Evaluating of chlorpyrifos-degrading by bacterial strains in mineral salt minimum and in the soil

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ABSTRACT

Four bacterial strains degraded chlorpyrifos, isolated from agricultural soil, were used as a source of bacteria to investigate their ability to decompose chlorpyrifos in mineral salt minimum and the soil. Barrientosimonas humi C4.3 was investigated for the decomposition of chlorpyrifos in this strain on different days (10, 20 and 30 days of culture) as supplemented and not supplemented TSB. At the same time, another experiment was carried out to evaluate the chlorpyrifos etherification of B. humi C4.3 and the four strains of Achromobacter xylosoxidans C3.1, B. humi C4.3, Microbacterium sp. C8.9, Staphylococcus pasteuri C9.2 in a soil environment. The experiment was carried out including 3 treatments, each treatment was repeated 3, two soil types (sterile soil and non-sterile soil) and bacteria (single bacteria and four bacterial species). The results showed that, in the same culture period of 30 days incubation, biodegradable chlorpyrifos of B. humi C4.3 in the mineral salt medium was more effective (63.07% biodegradable chlorpyrifos) than when grown in soil (21.4% biodegradable chlorpyrifos). Also, biodegradable chlorpyrifos of B. humi C4.3 that was cultured in sterile soil was higher than in non-sterile soil.

1. Introduction

Application of the pesticide on the crops is now a common practice and is an important factor of integrated pest management (IPM) strategies. It adversely affects the properties of the soil as well as alters the pH of the soil required for microbial activities of beneficial bacteria to act upon (Malinowski, 2000; Rahman & Motoyama, 2000). Some of these pesticides persist in the soil to form pollutants which may occasionally lead to surface and groundwater contamination. One of such pesticides is Chlorpyrifos. It is a broad-spectrum organophosphate insecticide and acaricide, which is widely used to control insect pests on grain, cotton, fruit, nut, and vegetable crops, as well as lawns and ornamental plants in Vietnam. The environmental fate of Chlorpyrifos has been studied extensively, and the reported half-life in the soil varies from 10 to120 days, with 3,5,6-trichloro-2-pyridinol (TCP) as the major degradation product

(Singh, Walker, Morgan, & Wright, 2003). The manufacture and formulation process of Chlorpyrifos also generate waste that contains the compound, and this has to be treated by physicochemical or biological means (Fulekar & Geetha, 2008). If Chlorpyrifos is not degraded or detoxified rapidly enough, the risk of their off-site migration may pose a health risk to humans. Increasing awareness of the potential adverse effects of pesticides has resulted in greater public pressure to assess, monitor and minimize off-site impacts. Enhanced degradation of chlorpyrifos by *Enterobacter strain* B-14 was reported by (Singh, Walker, Alum, Morgan, & Wright, 2004). *Alkaligenes faecalis* DSP3 was isolated, which is capable of degrading chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol (TCPy) (L. Yang, Zhao, Zhang, Yang, & Zhang, 2005). Six chlorpyrifos-degrading bacteria were isolated using chlorpyrifos as the sole carbon source by an enrichment procedure (C. Yang, Liu, Guo, & Qiao, 2006). One research has isolated four strains of bacteria from agricultural soil capable of decomposing chlorpyrifos in minimum mineral solution (Truong, Nguyen, & Duong, 2016). Therefore, to compare and evaluate the ability of four bacteria strains to disintegrate the chlorpyrifos in the minimum mineral solution and in the soil to provide more compelling data to be able to apply the potential bacterial strains practicality in the treatment of soil environmental pollution.

2. Materials and methods

2.1. Materials

Soil samples used in this experiment were taken at depths from 0-20cm from acid sulfate soil of paddy rice at Phung Hiep district, Hau Giang province. Soil characteristics: Clay 57%; Loam 42%; Sand 1%; Organic content 4.87% and pH 4.5. The soil used in the experiment are dried at room temperature, finely ground through a sieve 0.1 mm to create uniformity for processing soil samples and soil moisture at 60% by adding mineral salt minimum solution sterilized.

Chlorpyrifos ethyl (99,5%, Dr.Ehrenstorfer), Tryptose Soybean Agar (TSA), Acetone (99,8%), Toluen (99,9%).

Mineral Minimal Medium: 870mLQ-water, 25mL buffer solution (35 gram $Na_2HPO_4.2H_2O$, 4 gram KH_2PO_4), 100mL mineral salt solution [10 gram $(NH_4)2SO_4$, 2 gram $MgCl_2.6H_2O$, 1 gram $Ca(NO3)_2.4H_2O$], 5mL trace element.

2.2. Methods

2.2.1. Experiment 1: Evaluating of aerobic digestion chlorpyrifos by bacterial strain (PH_C4.3) in mineral salt minimum

The aim of the experiment is survey decomposition chlorpyrifos of bacterial strain (PH_C4.3) in temperatures, pH appropriate to evaluate the chlorpyrifos decomposition rate of bacterial strain (PH_C4.3) in different days (10, 20 and 30 days of culture) as supplemented and not supplemented TSB. The experiment was carried out including 3 treatments, each treatment was repeated 3, the components of each treatment are presented in Table 1.

Table 1

The components of experiment 1 design

Treatments	Ingredient	Target tracking
Control	4mL of mineral minimal medium, 20mg/L of chlorpyrifos	Chlorpyrifos of content and density of bacteria at the time of 0, 10, 20 and 30 days after incubation culture
1	3,9mL of mineral minimal medium, 20mg/L of chlorpyrifos, 100µL of bacteria strain (10^6 cells/mL).	
2	3,85mL of mineral minimal medium, 20mg/L of chlorpyrifos, 100µL of bacteria strain (10^6 cells/mL), 50µL of TSB (0,5%).	

Source: The researcher's data analysis

The experiment was arranged at 30^0C, pH 7 and mix continuously for up to the speed of 130 rpm.

2.2.2. Experiment 2: Evaluating of aerobic digestion chlorpyrifos by bacterial strain (PH_C4.3) and four bacterial strains (PH_C3.1, PH_C4.3, BM_C9.2, and BT_C8.9) in soil

The experiment was carried out to evaluate aerobic digestion chlorpyrifos by bacterial strain (PH_C4.3) and four bacterial strains (PH_C3.1, PH_C4.3, BM_C9.2, and BT_C8.9) in soil. Soil samples used in this experiment were taken at depths from 0-20cm from acid sulfate soil of paddy rice at Phung Hiep district, Hau Giang province (soil samples used to isolate bacterial strain PH_C4.3). Soil characteristics: Clay 57%; Loam 42%; Sand 1%; Organic content 4.87% and pH 4.5. The soil used in the experiment was dried at room temperature, finely ground through a sieve 0.1 mm to create uniformity for processing soil samples and soil moisture at 60% by adding mineral salt minimum solution sterilized.

The experiment was arranged in a vial (12mL) with 5 treatments, two factors were examined two soil types (sterile and non-sterile) and bacteria (one bacterial strain and four bacterial strains), each treatment was repeated 3 times. The components of each treatment are presented in Table 2.

Table 2

The components of experiment 2 design

Treatments	Ingredient	Target tracking
1-Control	3 g for sterile soil, 20mg/L for chlorpyrifos	Content of Chlorpyrif
2	3 g for sterile soil, 20mg/L for chlorpyrifos, 200µL for bacterial strain-PH_C4.3 (10^6cells/mL)	
3-Control	3 g for non-sterile soil, 20mg/L for chlorpyrifos	

Treatments	Ingredient	Target tracking
4	3 g for non-sterile soil, 20mg/L for chlorpyrifos, 200µL for bacterial strain-PH_C4.3 (10^6cells/mL)	os in the soil at the time of 30 days after incubation culture
5	3 g for non-sterile soil, 20mg/L for chlorpyrifos, 50µL for each bacterial strain (PH_C3.1, PH_C4.3, BT_C8.9, BM_C9.2) (10^6cells/mL).	

Source: The researcher's data analysis

The vial was vortex with speed 2000 rpm to the ingredients in a vial and exposed are distributed together. Bacteria in the sample were incubated at room temperature experiments (30^0C) and let stand. After 30 days of culture incubation, chlorpyrifos in the incubated soil samples was extracted, cleaned and analyzed. The content of Chlorpyrifos is determined on HPLC.

2.2.3. Methods of analysis and data processing

The data were processed using Microsoft Excel, Minitab 16 Statistical Software to analyze statistics and ANOVA.

3. Results and discussion

3.1. Evaluating of aerobic digestion chlorpyrifos by bacterial strain (Barrientosiimonas humi C4.3) in mineral salt minimum

The average density of the bacterial strain (*Barrientosimonas humi* C4.3) was presented in Figure 1.

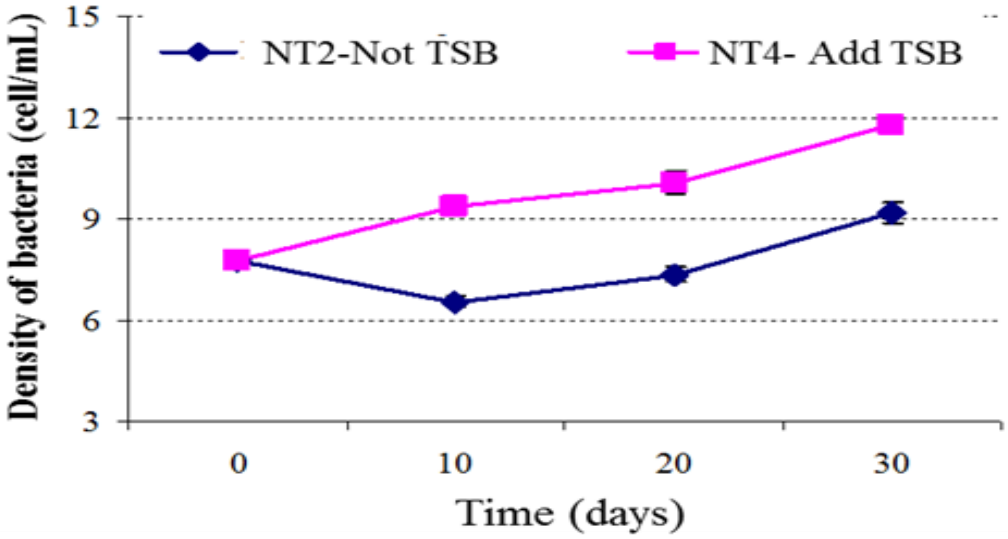

Figure 1. Density of Barrientosimonas humi C4.3

Besides TSB treatments, bacterial cell density higher than the density of the bacterial cells were always cultured in supplemented TSB environment.

Biodegradable chlorpyrifos, for no additional treatments TSB 30 days to the time remaining content of chlorpyrifos (6.8ppm), decreased by 12.1ppm (63.7%) and the difference was statistically significant compared to control. For additional treatments TSB, the time of 30 days remaining content of chlorpyrifos the difference was not statistically significant compared to control (Figure 2).

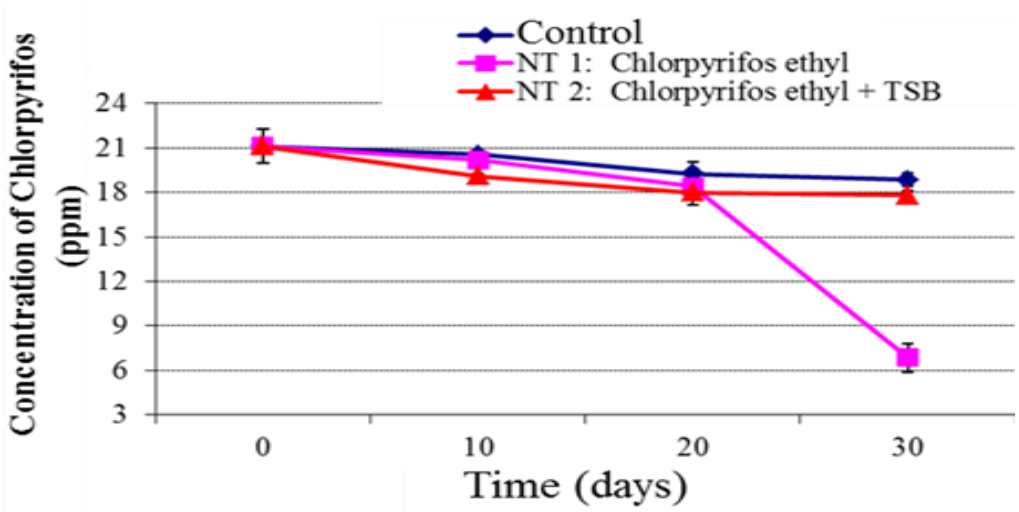

Figure 2. Biodegradable chlorpyrifos of Barrientosimonas humi C4.3

3.2. Evaluating of aerobic digestion chlorpyrifos by bacterial strains (Barrientosiimonas humi C4.3) and four bacterial strains in soil

After 30 days of culture incubated, *Barrientosimonas humi* C4.3 degraded chlorpyrifos in the two types of soil. On the other hand, when four bacterial strains were added to non-sterile soil, the level of biodegradable increased (28.4%) in comparison to single bacteria *Barrientosimonas humi* C4.3 (19.3%) (Figure 3).

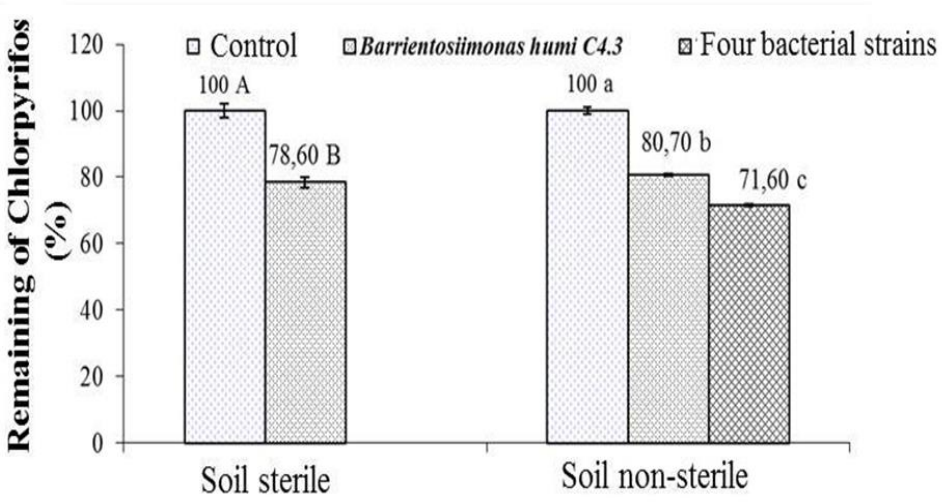

Figure 3. Biodegradable of chlorpyrifos of *Barrientosimonas humi* C4.3 and bacterial groups

Notes-Four bacterial strains include: Achromobacter xylosoxidans C3.1, Barrientosiimonas humi C4.3, Microbacterium sp. C8.9, Staphylococcus pasteuri C9.2

Thus, in the same culture period of 30 days incubation, biodegradable chlorpyrifos of *Barrientosimonas humi* C4.3 in the mineral salt medium was more effective (63.07% biodegradable chlorpyrifos) than when grown in soil (21.4% biodegradable chlorpyrifos). Also, biodegradable chlorpyrifos of *Barrientosimonas humi* C4.3 when cultured in sterile soil was higher than in non-sterile soil.

4. Conclusions

Biodegradable chlorpyrifos of *B. humi* C4.3 in the mineral salt medium was more effective than when grown in soil. Besides that, biodegradable chlorpyrifos of *B. humi* C4.3 when cultured in sterile soil was higher than in non-sterile soil.

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