

BIODEGRADATION OF ORGANOPHOSPHOROUS PESTICIDES BY TWO FUNGI ISOLATED FROM PESTICIDE CONTAMINATED SOILS

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Abstract

Two fungal species which degrade the organophosphorus pesticides (OPs) folimat, phoxim, methamidophos and glyphosate were isolated and their biodegradation pathways were explored. Cell morphology analysis and ITS rRNA gene sequencing showed that the strains as *Aspergillus oryzae* (Ahlburg) E. Cohn (ZHJ8) and *Cladosporium cladosporioides* (Fresen.) G.A. de Vries (ZHJ10). Both strains needed an external carbon source to degrade OPs, and ZHJ8 could use ethanol. Both strains could utilise nitrogen and phosphorus from OPs, both preferred to grow in acidic conditions, and both could degrade OPs efficiently at room temperature. The isolated strains could tolerate and degrade 2000 mg/l of methamidophos. Optimal conditions for methamidophos degradation by ZHJ8 were pH 5.0 - 5.5, 30°C, and 1.5% glucose, while pH 4.0 - 4.5, 25°C, and 1% glucose were optimal for ZHJ10. Based on the structure of the pesticides and the results of GC-MS, a possible biodegradation pathway was proposed.

Introduction

Organophosphorus pesticides (OPs) have been widely used for pest control. Thousands of organophosphorus compounds have been synthesized and screened of which over 100 marketed as pesticides on the basis of their low cost, easy synthesis, improved biodegradability and limited accumulation in living organisms. However, some OPs are highly toxic, and their residues in soil affect microbial activity and pose a threat to insects and mammals, including humans. OP contamination of ground and surface water is widespread and a significant water quality concern (Diez 2010). OPs are among the most commonly found organic impurities in the environment and food products (Mohammad *et al.* 2015).

In order to avoid the adverse impacts of OPs on human health, effective means to remove OP residues have been extensively studied. A number of methods, such as solar photocatalytic oxidation (Ahmad *et al.* 2015), catalytic oxidation (Mark *et al.* 2014), sonication (Pankaj *et al.* 2015), irradiation degradation (Yang *et al.* 2015) and biodegradation, have been developed to remove organophosphorus contamination from the environment. Among these techniques, biodegradation is an efficient, cost-effective environmental friendly process that is currently being investigated for a wide variety of chemical pollutants. Maya *et al.* (2011) studied the degradation of chlorpyrifos by bacterial communities comprising seven different isolates of pseudomonas, agrobacterium and bacillus strains (Shamsa *et al.* 2014).

Bacterial and fungal degradation of OPs only attracted interest in last two decades. The mechanism of fungal biodegradation of OPs is less established. Despite this, the characteristics of fungi, including high specific bioactivity and/or growth morphology, suggests filamentous fungi are better potential OP degraders than bacteria (Mollea *et al.* 2005). Zhao *et al.* (2010) isolated a

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Penicillium oxalicum strain that could degrade high concentrations of methamidophos, while Ramadev *et al.* (2012) reported high biodegradation of malathion by a filamentous fungus of *Aspergillus*.

Insecticides can affect the population and activity of beneficial microbial communities in soils, and continuous application of pesticides can influence microbial populations (Rachna *et al.* 2015). Pesticide treatment resulted in short-lived transient toxic effects on soil microbial biomass carbon, which increased over time in biopesticide-treated soil and stimulated agricultural production (Sonia and Sakshame 2013).

Recently, some workers have reported that filamentous fungi act synergistically with bacteria to enhance the degradation of pollutants by transforming them into an easier form for bacteria to degrade, thereby helping bacteria to metabolise otherwise inaccessible pollutants (Ellegaard-Jensen *et al.* 2014). It is therefore important to take advantage of the biodegrading capabilities of fungi, which have the ability to degrade a wide range of environmental contaminants (Oliveira *et al.* 2015).

Strains that tolerate and degrade highly toxic pesticides have enormous environmental benefit. In the present work, two fungi (*Aspergillus oryzae* (Ahlburg) E. Cohn) ZHJ8 and *Cladosporium cladosporioides* (Fresen.) G.A. de Vries) ZHJ10 were isolated from pesticide-contaminated soil by selective pressure of methamidophos, a water-soluble and highly toxic pesticide. The level of influence and optimal conditions for fungal degradation of pesticides were explored. The biodegradation pathway of methamidophos degradation was also investigated.

Materials and Methods

Soil samples were collected from nine pesticide factories in Baoding and Cangzhou Cities, Hebei province, China. Inorganic salt medium-1 (ISM-1) and inorganic salt medium-2 (ISM-2) were the basic media used in this study and consisted of the following: ISM-1 = 0.5 g/l K_2HPO_4 , 0.5 g/l K_2HPO_4 , 0.2 g/l $MgSO_4 \cdot 7H_2O$, 0.2 g/l NaCl, 0.1 g/l $CaCl_2$, 4.0×10^{-3} g/l $MnSO_4 \cdot H_2O$, and 2.0×10^{-3} g/l $FeCl_3 \cdot 6H_2O$; ISM-2 = 0.5 g/l KCl 0.50, 0.5 g/l $MgSO_4 \cdot 7H_2O$, 1.0×10^{-2} g/l $FeSO_4$, 1.0×10^{-2} g/l $CaCl_2 \cdot H_2O$ 1, and 1.0×10^{-2} g/l $MnCl_2 \cdot 2H_2O$ (Li and Zhong, 1999). Gause's No. 1 Medium without carbon source (GMCS) and Czapek's medium without carbon source (CMCS) were used to screen and isolate the strains and consisting of the following: GMCS = 20 g/l agar, 1 g/l KNO_3 , 0.5 g/l K_2HPO_4 , 0.5 g/l $MgSO_4 \cdot 7H_2O$, 0.5 g/l NaCl, and 1.0×10^{-2} g/l $FeSO_4$; CMCS = 20 g/l agar 20, 2 g/l $NaNO_3$, 1 g/l K_2HPO_4 , 0.5 g/l KCl, 0.5 g/l $MgSO_4 \cdot 7H_2O$, and 1.0×10^{-2} g/l $FeSO_4$.

Enrichment culture medium (ECM) and nutrient solid agar medium (NA) were used to enrich and isolate OPs-degrading strains. ECM was prepared by adding an appropriate volume of methamidophos to ISM-2. NA was obtained by adding 2% methamidophos and 2% agar to ISM-2.

Potato dextrose agar (PDA) was used to incubate the OPs-strains. The ingredients (per 100 ml) were 20 g white potato, 4 g agar, 2 g glucose, and 1.0×10^{-3} g methamidophos (Zhao *et al.* 2010).

Mixed soil samples (2.5 g) were transferred to a 250 ml Erlenmeyer flask containing 100 ml of ECM (with 100 mg/l methamidophos) and incubated at 30°C on a rotary shaker at 180 rpm. After 7 days of incubation, 10 ml aliquot was transferred to another 250 ml Erlenmeyer flask containing 100 ml fresh ECM (with 200 mg/l of methamidophos) and incubated in the same conditions. This incubation process was repeated 15 times in which fresh ECM contained 100 mg/l more methamidophos than the previous incubation, so that the final concentration of methamidophos was 1500 mg/l. After incubation, 2 ml aliquot was transferred to two NA plates, two CMCS plates and two GMCS plates, and plates were divided into two groups (one CMCS,

NA and GMCS plate in each group). One group was incubated at 37°C and the other at 30°C. After 3 days of incubation, pure colonies appeared on the plates, were isolated as potential OP-degrading strains.

Morphological properties were investigated using light microscopy (XSP-BM16C No. 6, Shanghai Optical Instruments, Shanghai, China). Identification was performed using ITS rRNA gene sequencing. Total DNA was extracted from fungal cells by milling with liquid nitrogen (Li *et al.* 2008). Fragments containing the ITS region were amplified using genomic DNA as template and a TaKaRa Fungi Identification PCR Kit (TaKaRa Biotechnology Co., Ltd. Dalian China). ZHJ8 and ZHJ10 ITS rRNA genes were amplified by PCR with forward and reverse primers (TaKaRa Biotechnology Co., Ltd. Dalian China). Reactions were performed in 50 µl mixtures containing 50 ng genomic DNA, 0.5 µl ITS forward primer, 25 µl PCR premix, 0.5 µl ITS reverse primer, and double distilled water. Amplification was typically performed in a DNA Engine Thermal cycler (PTC-200, BIO-RAD, USA) with a 4 min denaturation step at 94°C, followed by 30 cycles of 94°C for 5 min, primer annealing for 1 min at 52°C, extension for 1 min at 72°C, and a final elongation step for 7 min at 72°C. PCR products were sequenced by TaKaRa Biotechnology (Dalian) and deposited in the GenBank database. Alignment with different ITS rRNA gene sequences from Gen Bank was performed using MEGA version 5.2 software. A phylogenetic tree was built using the neighbour joining method. Each dataset was bootstrapped 1,000 times.

Experiment I: Methamidophos was dissolved in ISM-2 at a final concentration of 1000 mg/l, where methamidophos was the sole carbon, phosphorous and nitrogen source.

Experiment II: Methamidophos and glucose were dissolved in ISM-2 at a final concentration of 1000 mg/l and 1%, respectively, where glucose and methadiphos served as a carbon source, but only methamidophos provided a phosphorous and nitrogen source.

Experiment III: Methamidophos and glucose were dissolved in ISM-1 at a final concentration of 1000 mg/l and 1%, respectively, where glucose and methadiphos served as a carbon source, and only methamidophos provided nitrogen source.

Experiment IV: Methamidophos and sodium nitrate were dissolved in ISM-2 at a final concentration of 1000 mg/l and 1%, respectively, where only methamidophos provided a carbon source.

Other carbon or nitrogen sources including methanol, ethanol, glucose, fructose, lactose, sucrose, starch, dextrin, sodium nitrate, sodium nitrite and ammonium sulfate were also added to 45 ml of ISN-1 at a final concentration of 1%. Liquid medium was inoculated with 5 ml spore suspension at 30°C. Medium containing 5 ml boiled dead spore suspension was used as a control. All experimental procedures were carried out four times.

Methamidophos (50 mg), 5 g of glucose and 5 ml spore suspension (6×10^5 spore/ml) was added to 45 ml of ISM-1, and the effect of pH (3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0, adjusting with 0.01 M NaOH or HCl) on the biodegradation of methamidophos was investigated. The effect of temperature (15, 20, 25, 30 and 35°C) was also investigated with the initial concentration of methamidophos of 250, 500, 1000, 1250, 1500 and 2000 ppm.

By comparing the concentration of methamidophos with control experiments, the rate of methamidophos degradation was calculated. All experimental procedures were conducted for three times.

One ml samples was removed daily from degradation and control experiments, dehydrated with anhydrous sodium sulfate (4 g), and extracted five times by a mixture of 1 ml acetone and 1 ml ethyl acetate. Organic extracts were transferred to a 10 ml volumetric flask, made up to 10.00

ml and analyzed by gas chromatography. The degradation ratio was calculated according to the following equation:

$$\text{Degradation ratio} = (C_c - C_d)/C_c$$

where C_c is the concentration of methamidophos in the control experiment, and C_d is the concentration of methamidophos in the biodegradation experiment. After incubation, solutions were filtered using a 0.2 μm filter made of regenerated cellulose. Fungi were collected and dried to a constant weight in a drying cabinet at 80°C. Finally, the fungal biomass was obtained.

One ml aliquot of culture was mixed with 1 ml acetone, 1 ml acetic ether and 0.5 g NaCl in a 10 ml centrifuge tube and shaken for 1 min. The organic phase was transferred and filtered, and analysed by GC-MS (7890A, Agilent Corporation) (Zhao *et al.* 2009).

Six OPs (folimat, dursban, phoxim, mevinphos, dichlorfos and dipterex) were tested for degradation by ZHJ8 and ZHJ10. Five ml spore suspension and various quantities of pesticides were added to ISM-1 or ISM-2 medium at an initial concentration of 500, 1000 and 1500 mg/l. The degradation ratio was determined each day using GC. Control experiment was again included. Pesticides were also tested as sole carbon, nitrogen and phosphorus sources. All experiments were performed in triplicate, and statistical analysis was performed by ANOVA and post hoc Tukey tests in Origin 8.0.

Results and Discussion

A total of 15 fungal strains that can tolerate high concentrations of methamidophos (1500 mg/l) were isolated from long-term OP-polluted soil samples using the enrichment culture technique, and two strains (ZHJ8 and ZHJ10) that could degrade ~80% 1000 mg/l methamidophos in 8 days in ISM-1 medium during the initial screening were selected for further study.

ZHJ8 formed loose, filamentous and velvety colonies on PDA plates that were initially white but became yellow in the centre within two days, then dark green and circular with a diameter of 60 - 65 mm after 5 - 6 days. The ZHJ8 colonies appeared pale in the reverse side. Microscopic examination revealed conidia heads of 145 - 280 μm in diameter, and conidia (4 - 6 μm in diameter) were smooth and gathered into long chains. The tips of conidiophores were subsphaeroidal. Conidia were pear-shaped or oval when young, and spherical or nearly spherical at maturity. The isolate ZHJ10 formed loose, filamentous, flocculent colonies that were olive green and had a diameter of 0.5 - 1 cm after 5 days. Aerial hyphae were dark in colour and mycelia were colourless. Colonies were generally dark green in the reverse side. Conidiophores formed branched chains. Conidia were round or oval, and unicellular. Neither of the plates was extravagated.

ITS1 and ITS2 rRNA genes from strains ZHJ8 and ZHJ10 were deposited in the GenBank data library under accession numbers. ZHJ8 includes EF409787, EF669954, JF450778, FJ491662, JN882309, KC341712, FJ491473, JX406504, and EF652474. ZHJ10 includes AF393684, HM147998, HM148001, JN906978, AF393681, EF679334, JN906979, and JX845290. Based on morphology and ITS rRNA gene sequence analysis, we deduced that ZHJ8 belonged to *Aspergillus oryzae* and ZHJ10 belonged to *Cladosporium cladosporioides*. A phylogenetic tree was constructed based on the ZHJ8 and ZHJ10 ITS rRNA gene sequences.

Aspergillus oryzae strain ZHJ8 could degrade methamidophos when using ethanol, glucose, fructose, sucrose, lactose, starch and dextrin as a carbon and energy source. *Cladosporium cladosporioides* ZHJ10 could also use all these carbon sources except ethanol (Fig. 1).

Aspergillus oryzae ZHJ8 and *Cladosporium cladosporioides* ZHJ10 were incubated as described in experiments I - IV. The results showed that both strains could use methamidophos as

a sole nitrogen and/or phosphorus source, but not as a sole carbon source. ZHJ8 and ZHJ10 also could utilize other nitrogen and phosphorus sources for growth, such as sodium nitrate, sodium nitrite, ammonium sulfate and phosphate.

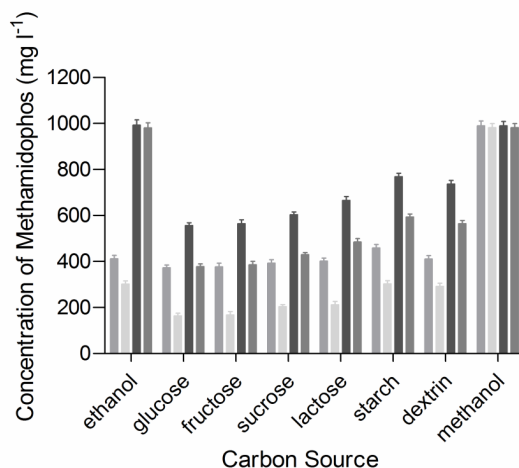


Fig. 1. Carbon source testing of the biodegradation of methamidophos by ZHJ8 and ZHJ10. Strains of treatments were as follows; ZHJ8-6d (■), ZHJ8- 8d (■), ZHJ10-6d (■), ZHJ10-8d (■).

Both ZHJ8 and ZHJ10 could effectively degrade methamidophos with an initial concentration of 1000 mg/l and pH in between 3.5 and 7.0. The optimum biodegradation pH for ZHJ8 was 5.0-5.5. Within 8 days, ZHJ8 had almost completely degraded methamidophos (Fig. 2a). The strain ZHJ10 tolerated more acidic conditions as low as pH 4.0 - 4.5, but its degradation efficiency was lower than that of ZHJ8; after 12 days, the degradation ratio reached 90% (Fig. 2b). The optimum degradation temperature was 25 - 30°C for ZHJ8. The strain ZHJ8 showed a stronger degradation capacity than ZHJ10, and could degrade > 99% methamidophos in 12 days. The most favourable degrading conditions for strain ZHJ8 was in inorganic salt medium at pH 5.0 and 25°C, with 1% glucose. The degradation efficiency significantly decreased when the temperature was less than 20°C (Fig. 2c). The optimal degradation temperature for ZHJ10 was 25°C, and the efficiency decreased more than 35°C (Fig. 2d). The strain ZHJ8 was less tolerant to lower temperatures than ZHJ10, but more tolerant to higher temperatures. Glucose concentration in between 0.5 - 2.0% had no significant effect on the degradation (Fig. 2e, f). The effect of methamidophos concentration was investigated between 250 and 2000 mg/l and the degradation ability decreased at higher concentrations. The strain ZHJ8 (after 8 days) and ZHJ10 (after 12 days) degraded 50% of the OP at an initial methamidophos concentration of 2000 mg/L (Fig. 2g, h). Although the degradation efficiency of ZHJ8 was higher, ZHJ10 could carry out effective degradation at lower pH and lower temperatures.

The relationship between the degradation efficiency and biomass production of ZHJ8 and ZHJ10 was also investigated. Initial methamidophos concentration of 1000 mg/l, and under optimal incubation conditions, the ZHJ8 biomass reached 0.014 g (2.0×10^3 g biomass per mol of methamidophos), and the ZHJ10 biomass reached 0.009 g (1.3×10^3 g biomass per molmethamidophos) after 12 days. Therefore, ZHJ8 grew faster than ZHJ10 when using methamidophos as a sole nitrogen and phosphorus source (Fig. 3).

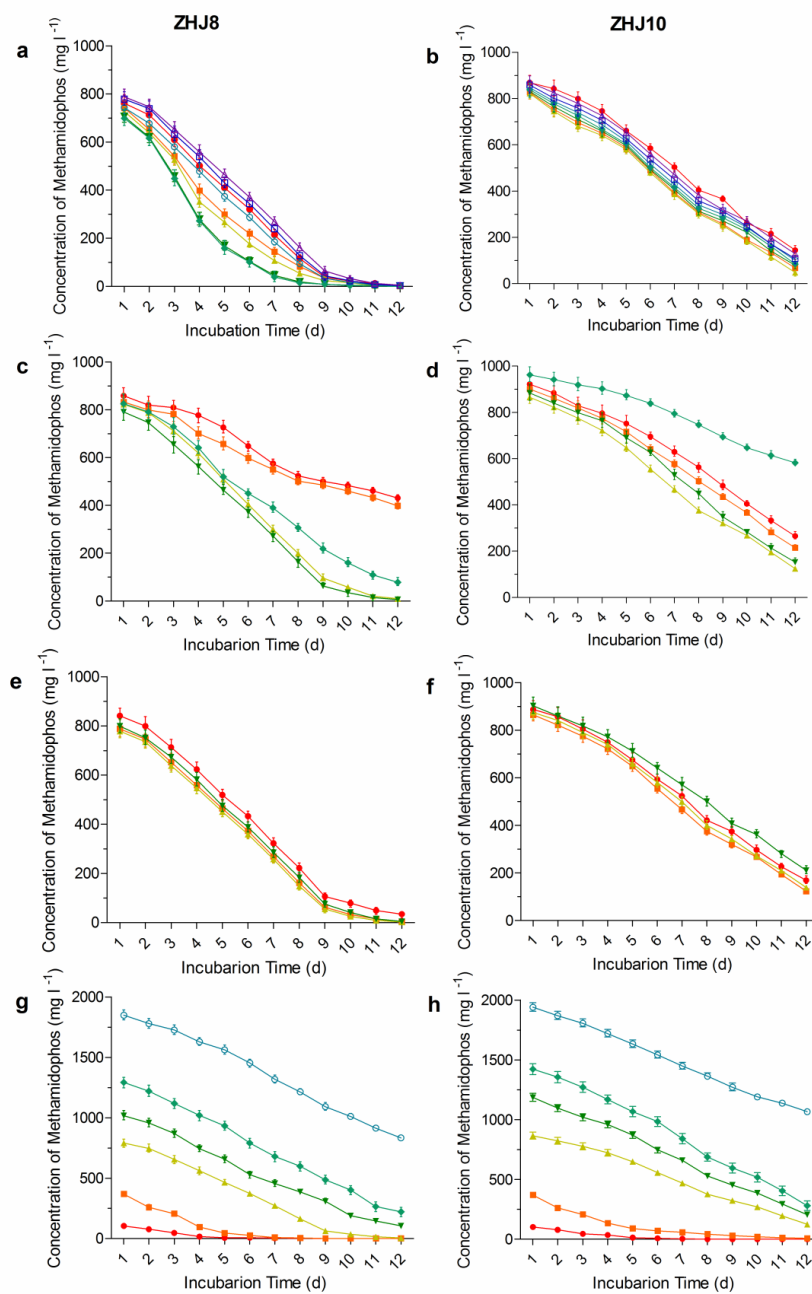


Fig. 2. Biodegradation of methamidophos by ZHJ8 and ZHJ10. a and b, pH (●) = 3.5, (■) = 4.0, (▲) = 4.5, (▼) = 5.0, (●) = 5.5, (○) = 6.0, (□) = 6.6, (□) = 7.0; c and d, temperature (●) = 15°C, (■) = 20°C, (▲) = 25°C, (▼) = 30°C, (●) = 35°C; e and f, glucose concentration (●) = 0.5%, (■) = 1.0%, (▲) = 1.5%, (▼) = 2.0%; g and h, initial methamidophos concentration (mg/l) (●) = 250, (■) = 500, (▲) = 1,000, (▼) = 1,250, (●) = 1,500, (○) = 2,000.

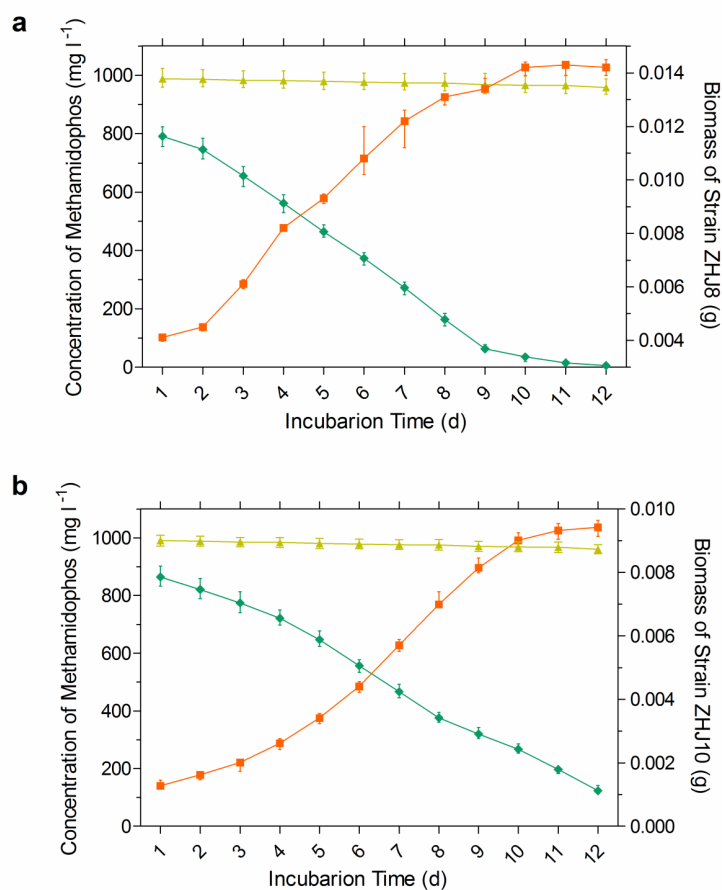


Fig. 3. Relationship between the concentration of methamidophos and the biomass. (a) ZHJ8 (□) = ZHJ8, (▲) = Control, (■) = Biomass of ZHJ8. (b) ZHJ10 (□) = ZHJ10, (▲) = Control, (■) = Biomass of ZHJ10.

By examining the degradation of different pesticides by ZHJ8 and ZHJ10 (Table 1), present authors inferred that the two fungal strains may degrade OPs by similar biodegradation pathways.

In further experiments, ZHJ8 and ZHJ10 were unable to degrade the pesticides chlorpyrifos, dichlorvos, phosdrin, and trichlorphon. However, in the absence of additional nitrogen or phosphate (ISM I without nitrogen source, and ISM II without nitrogen and phosphorous source), ZHJ8 and ZHJ10 could degrade the pesticides methamidophos, folimat, phoxim and glyphosate.

Chemical structures analysis of the tested pesticides (Fig. 4), we observed that those did not degrade (mevinphos, dichlorvos and trichlorphon) contain no nitrogen. Chlorpyrifos, which was also not degraded, contain nitrogen, but only in the aromatic ring. By contrast, methamidophos, folimat, phoxim and glyphosate all contain nitrogen that is not in the form of a heterocycle. This indicates that ZHJ8 and ZHJ10 could use nitrogen from these pesticides in their degradation function, but cannot use nitrogen contained in a heterocycle. Authors therefore deduced that ZHJ8 and ZHJ10 are able to cleave C-N and P-N bonds that are not part of aromatic systems.

Table 1. Biodegradation rate of organophosphorus pesticides by ZHJ8 and ZHJ10 after three days.

Strain	ZHJ8			ZHJ10			
	Initial concentration of OP (mg/l)	500 (%)	1,000 (%)	1,500 (%)	500 (%)	1,000 (%)	1,500 (%)
Methamidophos		70.2 ± 2.2	46.1 ± 1.0	31.1 ± 0.6	62.1 ± 2.3	28.0 ± 0.9	22.2 ± 0.6
Glyphosate		78.7 ± 1.9	62.4 ± 1.1	39.1 ± 0.7	54.2 ± 2.2	31.9 ± 1.1	17.1 ± 0.5
Folimat		34.1 ± 3.9	22.2 ± 1.5	11.6 ± 3.4	24.3 ± 2.9	10.3 ± 1.1	6.9 ± 0.5
Phoxim		17.8 ± 2.2	8.2 ± 1.1	2.5 ± 1.1	9.8 ± 2.2	5.0 ± 0.8	2.0 ± 0.4
Chlorpyrifos	-	-	-	-	-	-	-
Dichlorvos	-	-	-	-	-	-	-
Phosdrin	-	-	-	-	-	-	-
Trichlorphon	-	-	-	-	-	-	-

- indicates no degradation.

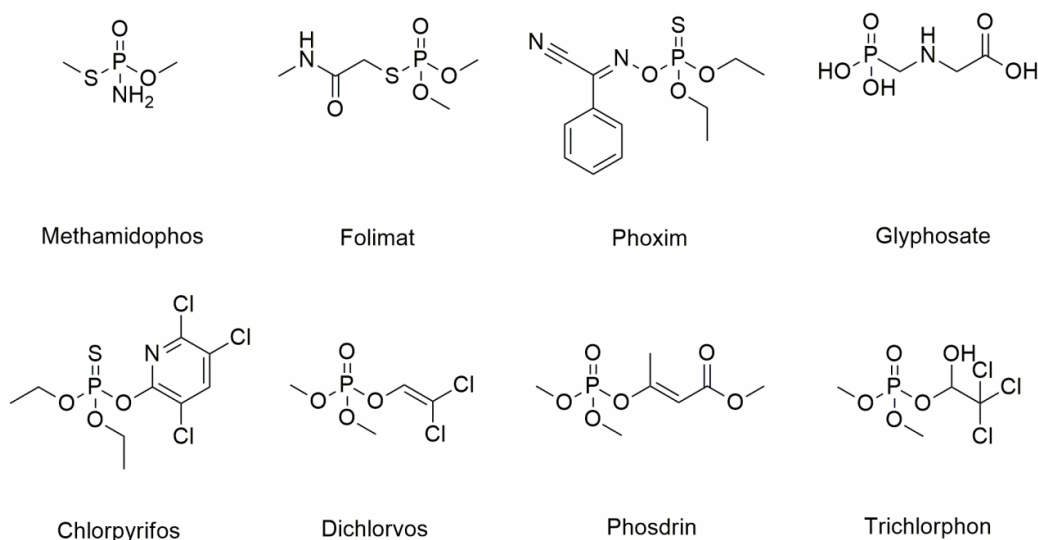


Fig. 4. Chemical structures of the tested phosphorus pesticides.

Media containing methamidophos was determined by GC-MS before and after incubation. Comparing the gas chromatography (GC) profiles before and after biodegradation, it was apparent that no new organophosphorus peaks were present after biodegradation, suggesting that even if new organophosphorus was produced, the concentration was not significant. Therefore, methamidophos may be completely degraded to inorganic phosphorus after incubation with ZHJ8 or ZHJ10 after eight days or 12 days, respectively.

The results showed that both ZHJ8 and ZHJ10 could utilize methamidophos as a nitrogen and phosphorous source. Methamidophos is a chlorine compound, and other microorganisms that can use it as a carbon source are defined as methylotrophs (Chae *et al.* 1994). However, neither ZHJ8 nor ZHJ10 were able to utilize methamidophos or methanol as a carbon source, suggesting they lack the enzymes that directly break down O-P and O-C bonds. ZHJ8, but not ZHJ10, was able to use ethanol as a carbon source, indicating an ability to use two-carbon sources.

Moreover, phosphorus from methamidophos can be used by ZHJ8 and ZHJ10, indicating that N-P cleavage promotes breakage of the S-P and O-P bonds to generate methanol and methyl mercaptan. However, since ZHJ8 and ZHJ10 are not methyl nutrition microorganisms, these could not be utilized, and remain in solution, consistent with the results obtained for *Saccharomyces rouxii* (Barnett *et al.* 1983). Based on the above analysis, we propose a methamidophos degradation pathway and mechanism that may be operable in ZHJ8 and HJ10 (Fig. 5).

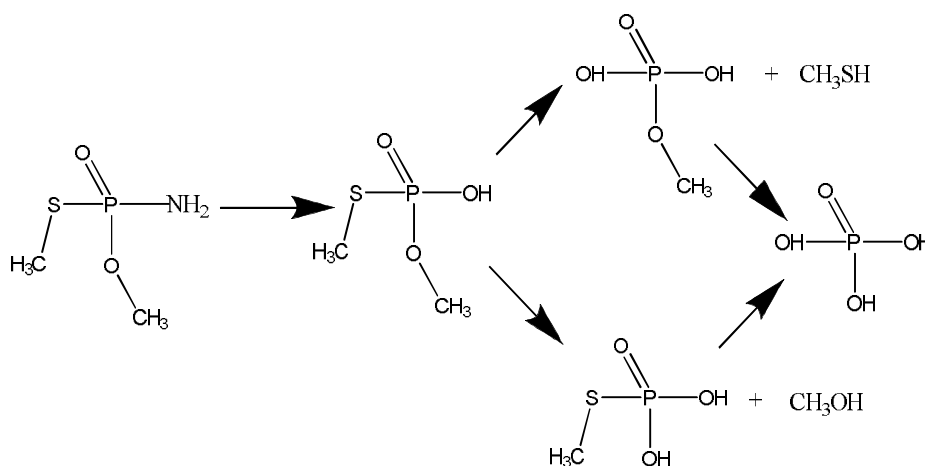


Fig. 5. Proposed methamidophos biodegradation pathway in ZHJ8 and ZHJ10.

Methamidophos can be biodegraded by a variety of bacteria. For example, *Hyphomicrobium* MAP-1 can use carbon, nitrogen and phosphorus from methamidophos degradation, and the inferred degradation mechanism is similar to that suggested in the present study. *Hyphomicrobium* MAP-1 can completely degrade 3,000 mg/l methamidophos in 84 hrs under optimal conditions (pH 7.0, 30°C), but this species is unable to utilize glucose, mannose, xylose, lactose, sucrose or starch as a sole carbon source (Wang *et al.* 2010). Although less tolerant to high concentrations of methamidophos than MAP-1, ZHJ8 and ZHJ10 are able to catalyse its degradation under acidic conditions.

Previous studies on the bacterial biodegradation of methamidophos have been reported. Mageong and Joseph (1994) found a phosphate esterase in *Escherichia coli* that slowly cleaves the S-P bond of methamidophos. Zheng *et al.* (2006) showed that *Acinetobacter* HS-A32 can use methamidophos as a sole carbon and nitrogen source, and thereby deduced the ability to cleave N-P, O-P and S-P bonds (Potin *et al.* 2004). Wu *et al.* (2005) reported that *Pseudomonas* S-2 can use methamidophos as a sole nitrogen source, and can therefore break P-O and N-P bonds.

These prokaryotes have no membrane-enclosed nucleus, and a single closed circular double stranded DNA, allowing them to adapt rapidly to biochemical and environmental changes. It is therefore unsurprising to find bacterial strains possessing biodegradation activity. The genomes of

fungi are more complicated than those of bacteria, and include active mechanisms to control spontaneous mutations during replication. Therefore, fungal organisms have a lower mutation rate and more stably maintain characteristics between generations. Although biodegradation activity is likely to emerge more rarely in fungi, once gained, it will be stably inherited by subsequent generations. Furthermore, filamentous fungi can quickly grow in soil at low pH, low nutrient levels and low humidity (Mollea *et al.* 2005), and fungal spores have strong adaptability. Thus, methamidophos-degrading fungi may be suitable for use in large contaminated areas, where environmental conditions may vary. Due to the strong ability of fungi to adapt to changing environments and stable genetic inheritance, ZHJ8 and ZHJ10 have great potential for bioremediation of OP-polluted environments.

Acknowledgements

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References

- Ahmad JJ, Mehdi SS, Yang JK, Mohammad NJ and Mehrdad F 2015. Photocatalytic degradation of diazinon with illuminated ZnO-TiO₂ composite. *J. Taiwan Inst. Chem. Eng.* **50**: 100-107.
- Barnett JA, Payne RW and Yarrow D 1983. *Yeasts: Characteristics and identification*. Cambridge University Press, Cambridge, UK.
- Chae MY, Postula JF and Raushel FM 1994. Stereospecific enzymatic hydrolysis of phosphorus-sulfur bonds in chiral organophosphate triesters. *Agri. Sci. Chin.* **4**(12): 1473-1478.
- Diez MC 2010. Biological aspects involved in the degradation of organic pollutants. *J. Soil. Sci. Plant. Nutr.* **10**(3): 244-267.
- Ellegaard-Jensen L, Knudsen BE, Johansen A, Albers CN, Aamand J and Rosendahl S 2014. Fungal - bacterial consortia increase diuron degradation in water-unsaturated systems. *Sci. Tot. Env.* **466-467C** (1): 699-705.
- Maya K, Singh RS, Upadhyay SN and Dubey SK 2011. Kinetic analysis reveals bacterial efficacy for biodegradation of chlorpyrifos and its hydrolyzing metabolite TCP. *Process Biochem.* **46**(11): 2130-2136.
- Li X, Zhang H, Wu M and Zhang Y 2008. Effect of methamidophos on soil fungi community in microcosms by plate count, DGGE and clone library analysis. *J. Env. Sci.* **20**(5): 619-625.
- Li S and Zhong Y 1999. Isolation of methamidophos-degrading bacterium and studies on its properties. *J. Anhui Uni. (Nat. Sci.)*. **18**(12): 564-567.
- Mageong YC and Joseph FP 1994. Stereospecific enzymatic hydrolysis of phosphorus-Sulfur bond in chiral organophosphate triesters. *J. Bio. Med. Chem. Lett.* **4**(2): 1473-1478.
- Mark M, Bailey JM, Heddleston JD, Jessica L, Staymates AR and Hight W 2014. Functionalized, carbon nanotube material for the catalytic degradation of organophosphate nerve agents. *Nano Res.* **7**(3): 390-398.
- Mohammad HR, Mina A and Mohammad RE 2015. Degradation of organophosphorus pesticide diazinon using activated persulfate: Optimization of operational parameters and comparative study by Taguchi's method. *J Taiwan Inst. Chem. Eng.* **57**: 77-90.
- Mollea C, Bosco F and Ruggeri B 2005. Fungal biodegradation of naphthalene: Microcosms studies. *Chemosphere* **60**(5): 636-643.
- Oliveira BR, Penetra A, Cardoso VV, Benolie MJ, Barreto-Crespo MT, Samson RA and Pereira VJ 2015. Biodegradation of pesticides using fungi species found in the aquatic environment. *Env. Sci. Poll. Res.* **22**(15):1-11.

- Pankaj N, Patil, Parag R and Gogate 2015. Degradation of dichlorvos using hybrid advanced oxidation processes based on ultrasound. *J. Water Pro. Engg.* **8**: e58-e65.
- Potin O, Rafin C and Veignie E 2004. Bioremediation of an aged polycyclic aromatic hydrocarbons (PAHs)-contaminated soil by filamentous fungi isolated from the soil. *Int. Biodeter. Biodegr.* **54**(1): 45-52.
- Rachna J, Veena G and Jyoti S 2015. Effect of an organophosphate pesticide, monocrotophos, on phosphate-solubilizing efficiency of soil fungal isolates. *Appl. Biochem. Biotechnol.* **175**(2): 813-824.
- Ramadevi C, Nath MM and Guru Prasad MG 2012. Mycodegradation of malathion by a soil fungal isolate, *Aspergillus niger*. *Int. J. Basic Appl. Chem. Sci.* **2**:108-115.
- Shamsa A, Sikander S and Michael K 2014. Bacterial community analysis in chlorpyrifos enrichment cultures via DGGE and use of bacterial consortium for CP biodegradation. *World J. Microbiol. Biotechnol.* **30**(10): 2755-2766.
- Sonia S and Saksham G 2013. Impact of pesticides and biopesticides on soil microbial biomass carbon. *Univ. J. Env. Res. Tech.* **3**: 326-330.
- Wang L, Wen Y, Guo X and Wang G 2010. Degradation of methamidophos by hyphomicrobium species MAP-1 and the biochemical degradation pathway. *Biodegradation* **21**(4): 513-523.
- Wu X, Wu Z, Wang Y, Dong C, Zhang L, Shi Y and Yang Y 2005. Study on degradation of methamidophos by *Pseudomonas* sp. S-2. *Biotechnology* **15**(1): 77-79.
- Yang J, Gao YP, Liu WH, Ge W and Zhao RB 2015. The Degradation and mineralisation of acephate by ionisation irradiation. *Envir. Pro. Sustain. Ener.* **34**(2): 2324-2332.
- Zhao RB, Bao HY and Liu YX 2010. Isolation and characterization of *penicillium oxalicum* ZHJ6 for biodegradation of methamidophos. *Agricul. Sci. Chin.* **9**(5): 695-703.
- Zhao RB, Bao HY and Liu YX 2009. γ -irradiation degradation of methamidophos. *Chin. J. Chem.* **27**(9): 1749-1754.
- Zheng YL, Liu DL, Gao Q and Wang W 2006. Isolation and characterization of a bacterium HS-A32 capable of degrading methamidophos. *Chin. J. Appl. Env. Biol.* **12**(3): 399-403.

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