Study on the Remediation of Phorate in Soil by Microbial Consortia

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Abstract. In order to bioremediating of phorate contaminated soil by microbial consortia, three degrading bacteria in different combinations were inoculated into the phorate contaminated soil. The residual phorate and its metabolites were determined. The results showed that in 42 days the degradation rates of phorate by any two bacteria (Brevibacterium frigoritolerans + Bacillus aerophilus, Bacillus aerophilus + Pseudomonas fulva and Brevibacterium frigoritolerans + Pseudomonas fulva) were 94.09%, 97.15% and 97.42%, respectively. However the degradation rate of phorate by three bacteria was 98.31%. The microbial consortia consisted of three degrading bacteria had the lowest content of phorate sulfone and phorate sulfoxide in soil. Therefore, the microbial consortia consisted of three degrading bacteria could significantly reduce the residual phorate and its metabolites in soil.

1. Introduction
Organophosphorus pesticides were widely used in agricultural production[1-3]. In the environment, microbial degradation was one of the important ways to remove organophosphorus pesticides. The microbial community composed of different strains has a strong degradation capacity for organophosphorus pesticides[4-8]. In the soil, the biodegradation of phorate results in the formation of phorate sulfoxide and phorate sulfone[6]. However, different microorganisms may produce different degradation products under different environmental conditions [4]. In this paper, three degradation bacteria were inoculated into the soil polluted by phorate in different combinations, and the residues of phorate and its metabolites (phorate sulfone, phorate sulfoxide and oxyphorate) were measured. The degradation ability of the mixed bacteria to phorate in the soil was evaluated, which provided a reference for the in-situ bioremediation of the soil polluted by phorate.

2. Experimental materials and methods
2.1. Experimental materials
Soil sample: the physical and chemical properties of sandy loam collected from a farmland were pH 7.66, sand 81.0%, organic carbon 0.38%, silt 10.0%, conductivity 0.015 s/m, clay 8.0%.

Phorate solution: dissolve phorate in acetone and prepare a solution with a concentration of 50μg / ml.

MSM: dissolve 0.6 g Na₂HPO₄, 4.0 g NaNO₃, 0.2 g KH₂PO₄, 0.01 g CaCl₂, 0.3 g MgSO₄, 0.01 g FeSO₄, 0.5 g yeast powder one by one, and fix the volume of distilled water to 1 L.
Source: three strains of bacteria were screened from the soil polluted by organic phosphorus, which were *Brevibacterium frigoritolerans*, *Bacillus aerophilus* and *Pseudomonas fulva*, which were preserved in our laboratory.

2.2. Biodegradation of phorate in soil

100 g of screened and sterilized soil samples were mixed with 300 mg/kg of phorate in a plastic bowl (18 cm in diameter and 15 cm in height). Inoculate 1mL of bacterial solution (bacterial density was 1.0 × 10^8 CFU /mL), and keep the water content at 60% - 70%. Three parallel groups were made in each group, and a group of blank control (without inoculation of bacterial solution) was set. The contents of phorate and its metabolites in the soil were determined by placing all pots at 35 ℃ and sampling every 7 days.

2.3. Determination of phorate and its metabolites

Dissolve 5 g of soil sample in 50mL of acetone and place overnight. Then, it was transferred to a 1 L separating funnel, added with a certain amount of hexane and dichloromethane for repeated concussion extraction, dehydrated with anhydrous sodium sulfate, and filtered by 0.45μm organic filter membrane. The dehydrated extract was further concentrated to a certain volume at < 35 ℃ by rotary vacuum evaporation.

3. Results and discussions

3.1. *Brevibacterium frigoritolerans* and *Bacillus aerophilus* Degradation of phorate in soil

When 0.5mL *Brevibacterium frigoritolerans* and 0.5mL *Bacillus aerophilus* were inoculated in turn in soil samples (the bacterial density was 1.0×10^8 CFU /mL), the degradation effect of phorate in soil was shown in Fig. 1. It can be seen from Fig. 1 that when *Brevibacterium frigoritolerans* and *Bacillus aerophilus* were inoculated into soil samples, with the increase of degradation time, the residue of phorate in the soil gradually decreases and the degradation rate gradually increases. When the degradation time was 42 days, the residue of phorate decreased from 284.16 mg/kg to 16.78 mg/kg, and the degradation rate reached 94.09%.

![Fig.1 Degradation of phorate in soil by *Brevibacterium frigoritolerans* and *Bacillus aerophilus*](image)

3.2. *Degradation of phorate in soil by Bacillus aerophilus* and *Pseudomonas fulva*

When 0.5ml of *Bacillus aerophilus* and 0.5ml of *Pseudomonas fulva* were inoculated successively in the soil sample (the bacterial density was 1.0×108 CFU /mL), the degradation effect of phorate in the soil was shown in Fig. 2. It can be seen from Fig. 2 that when two degradation bacteria, *Bacillus aerophilus* and *Pseudomonas fulva*, were inoculated in the soil sample, with the increase of degradation time, the residue of phorate in the soil decreases gradually and the degradation rate...
increases gradually. When the degradation time was 42 days, the residue of phorate decreased from 284.16 mg/kg to 8.09 mg/kg, and the degradation rate reached 97.15%.

Fig.2 Degradation of phorate in soil by *Bacillus aerophilus* and *Pseudomonas fulva*

### 3.3. Degradation of phorate in soil by *Brevibacterium frigoritolerans* and *Pseudomonas fulva*

When 0.5mL *Brevibacterium frigoritolerans* and 0.5mL *Pseudomonas fulva* were inoculated successively in the soil sample (the bacterial density was $1.0 \times 10^8$ CFU/mL), the degradation effect of phorate in the soil was shown in Fig. 3. It can be seen from Fig. 3 that when *Brevibacterium frigoritolerans* and *Pseudomonas fulva* were inoculated in the soil sample, with the increase of degradation time, the residue of phorate in the soil gradually decreases and the degradation rate gradually increases. When the degradation time was 42 days, the residue of phorate decreased from 284.16 mg/kg to 7.33 mg/kg, and the degradation rate reached 97.42%.

Fig.3 Degradation of phorate in soil by *Brevibacterium frigoritolerans* and *Pseudomonas fulva*

### 3.4. Degradation of phorate in soil by *Brevibacterium frigoritolerans*, *Bacillus aerophilus* and *Pseudomonas fulva*

When 0.33mL *Brevibacterium frigoritolerans*, 0.33mL *Bacillus aerophilus* and 0.33mL *Pseudomonas fulva* were inoculated into the soil samples successively (the bacterial density was $1.0 \times 10^8$ CFU/mL), the degradation effect of phorate in the soil was shown in Fig. 4, and the metabolites produced by phorate degradation were shown in Table 1. It can be seen from Fig. 4 that when *Brevibacterium frigoritolerans*, *Bacillus aerophilus* and *Pseudomonas fulva* were inoculated in the soil sample, with the increase of degradation time, the residue of phorate in the soil decreases gradually, and the degradation rate increases gradually. When the degradation time was 42 days, the residue of phorate in soil decreased from 284.16 mg/kg to 4.80 mg/kg, and the degradation rate reached 98.31%, which was
better than the degradation effect of any two kinds of degradation bacteria. The degradation rate of phorate in the blank control group was 33.76% at 42 days, which was lower than that in the experimental group inoculated with any degradation bacteria.

![Graph showing phorate degradation over time](image)

**Fig.4 Degradation of phorate in soil by Brevibacterium frigoritolerans, Bacillus aerophilus and Pseudomonas fulva**

It can be seen from Table 1 that phorate sulfone, the metabolite of phorate in soil, was detected at 7 days and reached the maximum value (28.61 mg/kg) at 21 days. Then, with the increase of degradation time, the content of phorate sulfone gradually decreased, reaching 1.31 mg/kg at 42 days. Phorate sulfone, the metabolite, was detected at 14 days and reached the maximum value (11.60) mg/kg at 28 days, and then with the increase of degradation time, the content of phorate sulfoxide decreased gradually, at 42 days, the content was 0.85 mg/kg, while the metabolite oxyphorate was not detected. In the 42 day degradation process, only phorate sulfone and phorate sulfoxide were detected in the metabolites of phorate in the soil, but no oxyphorate was detected, which was the same as the previous research results [4, 10]. Compared with the experimental group inoculated with any two kinds of degradation bacteria, the content of phorate sulfone and phorate sulfoxide was the lowest and the degradation was more thorough.

**Table 1. Metabolites from degradation of phorate in soil by Brevibacterium frigoritolerans, Bacillus aerophilus and Pseudomonas fulva**

| Time/d | Phorate sulfone/(mg/kg) | Phorate sulfoxide/(mg/kg) | Phorate oxychloride/(mg/kg) |
|--------|------------------------|--------------------------|-----------------------------|
| 0      | ND                     | ND                       | ND                          |
| 7      | ND                     | ND                       | ND                          |
| 14     | 3.89                   | ND                       | ND                          |
| 21     | 28.61                  | 5.30                     | ND                          |
| 28     | 23.05                  | 11.60                    | ND                          |
| 35     | 15.92                  | 10.80                    | ND                          |
| 42     | 1.31                   | 0.85                     | ND                          |

Note: ND is the detection limit lower than 0.01 mg/kg.

Different microorganisms produce different metabolites during the degradation of phorate, some of which were more lethal than the body [4]. It was reported that the mixed flora composed of Ralstonia eutropha, Pseudomonas aeruginosa and Enterobacter cloacae can degrade 55% of phorate in the soil,
while the content of toxic metabolite phora, sulfoxide and sulfanilamide increased \cite{10}. At present, the identified metabolites of phorate include dithiocarbophosphate sulfoxide, dithiosulfonate, triethylthiophosphate, formaldehyde, diethyl disulfide, hydrogen sulfate and diethylthiophosphate \cite{11}. In this study, the degradation of phorate was accompanied by the emergence of phorate sulfone and phorate sulfoxide, but no oxyphorate was generated, and the final residues of these metabolites in the soil were very small.

Therefore, three degradation bacteria \textit{Brevibacterium frigoritolerans}, \textit{Bacillus aerophilus} and \textit{Pseudomonas}. The mixed flora composed of fulva has a better degradation effect on phorate in soil, and the content of its metabolites, phorate sulfone and phorate sulfoxide, was lower. It has better bioremediation effect on the soil polluted by organophosphorus pesticide phorate, and was an ideal strain for the bioremediation of the soil polluted by phorate and its metabolites.

\section*{4. Conclusion}

Compared with the blank control group and the experimental group inoculated with any two kinds of degradation bacteria, the experimental group inoculated with three kinds of degradation bacteria had better degradation effect on phorate in soil, and the content of its metabolites, phorate sulfone and phorate sulfoxide, had better bioremediation effect on the soil polluted by organophosphorus pesticide, phorate and its metabolites Ideal strain for bioremediation.

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