Degradation of chlorpyrifos and BPMC by the bacteria isolated from contaminated shallot farm soil

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Abstract. Accumulation of insecticide residues is harmful to the environment and human living. The research was conducted to explore the chlorpyrifos and BPMC degrading bacteria from contaminated shallot farm soils and to formulate bacterial consortium to be applied as the insecticides bioremediation agent. Among nineteen bacterial isolates, K10 and K14 bacterial isolates could degrade up to 38.3% and 43.3% chlorpyrifos contained in its growth medium in 5 days, respectively. Two bacterial isolates namely B21 dan B17 could degrade BPMC up to 75.9% dan 77% in 5 days of incubation. Bacterial consortium of K10+K14, K10+B21, and B17+B21 isolates could enhance in-vitro degradation of chlorpyrifos up to 89.6%, 88.9%, and 88.1% respectively, while its BPMC degradation enhanced up to 75.9%, 70.3%, and 69.5% respectively. The highest in-vitro degradation was showed by K10+K14 bacterial consortium. It could degrade up to 79.9% for chlorpyrifos, and 71.9% for BPMC. Base on the 16S rDNA sequence analysis, the isolates have similarity 97.7% to A. baumannii, 96.3% to B. toyonensis, 94.4% to uncultured enterobacter sp. clone 150, and 78.08% to uncultured bacterium cloneck09g01c1 for K10, K14, B17, and B21 bacterial isolates, respectively.

Keywords: Insecticides biodegradation, A. baumannii, B. toyonensis, P. agglomerans, and S. marcescens

1. Introduction

The use of pesticides to manage pest and diseases in agronomic practices is still the main choice of the most Indonesian farmers. The farmers in the north coast of Java practice rotation system of shallot with rice cultivation. Diverse pesticides have been applied intensively in the crop cultivation. Consequently, many different pesticides residues also increase because of the application to control many different types of pests and diseases. Chlorpyrifos is commonly used to control armyworm pests (Spodoptera exigua) in shallot cultivation, while BPMC is often used to control brown plant hopper and stem borer in rice plants. Chlorpyrifos is an organophosphate pesticide, while BPMC is a carbamate pesticide.

Intensive and indiscriminate use of the agrochemicals in long term cause serious problem for environment [1], human [2], and animals [3]. Therefore, the study of these agrochemicals degradation is very important. Pesticide degradation studies are intended to convert pesticide residues in the environment into inactive, less toxic, or harmless compounds [4]. Microbial activity plays an important role in the degradation and reduction of pesticide residues in the environment. This research
was conducted to explore chlorpyriphos and BPMC degrading bacteria from a contaminated shallot farm soil and to formulate the bacterial consortium to be applied as the insecticides bioremediation agent.

2. Materials and methods

2.1. Isolation and selection of insecticides degrading bacteria
Five soil samples were taken from shallot farms in Patrol, an area of Indramayu district, West Java which were intensively applied insecticides in the farms. Each soil sample was diluted ten times in steril aquadest. A hundred microlitres of the solution was spreaded on 50 % nutrient agar (NA) plate which contained 100 ppm of appropriate commercial products of pesticides produced by PT. Bayer Indonesia and PT. Dow Agrosciences Indonesia respectively for BPMC and chlorpyrifos contained insecticides. The inoculated plates were incubated for 2–3 days at room temperature (25°C-27°C). The bacterial colonies grown on the plates were purified on the same media. The pure culture stocks of the isolates were kept on NA slant.

2.2. Screening of the insecticides degradation capability
One loop of the pure culture bacterial isolate was inoculated into 10 ml of 20% Nutrient Broth (NB) containing 100 ppm of the appropriate commercial insecticide. The was was incubated on a rotary shaker at 75 rpm for 48 hours at room temperature. Optical density of the culture were measured at 560 nm. Half milliliter of the culture (OD560: 1.0) was inoculated into 50 ml of the same media then incubated at room temperature on a rotary shaker (100 rpm) for 5 days. The cultures which showed growing well visually were determined its insecticides residues. The experiment was conducted in duplicate.

2.3. Compatibility test of the isolates
Compatibility or incompatibility potential between 4 selected isolates were examined by using cross streak method [5] on NA supplemented with 10 ppm of the insecticides.

2.4. Formulation of the insecticides biodegrading bacterial consortium
One loop of bacteria was innoculated into 50 ml LB and then incubated for 24-72 hours at room temperature on a rotary shaker. Two ml of the each culture was inoculated into a new of 250 ml NB and then incubated as described previously until its cell population reach up to 10^8 CFU/ml. The cultures were mixed with 1 kg of sterile talc or caolin which supplemented with 25 gram of yeast extract. The formula were dried until its humidity reach 35% before devided into 30 sealed plastic pouches and stored at room temperature (25°C-27°C).

2.5. In-vitro efficacy test of the formula
Thirty milliliters of each insecticides were added into 600 gram of sterile soil and mixed up well. The final concentration of the insecticides in the soil were 100 ppm. After 24 hours storage at room temperature, the soils were added with 30 ml solution of the formula (10 g/L) which has been store for a month at room temperature. Five days after inoculation, the insecticides residues in the soil were determined. The experiment was conducted in two replicates. The residues of BPMC and chlorpyrifos pesticides were determined by using the method which refered to National Pesticides Commission [6].

2.6. Characterization and identification of the selected isolates
The selected bacterial isolates were observed its colony morphology and their Gram staining reaction according to Benson [7]. Phytopathogenicity potential of the selected isolates were tested on tobacco plants [8].

DNA of the selected isolates was extracted using Presto™ Mini gDNA Bacteria Kit (Genaid). The 16S rDNA were amplified using AccuPower® PCR PreMix (Bioneer), and the primers and reaction
condition was conducted using the method as reported by Marchessi et al. [9]. The DNA amplification products were checked on agarose (1%) gel electrophoresis using 0.5x Tris Boric EDTA buffer, and stained with ethidium bromide before visualized on a UV transilluminator. The PCR products were send to DNA sequencing service company. The DNA sequences data were aligned with the data base in Gene Bank (NCBI) on http://www.ncbi.nlm.nih.org.

3. Results and discussion

3.1. The chlorpyrifos and and BPMC degrading bacterial isolates
One hundred and flivet nine of bacterial colonies were isolated and purified from 50% NA supplemented with 100 ppm chlorpyrifos (CP). However, only 10 isolates could grow in 20% NB containing 100 ppm CP. The bacterial isolates code of K17, K14, and K10 showed higher biodegradation activity of CP than that of the other isolates. CP degradation activity of the isolates were 43.7%, 43.3%, and 38.3% for K17, K14, and K10 isolates respectively.

Additionally, Ten of 105 bacteria isolates were isolated and purified from the isolation media supplemented with 100 ppm BPMC, and 9 isolates could grow in 20% NB containing 100 ppm BPMC. The highest biodegradation activity of BPMC was shown by the bacterial isolates code of B15-2, B17, and B21. They could degraded BPMC up to 75.9%, 77.0%, and 82.7% for B21, B17, and B15-2 respectively (table 1).

| No. | Isolate codes | Chlorpyrifos biodegradation activity (%) | No. | Isolate codes | BPMC biodegradation activity (%) |
|-----|---------------|------------------------------------------|-----|---------------|---------------------------------|
| 1.  | K2            | 36.5                                     | 1.  | B2            | 19.2                           |
| 2.  | K5-1          | 35.2                                     | 2.  | B5            | 1.3                            |
| 3.  | K5-2          | 20.5                                     | 3.  | B9            | 21.4                           |
| 4.  | K9            | 13.1                                     | 4.  | B10           | 33.2                           |
| 5.  | K10           | 38.3                                     | 5.  | B14           | 40.4                           |
| 6.  | K13           | 36.6                                     | 6.  | B15-1         | 19.7                           |
| 7.  | K14           | 43.3                                     | 7.  | B15-2         | 82.7                           |
| 8.  | K15           | 10.5                                     | 8.  | B17           | 77.0                           |
| 9.  | K17           | 43.7                                     | 9.  | B21           | 75.9                           |
| 10. | K21           | 36.5                                     | 10. | -             |                                 |

3.2. Compatibility of the insecticides biodegrading bacterial isolates
Compatibility test was carry out to determine the compatibly or incompatibility potential among the six selected isolates when live together in the same niche. The results showed that there are three combinations of the isolates that are mutually compatible, namely B17 + B21, K10 + B21, and K10 + K14 bacterial combinations, while other combinations showed incompatible reactions (table 2). Incompatible or antagonistic reaction among bacteria can be caused by excretion of enzymes [10] and or anti-microbial compounds such as antibiotics [11], organic acids [12], volatile organic compounds [13], and bacteriocins [14]. Therefore, only the compatible combinations that further used for combination and formulation experiments.

Combination of the compatible isolates increased significantly their biodegradability to chlorpyrifos and BPMC pesticides even though the increasing of BPMC degradation was smaller. Bacterial combination of K10 + K14 bacterial isolates increased biodegradation activity of chlorpyrifos and BPMC up to 89.6% and 75.9% for chlorpyrifos and BPMC residues in 5 days, respectively (figure 1). The degradation activity of this bacterial combination was higher than that of singel culture of K10 and K14 bacterial isolates which were 63.2% and 67.7% for K10 and K14,
respectively. Increasing trend of biodegradation activity also performed by bacterial combination of K10 + B21 isolates. Both combination also showed higher biodegradation activity compared to the single culture isolates. Chlorpyrifos and BPMC biodegradation activity of K10 + B21 bacterial combination was enhanced up to 88.9% and 70.3% respectively, that were higher than the insecticides biodegradation activities of the single culture of K10 and B21 isolates. As well as both the previous bacterial combinations, bacterial combination of B17+B21 isolates also shown increasing the insecticides biodegradation. Its chlorpyrifos degradation activity was 88.1%, and Its BPMC biodegradation activity was 69.5%. The increasing performed by the bacterial combination indicated that there was a synergy mechanism occuring among the isolates in the biodegradation processes of the pesticides.

Table 2. Result of compatibility assay of the six selected isolates.

| Combination     | Compatibility reaction |
|-----------------|------------------------|
| K10+K14         | +                      |
| K10+B17         | -                      |
| K10+B21         | +                      |
| K14+B17         | -                      |
| K14+B21         | -                      |
| B17+B21         | +                      |

3.3. Characteristics of the selected bacterial isolates

The result of Gram stained reaction of the selected isolate showed that K14 isolate is Gram positive bacteria, while the other bacterial isolates are Gram negative (table 3). The color of the K10 and B17 bacterial colonies on NA plates was cream, the bacterial colony of K14 isolate was white, and the bacterial colony of B21 isolate was red. Microscopic observation showed that cellulare shape of the isolates was rod shape except K10 isolate which was coccobacil. All of the isolates did not cause hypersensitive respons (HR) reaction on tobacco leaves when its were inflected to the plants (table 3). Therefore, there is no need to worry about phytopathogenicity potential of the isolates.

Based on the 16S rDNA sequence analysis, the isolates have similarity 97.7% to A. baumannii, 96.3% to B. toyonensis, 94.4% to uncultured enterobacter sp. clone 150, and 78.08% to uncultured bacterium clonenck09g01c1 for K10, K14, B17, and B21 bacterial isolates, respectively.

Figure 1. Degradation of chlorpyrifos (A) and BPMC (B) pertaincies by single and combination cultures of the selected bacterial isolates.
Table 3. Morphological characteristics of the four selected bacterial isolates.

| Isolate codes | Color of the colony | Gram reaction | Cells shape | HR |
|---------------|---------------------|---------------|-------------|----|
| K10           | Cream               | -             | Cocccobacil | -  |
| K14           | White               | +             | rod         | -  |
| B17           | Cream               | -             | rod         | -  |
| B21           | Red                 | -             | rod         | -  |

3.4. Effectivity of the bacterial formula in degrading the insecticides

Besides being influenced by the bacterial combination, effectivity of the formula to degrade the insecticide residues in the soil were also influenced by the capability of a carrier to maintain the bacterial viability. Compared to talc formulation, caolin formulation generally showed better biodegradation effectivity. The combination of K10+K14 bacterial isolates formulated in the caolin performed the best biodegradation effectivity. Biodegradation effectivity of the caolin formula reached up to 79.9% and 71.9% for chlorpyrifos and BPMC residues, respectively (Tables 4 and 5).

Table 4. Degradation of chlorpyrifos residue in the soil by the bioremediation agent formulas

| Carrier | K10 | K14 | B17 | B21 | K10+K14 | K10+B21 | B17+B21 |
|---------|-----|-----|-----|-----|---------|---------|---------|
| Caolin  | 75.4b| 66.1e| 69.9d| 72.0c| 79.9a   | 59.5f   | 70.0cd  |
| Talc    | 61.4f| 37.9i| 37.9i| 46.6h| 50.4g   | 48.1g   | 61.6f   |

The numbers followed by the same letter are not significantly different at the 0.05 test level

Table 5. Degradation of BPMC residue in the soil by the bioremediation agent formulas

| Carrier | K10 | K14 | B17 | B21 | K10+K14 | K10+B21 | B17+B21 |
|---------|-----|-----|-----|-----|---------|---------|---------|
| Caolin  | 55.6d| 65.4b| 43.1g| 39.6h| 71.9a   | 56.3d   | 60.1c   |
| Talc    | 52.2e| 42.9g| 29.9i| 20.4j| 10.6k   | 46.6f   | 51.4e   |

The numbers followed by the same letter are not significantly different at the 0.05 test level

4. Conclusion

A. baumannii K10 and B. toyonensis K14, B17 are potential to be developed as a bioremediation agent of chlorpyrifos and BPMC pesticides. Combination of the both bacteria increased the degradation activity of the insecticides. Caolin formulation of the bacteria is better than the talc formulation for maintaining the insecticide degradation activity of the bacteria.

Acknowledgments

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