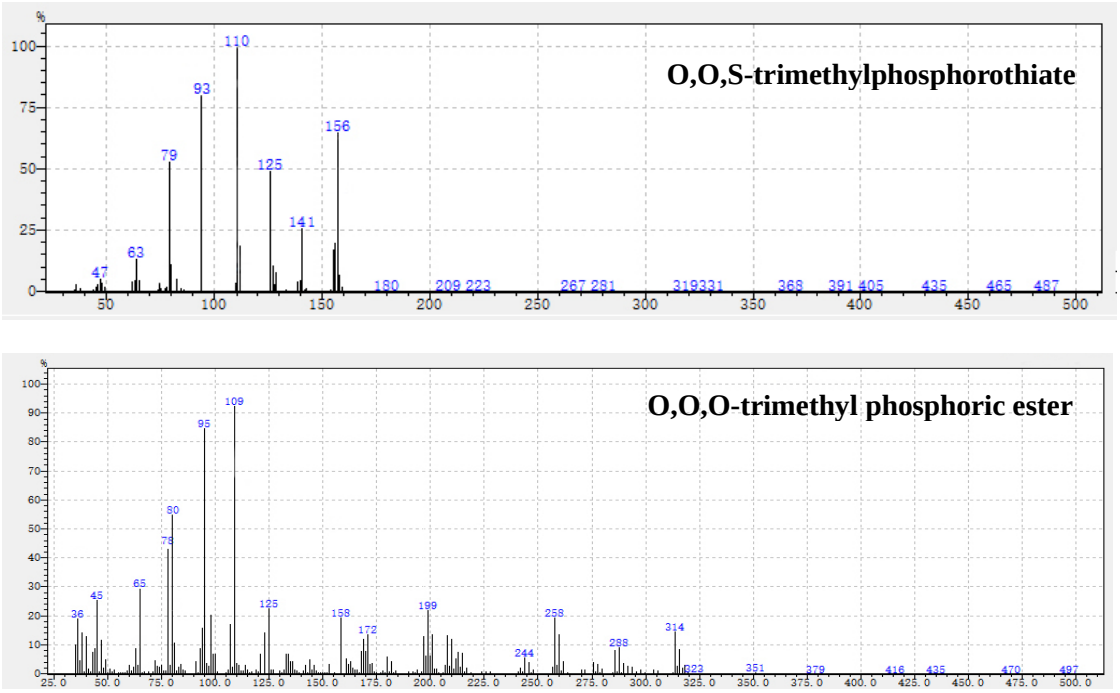


Figure S7. Mass spectrum of dimethoate degradation products.