Acephate

Pathways (Biodegradation):

Degradation through isolation, where you take a soil sample and treat it with acephate to find the best subculture

Using an *opd* (organophosphate degradation) gene to express mOPH or pre OPH that help with the biodegradation of OP's (Overview, Page 28)

OPH has optimal activity at higher pH

There is a opaA gene that has also shown the ability to degrade and perform the same functions, although it's vector is a chromosome (Page 31 of Overview)

Lateral transfer of organophosphate degradation (opd) genes among soil bacteria: mode of transfer and contributions to organismal fitness

Table 5. List of genes, their origin, vector, and gene products involved in degradation of organophosphorus compounds

Gene	Organism(s)	Location	Encoded enzyme	Reference
opd	*Pseudomonas diminuta*	Plasmid	OPH	Serder *et al.* (1989)
	Flavobacterium sp.	Plasmid	OPH	Mulbry *et al.* (1986)
	Flavobacterium balustinum	Plasmid	OPH	Somara & Siddavattam (1995)
	Pseudomonas sp.	Plasmid	OPH	Chaudry *et al.* (1988)
opaA	*Alteromonas* sp. JD6.5	Chromosome	OPAA	Cheng *et al.* (1996)
	Alteromonas haloplanktis	Chromosome	OPAA	Cheng *et al.* (1997)
	Alteromonas undina	Chromosome	OPAA	Cheng *et al.* (1996)
opdA	*Agrobacterium radiobacter*	Chromosome	OPDA	Horne *et al.* (2002b)
hocA	*Pseudomonas monteilli*	Chromosome	ND	Horne *et al.* (2002c)
mpd	*Plesiomonas* sp.	Chromosome	ND	Zhongli *et al.* (2001)
adpB	*Nocardia* sp. B-1	Chromosome	ADPase	Mulbry (1992)
PdeA	*Delftia acidovorans*	Chromosome	Phospho diesterase	Tehara & Keasling (2003)
PepA	*Escherichia coli*	Chromosome	AMPP	Jao *et al.* (2004)
Phn	*Escherichia coli*	Chromosome	Phosphonatase	Chen *et al.* (1990)
	Sinorhizobium meliloti	Chromosome		Parker *et al.* (1999)
glp A&B	*Pseudomonas pseuodomallei*	Chromosome	C–P lyase	Penaloza-Vazquez *et al.* (1995)
pehA	*Burkholderia caryophilli*	Chromosome	PEH	Dotson *et al.* (1996)

Table 4. Organophosphorus degrading microbial enzymes

				Bond cleavage			
Enzyme	Origin	MW	Structure	P–O	P–F	P–S	P–C
Bacterial							
OPH	*Pseudomonas diminuta*	72	Dimer	+	+	+	+
OPAA	*Alteromonas* spp.	50–60	Monomer	+	+	–	+
OPDA	*A. radiobacter*	70	Dimer	+	+	+	+
ADPase	*Nocardia* sp.	43	Monomer	+	ND	ND	–
AMPP	*Escherichia coli*	52	Tetramer	+	ND	ND	ND
HOCA	*Pseudomonas monteilli*	19	Monomer	+	ND	–	ND
SC-OPH	SC strain	67	Tetramer	+	ND	ND	–
NS-OPH	*Nocardiodes simplex*	45	Monomer	+	ND	ND	ND
PEH	*Burkholderia caryophilli*	58	Tetramer	–	–	–	+
C–P lyase	*Pseudomonas* spp.	200	ND	–	–	–	+
Phosphonatase	*Bacillus cereus*	37	Dimer	–	–	–	+
Fungal							
A-OPH	*Aspergillus niger*	67	Monomer	–	ND	+	ND
P-OPH	*Penicillium lilacinum*	60	Monomer	+	ND	+	ND
Laccase	*Pleurotus ostreatus*	ND	ND	–	–	+	ND

+, positive activity; –, no activity; ND, not determined; MW, molecular weight.

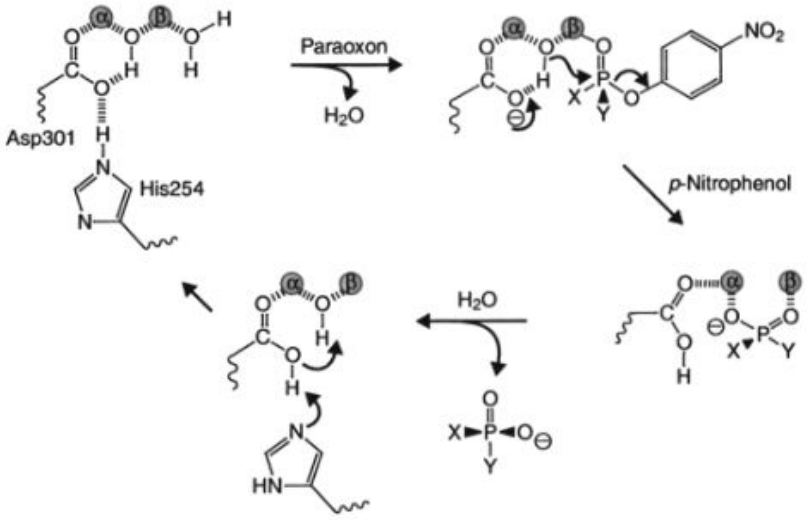


Fig. 12. Working model for the catalytic mechanism for hydrolysis of organophosphorus nerve agents by organophosphorus hydrolase (reproduced with permission from Raushel, 2002).

1) Biosafety Level → Depends on how lenient the teachers are. Remember, this is a neurotoxin to insects, and the metabolites can cause serious side effects.
2) Harmful Health Effects → There are very extreme side effects that can occur, such as seizures. It is labeled as a possible **carcinogen**, and acephate is absorbed through the skin, however it is the **metabolites that cause neural damage**. (Source)
3) How much past literature already exists? → The past literature is pretty solid, though they seem to follow the same formula

Half-life:

[Acephate half-life seems to be sensitive to temperature and pH and can vary (Source)]

Soil: (Sources vary)

< **3 days in aerobic soil** [studied in the lab and observed in in field studies, and this number can vary greatly depending on the type of soil] (Source)

Water:

Hydrolysis is slow but faster degradation rate at higher pH levels, degraded with a half life of 18 days at pH 9 (Source), (Source)

Notable information:

Details

Acephate converts to methamidophos, a strong and effective insecticide when consumed by insects (which is why it has selective toxicity and not as much health effects on mammals) (Source)

However, residue can cause harm to human health, as it inhibits an enzyme (AChE) that begins the breakdown of neurotransmitters (Source)

"Acephate is a general-use insecticide registered for use on food crops, agricultural seed and non-bearing plants, institutions and commercial buildings including public health facilities, sod, golf course turf, ant mounds, and horticultural nursery plants" (Source)

"Acephate is an organophosphate insecticide. The International Union of Pure and Applied Chemistry (IUPAC) chemical name for acephate is O,S-Dimethyl acetylphosphoramido-thioate2" (Source)

"Both [acephate and methamidophos] are very mobile in soil, moving with water easily… Acephate does not tend to off-gas from soil or water" (Source)

Degradation

Acephate was resistant to hydrolysis in distilled, buffered water at pH 4.0 to 6.9, but not at pH 8.2, held for 20 days at 20 or 30°C (Source)

Real World Applications

Acephate was considered in British Columbia in aerial applications but shut down due to fear of water contamination,

In 1988, a FDA study detected acephate in 33 of 1170 food items (Source)

In 2003, a survey detected acephate in 15 of 1039 food items [0.05 ppm, 2.0ppm) (Source)

Daily, the average intake for acephate 1988 was found by the FDA to be (in ug/kg body wt/day):
Infants: 0.0105
14-16 M: 0.0080
60-65 F: 0.0104
(Source) *However this most likely is considered "below the level of concern"

Honeybees that were treated with acephate at different doses, and the non lethal doses the bees were still negatively affected (Source)

In 2000 the USDA reported Acephate was the most commonly used insecticide making up for 40% of all insecticide use (Source)

A whole article on applications of organophosphates

Toxicity

"Acephate can cause cholinesterase inhibition in humans; that is, it can overstimulate the nervous system causing nausea, dizziness, confusion, and at very high exposures (e.g., accidents or major spills), respiratory paralysis and death" (Source)

At higher doses, it can inhibit the aceylchonsies enzymes in the nervous system, which leads to some very severe side effects, such as paralysis and seizures (Source)

Classified as possible carcinogen by EPA (Source)

Though Acephate is an insecticide, different insects are affected on various levels, for ex., this source shows that mayfly larvae was very sensitive while stonefly and other insects were less affected (Source)

Chemical/Physical Properties

"Technical grade acephate is a white or transparent solid
Vapor pressure: 1.7 x 10-6 mmHg at 24 °C
Octanol-Water Partition Coefficient (Kow)4: 0.13 at 25 °C
Henry's constant: 3.1 x 10-7 atm·m3/mol; 5.1 x 10-13 atm mole/m3
Molecular weight: 183.16 g/mol
Solubility (water): 79 - 83.5 g/100 mL
Soil Sorption Coefficient (Koc): 2; 2.7" (Source)
Chemical Formula: $C_4H_{10}NO_3PS$

Degradation Toxicity

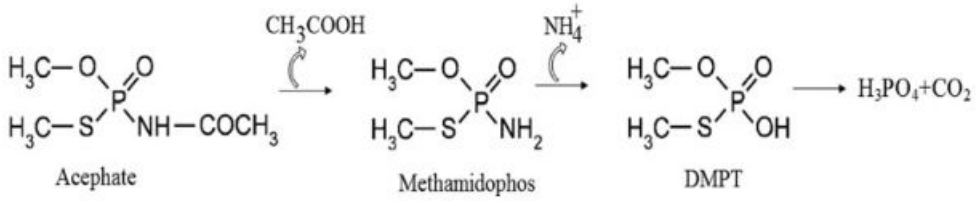
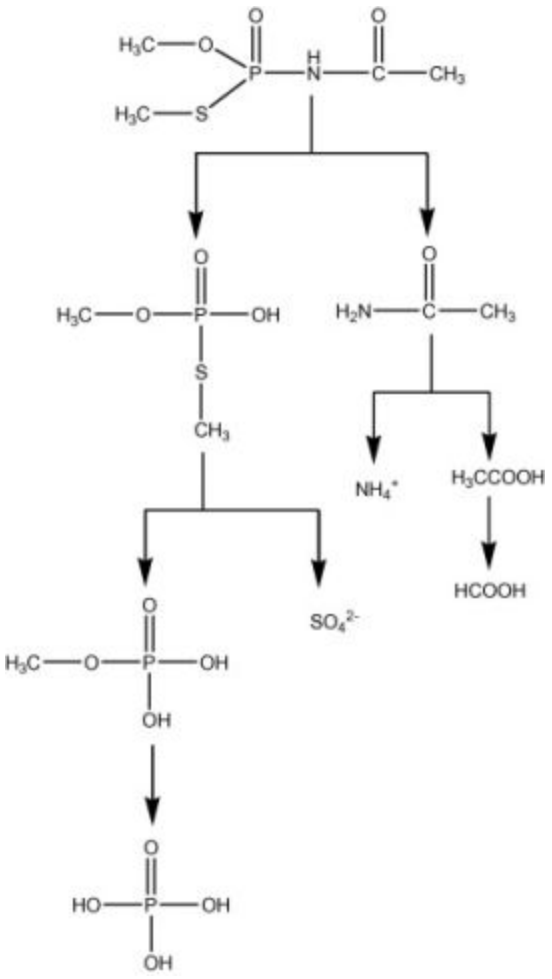
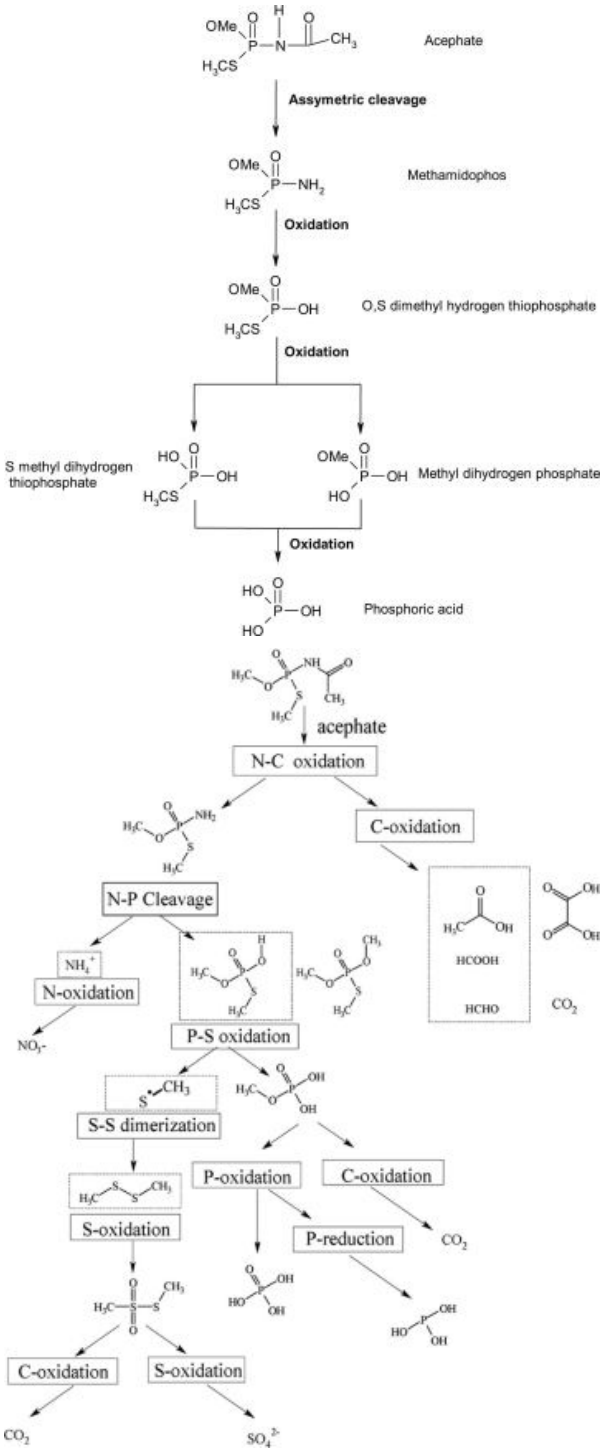


Fig. 5. Proposed pathway for the degradation of acephate by *Pseudomonas aeruginosa* strain Is-6.





The pathways usually biodegrade into Formic Acid or Phosphoric Acid.

Phosphoric acid seems to be hazardous in which it can causes severe skin burns and eye damage, but it depends on the concentration (Source)

Formic Acid acid also seems to be hazardous and cause the same symptoms as phosphoric acid (Source)

Prior Research

Biodegradation of acephate and methamidophos by soil bacterium Pseudomonas strain Is-6 (https://sci-hub.tw/10.1080/03601234.2013.836868)

Materials and Methods

M9 medium (containing Na2HPO4(6 g L−1), KH2PO4(3gL−1), NaCl(0.5 g L−1) and NH4Cl2 (1 g L−1) atp H (7.2±0.2))

Culture/enrichment was done in airtight flasks at 120 rpm and 30 degrees C

Soil samples originating from India (agricultural sites that have been treated w/ acephate for >8 years)

20g of soil were treated with 2 mg of acephate

Samples dissolved in 100μL of dichloromethane, incubated at room temperature for 5 weeks

10g of soil, 95 mL of 0.85% sterile saline was prepared in the shaker at 150 rpm

Diluted 10 fold, inoculated in 3mL M9 medium and 50 μg/mL acephate

Incubated until turbid in 26 degrees C, isolated using the pour plate method

Single colonies isolated until "homogenous colonies were observed" → the study had 6

Strains maintained at 4 degrees C

Sequencing of the 16S rRNA gene, extracted using lysis method

PCR was done using 7f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 492r (5′TACGGTTACCTTGTTACGACTT-3′) primers

Is-6 was prepared in M9 with 50 mg L^-1 acephate and and methamidophos (both)

Incubated overnight at 30 degrees C at 120 rpm

Centrifuged and washed four times

Cultures monitored spectrophotometer at 600 nm

Concentration of acephate and methamidophos "was measured by HPLC"

Degradation at different concentrations of the strain was studied, however there were duplicate flasks kept as controls

HPLC analysis was done in 24h intervals

Soil collected from India with a pH of 7.12 and OM content of 3.26%

"3 sets of fumigated soil and non-fumigated soil were inoculated with acephate degrading bacteria" (another set as the control)

Moisture was at 40% water holding capacity and incubated in the dark

Results

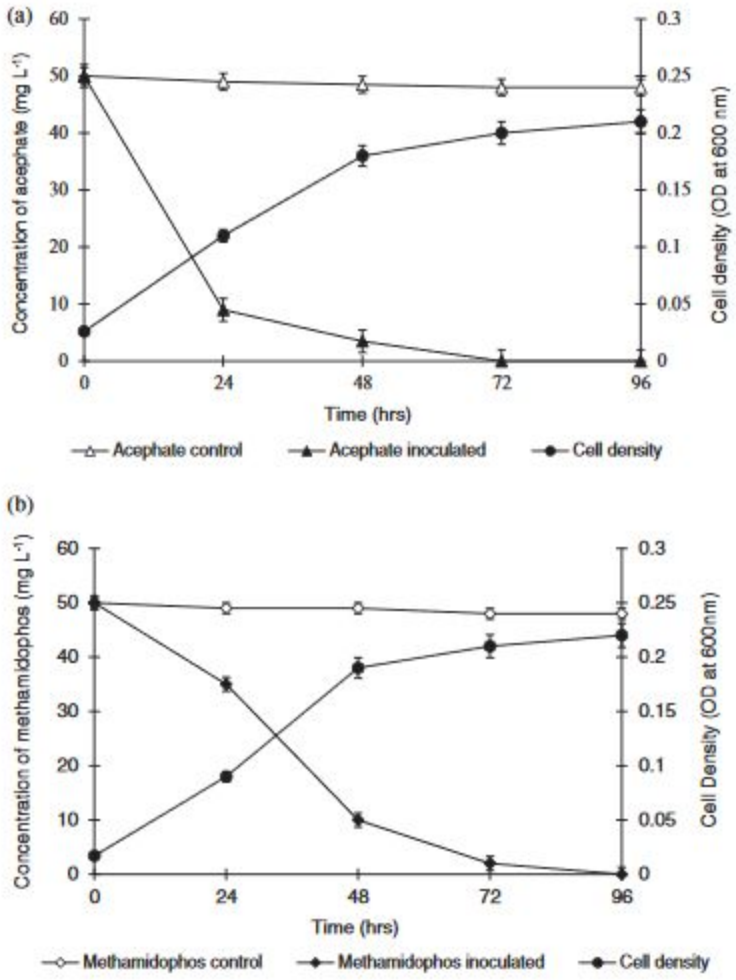
Out of the 6 cultures, Is-6 preformed the best, and is #HQ122657 in the EMBL Nucleotide Sequence database

Similar to Pseudomonas aeruginosa

“Strain Is-6 was a gram negative, aerobic, short rod and motile bacterium. It was positive tests for oxidase, catalase, nitrate reductase, citrate utilization and H2S production, but negative for Methyl-Red Test, Voges-Proskauer test, phenylalanine deaminase, ure-ase test, indole production, starch hydrolysis and gelatin liquefaction”

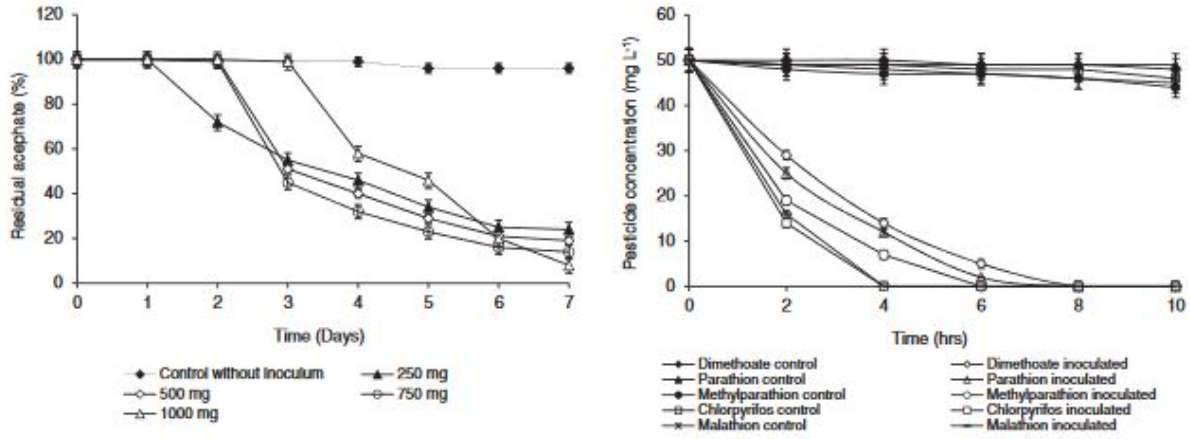
Is-6 could use acephate as a source of carbon, nitrogen, and phosphate, cell density grew in 96h, and 50 mg L^-1 was completely degraded in 96h

Biodegradation of acephate by new soil bacterium



Is-6 could also degrade at higher concentrations

Is-6 in the soil could degrade at a higher rate in fumigated soils than the uninoculated controls



Summary: Bacteria collected from acephate treated fields in India were isolated and their rRNA was amplified and classified. The bacteria was treated with acephate and 6 sub-cultures of bacteria were observed. Out of all of them, Is-6 proved to be the most efficient. And could degrade acephate and methamidophos at higher concentrations which was monitored by HPLC. In the fumigated soil, Is-6 performed better than the normal bacteria. Overall, Is-6 proved to be an efficient degradation method.

Biodegradation of acephate using a developed bacterial consortium and toxicological analysis using earthworms (Lumbricus terrestris) as a model animal
(https://sci-hub.tw/10.1016/j.ibiod.2011.11.013)

Materials and Methods

Isolation of bacteria from pesticide contaminated soil through enrichment culture techniques

MSM medium was prepared: 1) Nitrogen limiting, 2) Carbon limiting, 3) “Full strength”

2g of soil sample + 100 mg^-1 of acephate, incubation at 30 degrees C

Shaked at 120 rpm

Cultured after 5 days, with 100 mg l^-1 acephate, and the most efficient colony was selected after observation

16s rRNA sequence

The isolated bacteria was Exiguobacterium BCH4 (consortium ER) and another was borrowed from another experiment

Loopful inoculated (separately) into tubes with 5ml MSM

Incubated for 6h, 30 degrees C and a pH of 7

A range of acephate concentrations were added 50-250 mg l^-1

HPLC, FTIR, and GC-MS were among the few used

Earthworms were used to check their coelomocytes (used more to study toxicity than degradation)

Extracted coelomocytes were centrifuged at 4 degrees C, 3000g, for 10 min (discard supernatant)

Comet assay was performed

SOD activity was observed through photochemical reduction of nitro-blue tetrazolium

Results

Consortium ER degraded 78.85% of scephate in 6 days

Data suggests that acephate degradation was not by biosorption

Temperature played a role in degradation efficiency, with highest efficiency at 30 degrees celsius

Extreme pH also negatively impacted degradation, and neutral preformed the best

Different chemical formulas also inhibited degradation

Summary: Bacteria was isolated from pesticide treated agricultural soil and was prepared in three different types of MSM medium. Acephate was added to the solution and the colony that seemed to degrade acephate the best was selected and the 16s rRNA sequence, discovered to be a type of Exiguobacterium. Biodegradation was monitored by HPLC, and different concentrations of acephate were added to the solution. HPLC, FTIR, and GC-MS were used to analyze the degradation of the acephate. The solution best had a degradation rate of 75.85% at 30 degrees C and a neutral pH over 6 days.

Degradation of acephate by Enterobacter asburiae, Bacillus cereus and Pantoea agglomerans isolated from diamondback moth plutella xylostella (L), a pest of cruciferous crops (http://www.jeb.co.in/journal_issues/201607_jul16/paper_20.pdf)

Materials and Methods

Diamondback moths, which harbors a plethora of microbiota that plays an essential role in growth and development, pathogenesis, and environmental adaptation of host insects. These gut microbiota can potentially be used to deoxidize and degrade insecticides.

- To start, third-instar diamondback moths larvae were collected (around 200 larvae from each cabbage crop location)
- Larvae were surface-sterilized with sodium hypochlorite (0.1%) and ethanol (70%) for 5 sec to remove adhering contaminants, especially external microflora, and underwent homogenization
- Homogenate place in sterile LB agar media, incubated in 30 degrees C for 48 hrs to triplicate, then colonies that grew on acephate were streaked onto LB broth and cycle was done several times for purity

- MM (minimal media) tests were done to see how well the bacteria were able to use carbon, sulfur, nitrogen
- The MM with bacterial inocula (control for growth studies), MM1, MM2 were all incubated and
- Carbon:Nitrogen:Sulfur ratio was 4:1:1, shows bacteria was able to use acephate as a source for these resources
- Bacterial growth was measured spectrophotometrically at aliquot time points

Results

Analysis showed that the Eneterobacter strain PXE had the closest match to E. asburiae AAB08 and E. asburiae YMC

E. asburiae and B. cereus, and O. agglorans showed potential to use acephate as a sole carbon source

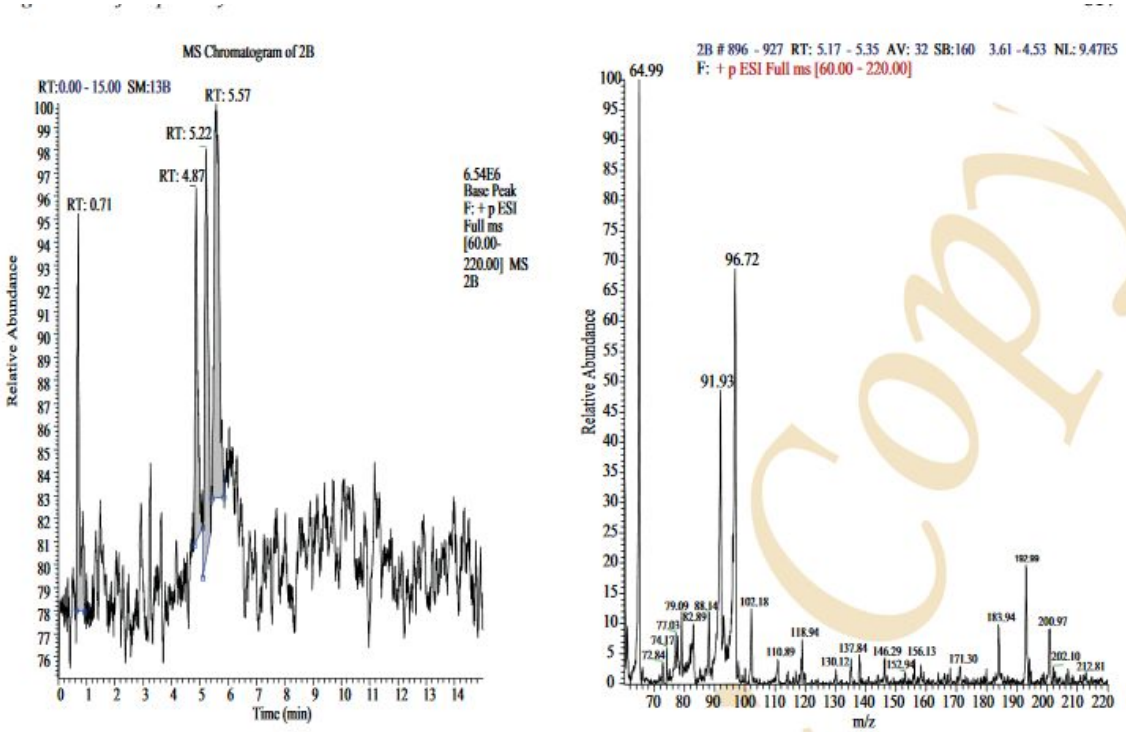


Fig. 6 : Chromatogram and mass spectra for acephate degradation by *P. agglomerans*

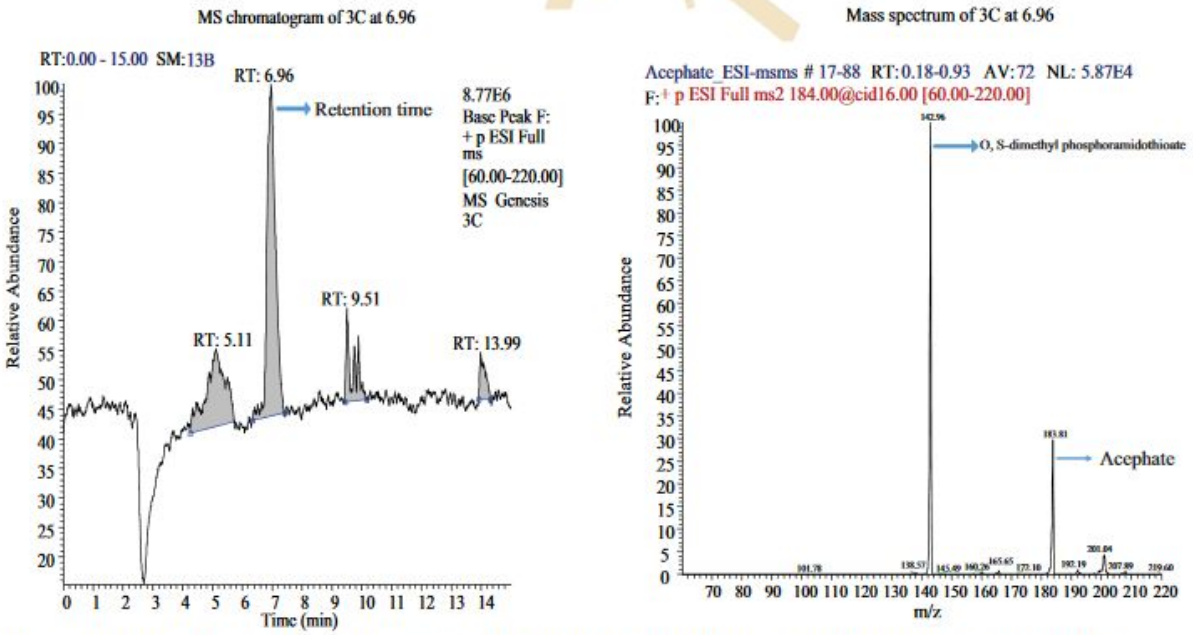
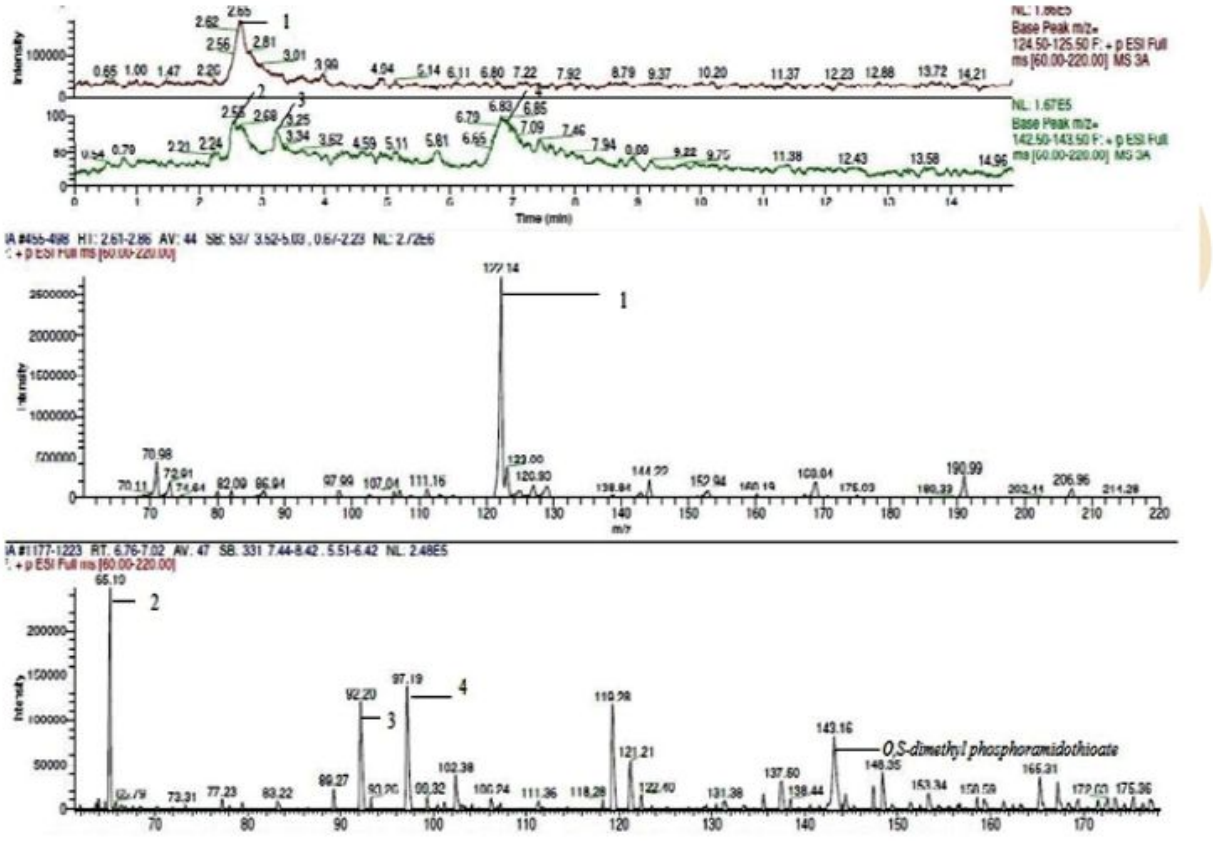


Fig. 5 : Chromatogram and mass spectra showing degradation of acephate into O,S-dimethyl phosphoramidothioate by *B. cereus* (PX-B.C.Or)



Summary: Several microorganisms can degrade organophosphorus chemical substances and use these as a source of carbon, nitrogen and sulfur. The results of the bacteria tested were shown to

be able to use acephate as a source for carbon, nitrogen and sulfur, but some bacteria were not able to get enough nitrogen and sulfur to be their only sources to strive. Each bacteria in the testing had different amounts of the components and some were unable to survive solely by acephate.

Isolation and Determination of Efficacy of Acephate Degrading Bacteria from Agricultural Soil (https://pdfs.semanticscholar.org/e6e3/1ab863b90c5427b0e4951790e9fd2696fcf2.pdf)

Materials and Methods

Soil was collected from soil in India (stored at 4 degrees C)

5g of soil inoculated into 50 ml MSM medium with 50 ppm acephate in a flask

Incubated at 37 degrees C, 72h in a shaker water bath at 100 rpm

5ml was transferred into 45ml MSM with 50 ppm

Both flasks were plated on MSM aga with 50ppm acephate

Plates incubated at 37 degrees for 72 hours

5 isolates were chosen and were further analyzed and their 16s rRNA were sequenced through PCR

The 5 isolates were inoculated into 5ml of sterile saline and incubated at 37 degrees C for 3h

Different concentrations of acephate were prepared, and one set was used as a control

0.1 ml of bateria culture were inoculated into 5ml MSM tubes, incubated for 37 degrees C for 7 days

300ppm concentration centrifuged at 14000rpm for 10 min and was analyzed with HPLC

Results

Turbidity were observed in all concentration

It appears that Bacillus cereus ADI-10 had the highest optimal density over a 7 day period

Lysinibacillus fusiformis, Pseudomonas pseudoalcaligenes, Pseudomonassp., Bacillus cereus all had the ability to use acephate as a carbon source

Bacillus preformed better than Pseudomonas, and Pseudomonas better than, following Lysinibacillus fusiformis, Pseudomonas pseudoalcaligenes, Pseudomonas sp.ADI-04, and Pseudomonas pseudoalcaligenes

Summary: Bacteria were isolated from soil and treated with acephate until 5 colonies were selected for their degradation abilities. Over a 7 day period, these different isolates were subjected to different concentrations of acephate and 4 were able to utilize the acephate as a carbon source, with the Bacillus strain having the best performance.

Expression and subcellular localization of organophosphate hydrolase in acephate-degrading Pseudomonas sp. strain I nd01 and its use as a potential biocatalyst for elimination of organophosphate insecticides
(https://sfamjournals.onlinelibrary.wiley.com/doi/pdf/10.1111/lam.12080)

Materials and Methods

Cells were grown in LB or MSM with 10 mmol l^-1 acephate

Antibiotics chloramphenicol and kanamycin were used when needed

pMMB206 was used for a vector for preOPH and mOPH, and resistance gene for kanamycin resistance (cell already resistant to chloramphenicol) (Diagram)

opd genes from pSM5 and pHLNS400

Plasmids (pre and mature) transformed into E. coli S17-1, which then mated with Pseudomonas sp. Ind01

Selected with kanamycin and chloramphenicol plates at 30 degrees C for a day

Grown at 30 degrees C until optical density of 0·5 at 600 nm

Induced to express OPH by adding IPTG with a concentration of 1 mmol 1^-1

1ml drawn every ½ h to see OPH activity using methyl parathion

Cells lysed (prep by sonication) at 13800g for 10 min, then centrifuge crude extract at 173,037g for 1h

Western blots performed to detect OPH with anti-OPH antibodies

Results

OPH, a membrane-associated protein, in which degradation of organophosphate pesticides requires OPH to be anchored into the membrane (at least within Pseudomonas sp. strain I nd01).

Cultures with pre-POH expression showed OPH expression only in the membrane, meaning the membrane was "successfully" targeted.

Cultures expressing mOPH (mature OPH) did not target the membrane, but it was able to degrade methyl parathion

"mOPH expressed in Escherichia coli was active and noted elevated synthesis of mOPH upon removal of an inverted repeat sequence that could generate a secondary structure at the 5′ region of the opd-specific mRNA"

The modified cell grew like normal wild-type cells while still using acephate as the sole carbon+nitrogen source

OPH activity was similar to B. diminuta cells (wild-type)

The OPH allowed the cell to degrade organophosphate, which means it can degrade acephate

They grew like wild-type cells using acephate as sole source of carbon and nitrogen

Summary: *Opd* genes that coded either for preOPH or mOPH were used in which pMMB206 was used as a vector and transfected into E. coli. E. coli then mated with Pseudomonas sp. strain Ind01, a reported acephate degrading strain. Using kanamycin and chloramphenicol plates for selection, the OPH expression was then analyzed. OPH has been proven before to help degrade OP's, for reference:
https://pubmed.ncbi.nlm.nih.gov/16766477/ → OPH has the ability to biodegrade OP neurotoxins
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC123893/ → Looking for more effective OPH varaints
https://onlinelibrary.wiley.com/doi/abs/10.1002/jctb.6428?af=R→ Using BiOBr as a catalyst for OPH degradation
https://academic.oup.com/peds/article/19/3/99/1524401
https://pubmed.ncbi.nlm.nih.gov/26861877/

Degradation of methamidophos by Hypho Species Map-1 and the biochemical degradation pathway
(https://sci-hub.tw/10.1007/s10532-009-9320-9)

Transposon-Like Organization of the Plasmid-Borne Organophosphate Degradation (opd) Gene Cluster Found in Flavobacterium sp
(https://aem.asm.org/content/69/5/2533)

Further Reading

The fate of acephate and carbaryl in water
(https://sci-hub.tw/https://www.tandfonline.com/doi/pdf/10.1080/03601237909372157?needAccess=true)

Studies on the toxicity, metabolism, and anticholinesterase properties of acephate and methamidophos
(https://sci-hub.tw/https://www.tandfonline.com/doi/abs/10.1080/03601238509372472)

Summary: The toxicity of acephate was tested on 4 different aquatic insects and how dangerous it was, rats were also given acephate to study its metabolistic effects and how cholinesterases were inhibited. The cholinesterase-inhibiting properties of acephate and methamphidos were then compared *in vitro* to paraoxon on 4 different enzymes from living creatures (paraoxon is already known to be a strong anticholinesterase). In most cases, the acephate was significantly weaker (6 degrees weaker in acephate and 3 for methamidophos) than paraoxon, except in trout.

Acephate Insecticide Toxicity: Safety Conferred by Inhibition of the Bioactivating Carboxyamidase by the Metabolite Methamidophos
(https://pubs.acs.org/doi/10.1021/tx9601420)

Toxic effects of acephate on Paramecium caudatum with special emphasis on morphology, behaviour, and generation time
(https://sci-hub.tw/https://www.sciencedirect.com/science/article/abs/pii/S004835750600037X)