# Pesticide Degrading Ability of Indigenous-Bacteria from Contaminated Cropland in Kersana, Brebes Regency, Indonesia

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**Abstract.** Kersana is one of the central shallot-producing districts in Brebes Regency. Synthetic pesticides are widely and usually used in this cropland, and it has caused pollution of soil and water. Bioremediation can be one of the solutions to overcome this problem by using indigenous-bacteria from contaminated soil and water as biological agents. This research was purposed to isolate and identify pesticide degrading bacteria from shallot fields in Kersana, Brebes regency. Twenty-three (23) isolates were obtained in the isolation process. Those isolates were tested for biochemical characterizations which are potassium hydroxide test, proteolytic test, amylolytic test, and catalase test and potential test which are hypersensitive reaction test and pesticide degradation ability test. Based on the biochemical test and pesticide degradation ability test, all isolates have an ability to live under a pesticide contaminated environment and thirteen (13) isolates were suspected to be safe for application in the field. These results suggest that indigenous bacteria can help reduce pollution due to the use of pesticides in an environmentally friendly way.

**Keywords:** Pesticide Residue · Bioremediation · Sustainable Farming · Eco-Friendly Agriculture

## 1 Introduction

Shallots (*Allium cepa* L.) are a superior horticultural commodity with many benefits. This plant is widely used for cooking purposes as herb by residents. Several shallot production centers include Central Java, East Java, West Java, and West Nusa Tenggara [1]. One of the leading shallot-producing areas in Indonesia is located in Brebes Regency. Kersana District is one of the shallot-producing areas in Brebes Regency. This sub-district was recorded in the DPKP of Brebes Regency in 2021 to produce 109,140 quintals of shallots. Kersana is an area where farmers generally use pesticides by mixing 3–5 types of pesticides with a frequency of application almost every day, especially during the rainy season [2].


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Pesticides are often used because of its effectiveness, practicality, and ability to bring significant economic benefits to farmers. One type of pesticide that farmers often choose is a chemical pesticide. Excessive use of chemical pesticides can harm the environment and farmers' health. Excessive application of pesticides can be found on shallot cropland in Kersana District, which causes cracked soil conditions and turbid green water, which may indicate the land has been polluted.

The type of pesticide widely used by shallot farmers is organophosphate. Organophosphates are phosphoric acid or thiophosphoric acid ester insecticides with very toxic properties [3]. Several types of pesticides are included in the pesticide group, such as Azinophosmethyl, Chloryfos, Demeton Methyl, Dichlorovos, Dimethoate, Disulfoton, Ethion, Palathion, Malathion, Parathion, Diazinon, and Chlorpyrifos [4].

Organophosphate pesticides are recommended in the agricultural sector because they are easily decomposed in nature. However, it has a negative impact, one of which is leaving residues on agricultural products, which can endanger consumer's health. The solution to overcome this impact is bioremediation. Bioremediation is a process that relies on biological mechanisms to reduce pollutants or contaminants to lower levels [5].

Using indigenous bacteria as a bioremediation agent is one of the solutions for pollution issues caused by organophosphate pesticides. Indigenous bacteria are isolated and used for environmental problems in their places of origin [6]. This study collected bacteria from shallot cropland to be identified using biochemical characterizations, plant hypersensitivity response, and pesticide degrading ability test. Potential isolates are expected to be able being applied as pesticide bioremediation agents.

## 2 Materials and Methods

### 2.1 Isolation and Sample Collection

The soil and water samples were taken from shallot cropland in Kersana District, Brebes Regency. Dilution was done by using 1 g of soil sample suspended in 9 ml of sterile water and 1 ml of water sample suspended in 9 ml of sterile water until 10–11 dilution. The dilutions used are 10–7, 10–8, 10–9, 10–10, and 10–11 dilutions with two repetitions which will be poured on YPA (Yeast Peptone Agar) media and incubated for 48 h or two days. Isolation was done by using modified YPA media by dissolving 10 g of Bacto Yeast Extract, 20 g of Bacto Yeast Peptone, 10 g of D-Glucose, and 22 g of Bacto Agar in 1 L of sterile water.

### 2.2 Bacterial Characterization

Resulted isolates were characterized using biochemical tests to identify the type of potential isolates. Gram test, amylolitic test, proteolytic test, catalase test, hypersensitivity reaction test, and pesticide degradation ability test were used in this research. Bergeys's Manual of Determinative Bacteriology was used to specify bacteria characteristic based on biochemical tests.

### 2.2.1 Gram Test

Potassium hydroxide or KOH 5% was used to identify the type of bacteria either gram-positive or gram-negative. The test was carried out by taking the isolate with a wire loop and placing it on a glass slide. Then, 5% KOH solution was dripped on the glass slide. The isolate was emulsified to 5% KOH and mixed well for 1 min. Isolates with positive response (gram-negative) adhered to the loop and formed a string approximately 1–5 cm long within the first 30 s when it was lifted.

### 2.2.2 Amylolytic Test

The starch agar media composed of 2 g of glucose, 2 g of peptone, 0.2 g of casein hydrolyzate, 0.4 g of starch, and 4 g of agar in 200 ml of sterile water. The observation was done by giving 2–3 drips of lugol so that a clear zone is formed around the colony, while other areas are dark blue. The clear zone around the colony area shows that the starch content in the media is degraded by the amylase enzyme produced by bacteria into simple sugar compounds [7].

### 2.2.3 Proteolytic Test

The proteolytic test was aimed to determine the ability of bacteria to produce hydrolyze protein marked by forming a clear zone on the media and around the colony due to the hydrolysis of casein into dissolved nitrogen. The materials used to manufacture proteolytic test media are mixing 0.1 g of peptone, 0.5 g of NaCl, 2 g of agar, and 10 g of skim milk powder in 100 ml of boiled water. Furthermore, the plating was carried out using four sterile petri dishes. The next stage is waiting for the media to harden, followed by bacterial inoculation.

### 2.2.4 Catalase Test

The catalase test was tested by dripping hydrogen peroxide (H2O2) on the object glass and the isolates were inoculated to H2O2 and mixed slowly using inoculating loop. Air bubbles will form on the object glass showing the characteristic of gram-positive bacteria.

## 2.3 Hypersensitivity Reaction Test

Hypersensitivity reaction test is a bacterial test on tobacco plants that can be an antibiotic for some bacteria [8]. Hypersensitivity test was aimed to determine whether bacterial isolates belong to pathogenic or non-pathogenic. Bacteria leading a positive response are bacteria with high sensitivity to plants, indicating they are plant pathogens. The hypersensitivity test of bacterial isolates to tobacco plants was carried out by inoculating bacterial colonies with a density of approximately 108 cells into a microtube containing 1 ml of distilled water and homogenized using a vortex. The colony mixture was injected into the leaves of tobacco plants using a syringe without a needle.

# 3 Result and Discussion

## 3.1 Bacterial Characterization

Results of biochemical characterizations are shown in Table 1. It is included the identification of bacteria based on Bergeys's Manual of Determinative Bacteriology.

### 3.1.1 Gram Test

In this test, the isolates were characterized with the presence of mucus (Fig. 1). Gram-positive (+) bacteria has thick cell walls and thin peptidoglycan, while gram-negative (−) bacteria has thicker peptidoglycan than gram-positive bacteria and thin cell walls in periplasm. KOH 5% will break down gram-negative (−) cells and release genetic material (DNA). Based on the research, KOH tested by T2, T3, and T7 changed to slimy form when lifted using a wire loop that showed characteristic of gram-negative bacteria, while other isolates showed gram-positive (+) bacteria by not changing KOH to slimy form. Due to the sticky strings of DNA molecules, it looks like mucus when the inoculum needle is lifted [9]. Gram-negative (−) bacteria produce mucus because they have cell walls that are more sensitive than gram-positive bacteria (+), and there is no resistance to alkaline inhibitors such as KOH solution [10].

### 3.1.2 Amylolytic Test

The amylolytic test was used for capturing the extracellular amylase enzyme activity in bacteria using starch agar media. The test was carried out to differentiate bacteria ability producing amylase enzyme that is marked with the presence of clear zone on starch agar media. Bacteria that have the ability to produce amylase enzyme based on the result of amylolytic test by producing a clear zone after dripping lugol on the media are bacterial isolates T1–T16 and A1, A2, A3, A5, A6, A7.

Starch is used as a carbon source by amylolytic bacteria as an energy source. The reshuffle of starch structure occurs due to the activity of amylase which is marked by a clear zone around the amylolytic bacteria. The media that does not show a clear zone and is blue when after dripping lugol indicates that the bacteria do not produce amylase

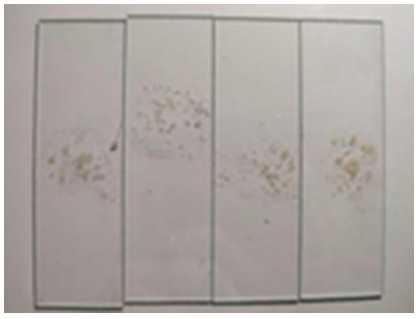

**Fig. 1.** KOH test on soil isolates.

**Table 1.** The result of biochemical tests and the identification of bacteria

| Code | Source of Isolates | Result | | | | | |
|---|---|---|---|---|---|---|---|
| | | Gram (KOH) | Proteolytic | Amylolytic | HR | Catalase | Identification of Bacteria |
| T1 | Soil | + | + | + | + | + | Non-potential |
| T2 | Soil | − | + | + | − | + | *Pseudomonas sp.* |
| T3 | Soil | − | + | + | + | + | Non-potential |
| T4 | Soil | + | + | + | − | + | *Planococcus sp.* |
| T5 | Soil | + | + | + | + | + | Non-potential |
| T6 | Soil | + | + | + | + | + | Non-potential |
| T7 | Soil | − | + | + | + | + | Non-potential |
| T8 | Soil | + | + | + | + | + | Non-potential |
| T9 | Soil | + | + | + | − | + | *Planococcus sp.* |
| T10 | Soil | + | + | + | − | + | *Planococcus sp.* |
| T11 | Soil | + | + | + | + | + | Non-potential |
| T12 | Soil | + | + | + | − | + | *Planococcus sp.* |
| T13 | Soil | + | + | + | − | + | *Planococcus sp.* |
| T14 | Soil | + | + | + | − | + | *Planococcus sp.* |
| T15 | Soil | + | + | + | − | + | *Planococcus sp.* |
| T16 | Soil | + | + | + | − | + | *Planococcus sp.* |
| A1 | Water | + | + | + | − | + | *Planococcus sp.* |
| A2 | Water | + | + | + | − | + | *Planococcus sp.* |
| A3 | Water | + | + | + | + | + | Non-potential |
| A4 | Water | + | + | − | + | + | Non-potential |
| A5 | Water | + | + | + | + | + | Non-potential |
| A6 | Water | + | + | + | − | + | *Planococcus sp.* |

(*continued*)

**Table 1.** (*continued*)

| Code | Source of Isolates | Result | | | | | |
|---|---|---|---|---|---|---|---|
| | | Gram (KOH) | Proteolytic | Amylolytic | HR | Catalase | Identification of Bacteria |
| A7 | Water | + | + | + | – | + | *Planococcus sp.* |

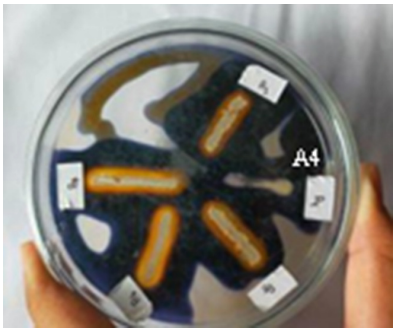


**Fig. 2.** Amylolytic test on water isolates.

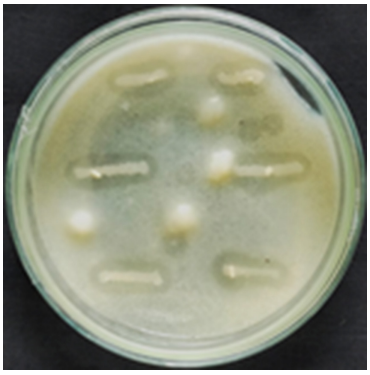

**Fig. 3.** Proteolytic test on water isolates.

enzyme [11]. The bacteria isolates that did not show a clear zone around is isolate A4 (Fig. 2).

### 3.1.3 Proteolytic Test

Proteolytic test was carried out to determine whether the isolated sample could produce proteases by forming a clear zone around the colony (Fig. 3). It is known that isolates T1-T16 and A1-A7 can have the ability to produce proteases. Proteolytic bacteria are bacteria that have the ability to degrade proteins, produce extracellular protease enzymes. Several

genera of bacteria include proteolytic bacteria, namely the genus Bacillus, Pseudomonas, Proteus, Streptobacillus, Staphylococcus, and Streptococcus [12].

### 3.1.4 Catalase Test

Catalase test was carried out to identify groups of bacteria that can produce the catalase enzyme [13]. Catalase is an enzyme catalyzing the breakdown of hydrogen peroxide into H2O and O2. The catalase enzyme functions as a decomposition of hydrogen peroxide (H2O2) which is toxic so that cell death does not occur [14]. Hydrogen peroxide is formed during aerobic metabolism, so microorganisms growing in an aerobic environment must decompose the material. Based on the research, it was found that the bacteria isolated from soil samples (T1–T16) and bacteria isolated from water samples (A1–A7) had positive catalase results which could be seen on the object glass produce air or gas bubbles.

## 3.2 Identification of Bacteria

Based on the results of the hypersensitivity reaction (HR) test using tobacco leaves and pesticide degradation ability test, 13 potential isolates were found, namely T2, T4, T9, T10, T12, T13, T14, T15, T16, A1, A2, A6, and A7 (Table 1). Those isolates were then used to identify bacteria using Bergey's Manual of Determinative Bacteriology based on the results of characterization with biochemical tests. The T2 isolate was suspected to be Pseudomonas sp. with gram-negative characteristics, round (coccus) or rods (rods), and obligate aerobes [15]. It is also mentioned in Bergey's Manual of Determinative Bacteriology (1994) that Pseudomonas sp. have positive catalase characteristics. Meanwhile, isolates T4, T9, T10, T12, T13, T14, T15, T16, A1, A2, A6, and A7 were suspected as Planococcus sp. with the characteristics of spherical (coccus), aerobic, catalase positive, and gram-positive bacteria [16].

## 3.3 Potential of Bacteria and Pesticide Degrading Ability

Based on the hypersensitive reaction (HR) test (Table 1), 13 isolates resulted in a negative response. So, they were supposed to be applied in the cropland safely. On the other hand, there were 10 isolates that showed the positive response and it could be indicated as a characteristic of phytopathogenic. According to the disclosure, all of the phytopathogenic bacteria lead the hypersensitive reaction in the leaf mesophyll tissues [17] which is indicated by cell death to deprive microbial growth and nutritional supply [18].

Plant's induced hypersensitive reaction is resulted by pathogen elicitors-producing as known as microbe-associated-molecular patterns or pathogen-associated-molecular patterns (MAMPs or PAMPs). MAMPs are recognized by Pattern Recognition Receptors (PRRs) localized on the cell surface so that the first defense induction called MAMPs-Triggered Immunity (MTI) is formed. Injection of bacterial effector proteins into host cytoplasm via pathogens type III secretion system (TTSS) leads to MTI suppression and results in Effector-Triggered Susceptibility (ETS). The effector recognition troughs a set of plant resistance (R) gene products. It activates Effector-Triggered Immunity

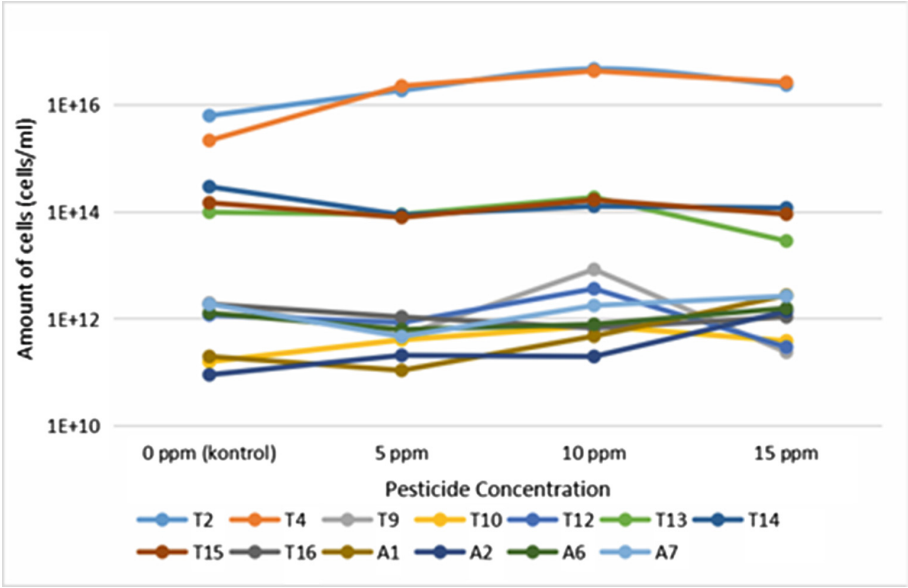


**Fig. 4.** Amount of cells from the isolates living under organophosphate contamination.

(ETI) as the advance defense. Most cases, ETI causes localized cell death known as hypersensitive reaction [18].

Based on Fig. 4, the result of the pesticide degrading ability test shows that isolates of T2 and T4 were resistant to pesticide contamination. These are indicated by graphical increase at 5 ppm and 10 ppm of pesticide stress. Although there is a decrease at 15 ppm, both of the isolates show a higher amount of cells than the other isolates, these are approximately $2.4 \times 10^{16}$ and $2.7 \times 10^{16}$ cells/ml. The number of soil bacteria living at contaminated soil by organophosphate is lower than soil bacteria living under contamination of pesticide with other chemical compounds [19]. However, the result of this research is according to the study of Istiqomah et al. that the soil isolate showed a stable-growth than the water isolates under organophosphate treatment [20].

Pesticide degradation of indigenous-bacteria occurs via enzymatic reaction. In this case, the compound of pesticide can be used as a nutritional source which is broken down into smaller molecules. Phosphatase and esterase, the hydrolysis enzyme of microbe, play a role in breaking down the pesticide compound built by unstable chains, such as organophosphate [19]. As the mechanism of degrading pesticide goes through 3 steps, they are absorption of pesticide compound on the surface of cell membrane, entering pesticide compound into cell membrane, and breaking down pesticide compound into the less toxicity or non-toxicity compounds [21].

## 4 Conclusion

There are 13 isolates that can be utilized as bioremediation agent which are T2, T4, T9, T10, T12, T13, T14, T15, T16, A1, A2, A6, and A7. Those isolates were then identified

as Pseudomonas sp. for T2 and Planococcus sp. for T4, T9, T10, T12, T13, T14, T15, T16, A1, A2, A6, and A7. These 13 isolates are also potential for degrading pesticide contamination based on positive result of pesticide degradation ability test. Nevertheless, before used as bioremediation agent, the isolates need to be tested further to ensure if those isolates are safe for the environment and human.

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