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Exploration of Symbiotic Bacteria with the Potential to Degrade Chlorpyrifos Insecticide in the Digestive Tract of the Beet Armyworm (*Spodoptera exigua*) (Lepidoptera: Noctuidae) in Shallot Fields, Probolinggo

Eksplorasi dan Karakterisasi Bakteri Simbion Pendegradasi Insektisida Klorpirifos Pada Larva *Spodoptera exigua* Di Lahan Bawang Merah, Kabupaten Probolinggo

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ABSTRACT

The beet armyworm, *Spodoptera exigua* is a notorious pest that inflicts significant damage to shallot crops, especially leeks, causing leaves to wither due to brown spots and transparency. This issue often leads to excessive chemical control measures, which, in turn, promote insecticide resistance. This study hypothesized that symbiotic bacteria within *S.exigua* have the capacity to degrade chlorpyrifos-based insecticides. *S.exigua* samples were collected in Dringu, Gending, and Leces districts, Probolinggo, and subsequently examined for symbiotic bacteria with biodegradation potential. Bacterial exploration and identification were conducted from April to October 2023 at the Pesticide Toxicology Laboratory, Brawijaya University. The study analyzed the clear zone diameter produced by bacteria in media contaminated with chlorpyrifos-based insecticides as an indicator of biodegradation. Out of 41 surviving bacterial isolates, 10 demonstrated notable biodegradation capabilities. The Genus identification revealed that *Erwinia* sp., *Pantoea* sp., and *Coryneform* sp. were among the contributing bacteria. This research emphasizes the potential role of *S.exigua*-associated symbiotic bacteria in managing chlorpyrifos-resistant pest populations, offering a promising avenue for sustainable pest control methods.

keywords: Biodegradation; Chlorpyrifos; *Spodoptera exigua*; Symbiotic bacteria

INTRODUCTION

Spodoptera exigua, commonly known as onion caterpillars, is a pest that damages shallot plants. *S.exigua* can be said to be the main pest of shallot plants. *S.exigua* has the characteristics of a green body with a body length of around 3-5 cm. *S.exigua* attacks red onion leaves, causing the red onion leaves to become transparent, dry, with brownish spots until the leaves wilt completely.

According to Marsadi et al. (2017), yield losses caused by the *S.exigua* pest in 2017 could reach 100%, so it can be said that the *S.exigua* attack could cause total crop failure for shallot farmers. In 2021, the shallot harvest area in Probolinggo Regency reached 9,267 hectares, but in 2022 the

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shallot harvest area in Probolinggo Regency decreased, namely 9,038 hectares. This shows that the decrease in shallot harvest area was also followed by a decrease in shallot harvest. Chemical control is one of the control techniques commonly used by farmers. Chemical control is carried out by farmers because it provides fast results for the death of the target pest. However, it is known that chemical control can cause various negative impacts. According to Dharmadewi and Suryatini (2022), the use of chemical pesticides can cause the accumulation of chemical residues in the environment which can cause poisoning not only to target pests, but also to natural enemies and even humans.

One of the active ingredients of chemical pesticides applied by farmers is the active ingredient chlorpyrifos. Based on research by Moekasan and Basuki (2007), *S.exigua* caterpillars have resistance to pesticides containing the active ingredient chlorpyrifos. Based on research by Hadi et al. (2021), it is known that there is a role for symbiotic bacteria in *Plutella xylostella* caterpillars in the degradation of the active ingredient of the pesticide chlorpyrifos. 15 symbiotic bacteria were found with the ability to degrade the active ingredient chlorpyrifos. This research aims to characterize symbiotic bacteria found in the digestive tract of Spodoptera exigua larvae in shallot plantations in Probolinggo Regency. Thus far, research on symbiotic bacteria in *S.exigua* remains scarce, making this study highly relevant and impactful in exploring potential solutions for managing this pest.

MATERIALS AND METHODS

Time and Place

The research was conducted from April to October 2023. The research was carried out in two stages. Stage 1 involved the sampling of *S.exigua* in three districts: Leces District with coordinates -7° 48' 43", 113° 13' 57", Dringu District with coordinates -7° 48' 40", 113° 14' 22", and Gending District with coordinates -7° 48' 3", 113° 18' 18", located in Probolinggo Regency. Stage 2 was a follow-up study conducted in the Pesticide Toxicology Laboratory, Brawijaya University, Malang City.

Sampling of *S.exigua*

The sampling activity began with the collection of *S.exigua* in the designated shallot fields. The sampling of *S.exigua* followed the method by Xia et al. (2013), which involved randomly collecting larvae in the fields without considering their gender. The caterpillars were selected based on their body size, specifically targeting those in the third instar. Approximately 50 *S.exigua* were collected from each shallot field in three districts of Probolinggo Regency. The *S.exigua* were manually collected using hands, placed in perforated storage boxes, provided with food and moist tissue, and then kept for further maintenance and study in the laboratory.

Selection of *S.exigua* Samples

The selection of *S.exigua* was carried out by rearing the *S.exigua* in perforated boxes to allow air circulation, and they were fed with shallot leaves contaminated with chlorpyrifos insecticide. Shallot leaves were dipped in a chlorpyrifos insecticide solution at the recommended dose of 1 ml/l. The *S.exigua* that had been fed were then incubated for 24 hours. After 24 hours, larvae that were still alive and approximately in the third instar were chosen and separated into another storage box.

Preparation of Culture Media

Sterilization of the media was conducted by preparing the necessary equipment and materials. The equipment used included disposable Petri dishes, Erlenmeyer flasks, a stirrer, plastic wrap, an autoclave, and a laminar flow cabinet

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(LAFC). The materials used included Nutrient Broth (NB) media, agarose, and distilled water (aquades). To prepare 200 ml of media, 2 grams of NB media, 4 grams of agarose, and 200 ml of distilled water were needed. The media was sterilized in the autoclave for 30 minutes at a temperature of 121°C. After sterilization, the media was poured into Petri dishes and stored at room temperature for 24 hours before use. The preparation of the culture media was based on research by Napitupulu et al. (2019), which involved mixing 2 grams of NB media and 4 grams of agar with 200 ml of distilled water to create the nutrient broth media.

Isolation of *S.exigua* Larvae

The isolation of *S.exigua* larvae was carried out by sterilizing the larvae in 70% alcohol, followed by rinsing them three times with distilled water (aquades). Afterward, the *S.exigua* larvae were placed on plasticine to facilitate piercing and larval surgery. The surgery of *S.exigua* larvae was conducted based on the method employed in the study by Hadi et al. (2021). The larvae were dissected in the abdominal region to extract the digestive tract. The digestive tract was collected using a micro pipette and then placed into a solution of 9 ml of distilled water with a 10-1 dilution. Subsequently, the digestive tract was vortexed for approximately 30 seconds until the midgut ruptured. The resulting mixture was collected in a volume of 100 µl using a micro pipette and spread on NA media with the spread plate method (Varghese & Joy, 2014). The isolates were incubated for 24-48 hours until bacterial growth occurred.

Purification

Bacterial purification is performed by selecting bacterial isolates from the isolation results. The selection of bacterial isolates is based on the morphology of the bacteria, such as the isolate's color, size, elevation, shape, and edges. Purification is carried out by streaking a plate using the streak plate method, specifically the "streak T" technique, on NA (Nutrient Agar) media, using an inoculating needle.

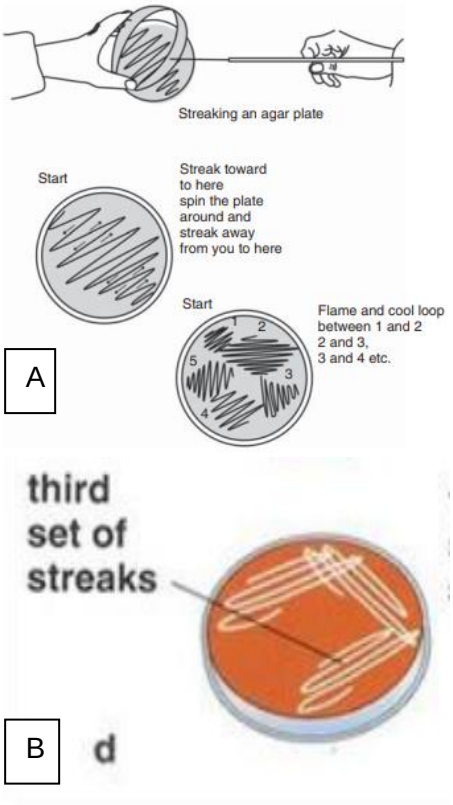

Figure 1. (a) streak plate methods (Bisen et al., 2012), (b) T streaks illustration (Varghese & Joy, 2014).

Biodegradation Test of Insecticides Containing the Active Ingredient Chlorpyrifos

The biodegradation test, or degradation ability, was carried out using a Completely Randomized Design (CRD) method with the number of treatments based on the bacterial isolates obtained and repeated four times. Based on research by Wiguna (2019), the biodegradation test involved one control, which was performed by soaking Whatman paper in aquades without the addition of bacterial isolates. The Whatman paper was then placed in pesticide-contaminated media that had been treated with chlorpyrifos insecticide.

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Preparation of the contaminated test media was done by preparing NA (Nutrient Agar) solution contaminated with chlorpyrifos insecticide and adding Congo red powder as a colorant to the NA media. According to Lesmana et al. (2019), adding Congo red to solid media can be done to change the color of the originally white NA media to red. The NA media was sterilized first in an autoclave for 30 minutes at 121°C. After sterilizing the media, chlorpyrifos insecticide was added at the recommended dosage on the packaging, which is 1.5 ml/l. The media was plated in Petri dishes and incubated for one day (24 hours).

Sterile liquid NB (Nutrient Broth) was then added to Eppendorf tubes, 1 ml in each Eppendorf tube. In each Eppendorf tube, one isolate of bacteria was added, and it was shaken for one day. Then, Whatman paper with a diameter of 0.6 cm was placed in each Eppendorf and left for 1 hour. After an hour, the Whatman paper was taken and inoculated on the surface of the contaminated media. The degradation ability of the bacteria was determined based on the clear zones formed on the Petri dishes, and the diameter of these clear zones was measured with a ruler. Observations were conducted by measuring the diameter of the clear zones formed on media contaminated with chlorpyrifos insecticide. The formation of clear zones was observed for 96 hours and then calculated using the formula based on Lestari (2019):

Clear Zone Diameter = Inhibition Zone Diameter - Filter Paper Diameter.

The calculation of bacterial degradation percentage in the contaminated media was performed to assess the level of bacterial degradation of chlorpyrifos insecticide. The larger the diameter of the bacterial clear zone, the higher the percentage of bacterial degradation of chlorpyrifos. The calculation of the bacterial degradation percentage was done using the formula:

$$\frac{Treatment\ diameter - control\ diameter}{Treatment\ diameter} \times 100$$

The broader clear zone formed, the greater bacterial degradation ability. According to Rundengan et al. (2017), the inhibitory power based on the size of the inhibition zone can be categorized as follows:

a. Inhibition zone > 2 cm: very strong category
b. Inhibition zone 1-2 cm: strong category
c. Inhibition zone 0.5-1 cm: moderate category
d. Inhibition zone < 0.5 cm: weak category

Characterization of Symbiotic Bacteria

Bacterial characterization is performed to determine the characteristics of the test bacteria. Bacteria selected based on their degradation ability are further characterized for their morphology. Morphological characterization involves observing colony shape, colony color, colony margin, and elevation (Wiguna, 2019). Then, physiological characterization is based on the physiology of the bacteria. Tests include Gram staining, 3% KOH solubility test, catalase test, spore staining, oxidative-fermentative test, and tests on YDC media. These tests are based on Bergey's Manual of Determinative Bacteriology (Holt et al., 1994) and (Schaad et al., 2001).

Gram Staining Test

The Gram staining test is conducted by examining bacteria that have been grown for 24 hours. Bacterial isolates are taken with a sterile loop and mixed with sterile distilled water. They are then placed on a sterile glass slide and dried using a Bunsen burner. Once the bacteria are dry, they are stained with one drop of crystal violet and left for 30 seconds. After that, they are rinsed with running water and dried using a Bunsen burner. Next, one drop of iodine solution (Lugol) is added and left for 30 seconds. The glass slide is cleaned with 90% alcohol and left for 30 seconds before being rinsed with

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running water and dried using a Bunsen burner. Subsequently, one drop of safranin is applied and left for 30 seconds before rinsing with running water and drying. The observation process on the glass slide is performed using a microscope at 1000x magnification with the addition of one drop of immersion oil to enhance the observation results. Gram-negative bacteria will appear red, while Gram-positive bacteria will appear blue or purplish-blue.

KOH 3% Solubility Test

The KOH 3% solubility test is conducted using pure bacteria that have been grown for 24 hours. A small amount of bacteria is taken and suspended on a glass slide. Then, one drop of 3% KOH solution, which has been mixed with sterile distilled water, is added to the suspension. The resulting suspension is stirred by rotating the loop and drawn upwards using a sterile needle. If it forms a slimy thread, the result is negative; if it does not form a slimy thread, the result is positive.

Catalase Test

The catalase test is conducted using pure bacteria that have been grown for 24 hours. A small amount of bacteria is taken and suspended on a glass slide. Then, one drop of hydrogen peroxide (H_2O_2) solution is added to the suspension. If the suspension forms air bubbles, the result is positive; if it does not form air bubbles, the result is negative.

Sporulation Staining

Bacterial spore staining is performed using bacteria that have been grown for 24-48 hours. A small amount of bacteria is taken and smeared on a sterile glass slide, then one drop of distilled water is added and it's dried using a Bunsen burner. Once the bacteria is dry, one drop of 5% malachite green solution is applied, and it's left to sit for 10 minutes. The glass slide is rinsed with distilled water, dried again with a Bunsen burner, and after it's dry, one drop of safranin is added for 15 seconds. Then it's rinsed and dried once more. Observation is conducted under a microscope with 1000x magnification. If the bacteria produce spores, they will appear green, but if they do not produce spores, the bacterial cells will appear red.

Oxidative-Fermentative (OF) Test

The oxidative-fermentative test is performed by growing selected bacteria on glucose fermentation media with a pH of 7 - 7.1 in test tubes. The glucose fermentation medium is composed of 2 grams of peptone, 5 grams of NaCl, 0.3 grams of KH_2PO_4, 3 grams of agar, and 3 ml of Bromthymol blue (1%). These ingredients are mixed and dissolved in 1 liter of sterile distilled water and then sterilized using an autoclave. Next, 0.5 ml of 10% glucose solution is added aseptically to each test tube using a micropipette.

Each culture of pure bacteria, aged 24 hours, is then inoculated into two test tubes filled with oxidative-fermentative solid media. One of the test tubes is sealed with paraffin and incubated for 7-14 days. If there is a color change in the medium to yellow in the test tube that is not sealed with paraffin, the reaction is oxidative. If a color change occurs in both test tubes, the reaction is fermentative.

YDC Medium Growth Test

The YDC medium growth test is conducted by cultivating bacteria on YDC medium with the following composition: 10 grams of yeast, 20 grams of glucose, 20 grams of $CaCO_3$, and 15 grams of agar. These ingredients are mixed, dissolved in 1 liter of sterile distilled water, and then sterilized using an autoclave. Subsequently, the YDC medium is poured into Petri dishes and incubated for one day.

Pure bacteria aged 24 hours are grown on YDC medium using the streak plate method. If the bacteria produce yellow colonies, the

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result is considered positive. If the bacteria produce white colonies, the reaction is considered negative.

Data Analysis

The results of the biodegradation test of the insecticide with the active ingredient chlorpyrifos include the average diameter of the clear zones that appeared on 10 test bacterial isolates suspected to have degradation capabilities. The biodegradation test data obtained is tabulated using Microsoft Excel. Subsequently, the data is analyzed using ANOVA, and if the results are significantly different, it is followed by a 5% level DMRT test using the IBM SPSS Statistics 25 application.

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RESULTS AND DISCUSSIONS

Exploration and Isolation of Bacteria in the Midgut of *S. exigua*

Based on the isolation of bacteria in the midgut of *S.exigua* larvae, a total of 41 bacterial isolates were obtained. The diluted samples were then cultured on NA media and incubated for up to 48 hours. In the results of the bacterial isolation, different numbers of bacterial isolates were obtained after purification at each location in Probolinggo Regency. The bacterial isolates found in the Gending location amounted to 14 isolates, in Leces amounted to 14 isolates, and in Dringu amounted to 13 isolates. The presence of bacterial isolates in the midgut of larvae has been confirmed, where 66 bacterial isolates were found in the midgut of Plutella xylostella in both organic and conventional fields (Hadi et al., 2021).

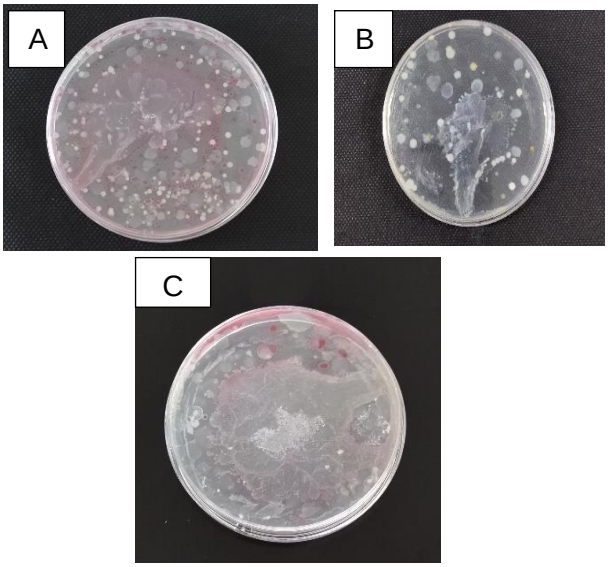

Figure 2. Results of bacterial isolation on *S.exigua* : (a) Bacterial isolation results of *S.exigua* from Leces,(b) Bacterial isolation results of *S.exigua* from Dringu, (c) Bacterial isolation results of *S.exigua* from Gending.

Biodegradation Test of Chlorpyrifos Insecticide Active Ingredient

In the biodegradation test, it was found that there are 10 bacterial isolates capable of producing clear zones on contaminated NA media. Based on the analysis of variance (ANOVA) results and further DMRT test at a 5% level, it was found that the treatment had a significantly different effect. The observation results of the biodegradation test at 96 hours revealed that all isolates were able to grow on contaminated NA media. However, only 10 isolates could produce clear zones, indicating the ability to degrade chlorpyrifos insecticide.

Table 1. Results of bacterial isolate biodegradation test

Isolates code	Tested on NA media contaminated with 1.5 ml/L chlorpyrifos	Isolates code	Tested on NA media contaminated with 1.5 ml/L chlorpyrifos
Kontrol	-	LC7	-

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Isolates code	Tested on NA media contaminated with 1.5 ml/L chlorpyrifos	Isolates code	Tested on NA media contaminated with 1.5 ml/L chlorpyrifos
GD1	-	LC8	-
GD2	-	LC9	-
GD3	+	LC10	-
GD4	-	LC11	-
GD5	-	LC12	+
GD6	-	LC13	+
GD7	+	LC14	-
GD8	+	DR1	-
GD9	-	DR2	-
GD10	-	DR3	+
GD11	-	DR4	-
GD12	-	DR5	-
GD13	-	DR6	-
GD14	-	DR7	+
LC1	+	DR8	-
LC2	-	DR9	-
LC3	-	DR10	-
LC4	-	DR11	-
LC5	-	DR12	+
LC6	-	DR13	+

Description: (+) if the bacterial isolate is capable of producing a clear zone on the contaminated media, (-) if the bacteria cannot produce a clear zone on the contaminated media.

In Leces District, the bacterial isolate codes that produced clear zones were LC1, LC12, and LC13. In Dringu District, bacterial isolate codes that produced clear zones were DR3, DR7, DR12, and DR13. In Gending District, bacterial isolate codes that produced clear zones were GD3, GD7, and GD8 (Table 1).

Table 2. Mean diameter of clear zones in the biodegradation test of chlorpyrifos insecticide.

Isolates code	Mean diameter of clear zones in the observation at 24-96 hours (cm)			
	24 hours ($\bar{x}$ ± SD)	48 hours ($\bar{x}$ ± SD)	72 hours ($\bar{x}$ ± SD)	96 hours ($\bar{x}$ ± SD)
Control	0.00 ± 0.00 a	0,00 ± 0.00 a	0,00 ± 0.00 a	0,00 ± 0.00 a
LC1	0.00 ± 0.00 a	0,17 ± 0.15 ab	0.73 ± 0.05 def	0.95 ± 0.10 d
LC12	0.00 ± 0.00 a	0,50 ± 0.24 d	0.83 ± 0.13 ef	0.95 ± 0.24 d
LC13	0.00 ± 0.00 a	0,35 ± 0.17 bcd	0.70 ± 0.14 de	0.78 ± 0.10 d
DR3	0.00 ± 0.00 a	0,02 ± 0.05 a	0.08 ± 0.05 ab	0.33 ± 0.22 bc
DR7	0.00 ± 0.00 a	0,15 ± 0.10 ab	0.25 ± 0.17 bc	0.33 ± 0.22 bc
DR12	0.00 ± 0.00 a	0,23 ± 0.17 a	0.35 ± 0.29 c	0.48 ± 0.32 c
DR13	0.00 ± 0.00 a	0,45 ± 0.10 cd	0.68 ± 0.13 de	0.83 ± 0.10 d
GD3	0.00 ± 0.00 a	0 ± 0.00 a	0.05 ± 0.06 ab	0.15 ± 0.17 ab
GD7	0.00 ± 0.00 a	0,78 ± 0.10 e	0.95 ± 0.13 f	1.33 ± 0.10 e
GD8	0.00 ± 0.00 a	0,33 ± 0.26 bcd	0.58 ± 0.24 d	1.08 ± 0.26 de

Explanation: Numbers followed by different letters in the same column indicate significantly different results based on the Duncan test at a 5% error level. (SD = Standard Deviation)

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Observations were made at 24-96 hours. In the observation results, it was found that the clear zone in 9 treatment isolates grew at the 48-hour observation. The highest average diameter of the clear zone in the 48-hour, 72-hour, and 96-hour observations was in the GD7 isolate with respectively average diameters of 0.78 cm, 0.95 cm, and 1.33 cm. Meanwhile, the lowest result was in the GD3 isolate with average clear zone diameters in the 48-hour, 72-hour, and 96-hour observations of 0 cm, 0.05 cm, and 0.15 cm, respectively (Table 2). These average clear zone diameter results indicate significant differences in each observation compared to the control.

Table 3. Percentage of degradation of chlorpyrifos insecticide

Isolates code	Percentage of Degradation of Chlorpyrifos Insecticide (%)			
	24 hours	48 hours	72 hours	96 hours
Control	0.00	0.00	0.00	0.00
LC1	0.00	20.71	54.67	61.18
LC12	0.00	43.17	57.66	57.03
LC13	0.00	35.42	53.46	56.21
DR3	0.00	3.57	10.71	31.36
DR7	0.00	18.75	26.67	31.36
DR12	0.00	24.58	31.79	38.46
DR13	0.00	42.50	52.57	57.75
GD3	0.00	0.00	7.14	17.14
GD7	0.00	56.21	61.09	65.13
GD8	0.00	31.62	47.50	59.77

On the results of the percentage degradation of chlorpyrifos insecticide, the lowest percentage was obtained for treatment isolate GD3 with calculations at 24 hours, 48 hours, 72 hours, and 96 hours being 0%, 0%, 7.14%, 17.14% respectively. Meanwhile, the highest calculation results were for treatment isolate GD7 with results at 24 hours, 48 hours, 72 hours, and 96 hours being 0%, 56.21%, 61.09%, 65.13% respectively (Table 3). Based on the average diameter of the clear zone, according to the inhibition zone ability criteria by Rundengan et al. (2017) it is known that at the 96-hour observation, bacterial isolates LC1, LC12, LC13, DR3, DR7, DR12, DR13, and GD3 have a moderate inhibitory power. Isolates GD7 and GD8 have strong inhibitory power. The larger the diameter of the clear zone, the higher the ability of a bacterium to inhibit a substance. This statement is in line with the statement by Rahmawati et al. (2014), that the larger the clear zone formed, the greater its inhibitory ability.

Based on the statement by Ratnaningsih et al. (2020), microorganisms play a crucial role in reducing or degrading chemical active ingredients. Microorganisms degrade pesticides through the biodegradation process. According to Siddiqui et al. (2022), microbial digestion produces enzymes that detoxify pesticides such as pyrethroids, carbamates, diamides, and organochlorines. Some groups of bacteria from both gram-positive and gram-negative categories can degrade pesticides, one of which is chlorpyrifos.

According to Hadi et al. (2021), the ability of bacteria to degrade insecticides with chlorpyrifos as the active ingredient is by utilizing hydrocarbon compounds as an energy source for the bacteria. Chlorpyrifos belongs to hydrocarbon compounds, so bacteria that can degrade it make this compound their source of energy. The group of bacteria that can degrade insecticides more effectively is the Gram-Negative bacteria group compared to the Gram-Positive bacteria group. This is in line with

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the results of the biodegradation test, which identified bacterial isolates that could growth the clearest zones mainly belonged to the Gram-Negative bacteria genus.

In the identification results of bacterial isolates capable of degrading chlorpyrifos insecticide, several isolates were found with the genera Pantoea sp., Erwinia sp., and Coryneform sp. Based on previous research by Wiguna (2019), the Pantoea sp. genus was found to be able to degrade the insecticide with the active ingredient profenofos at concentrations of 0.75 ml/L, 1.5 ml/L, and 3 ml/L. In the study by Hariadi (2018), it was reported that Erwinia sp. bacteria are potentially bioremediators against the herbicide glyphosate. According to Dewi (2017), Erwinia sp. bacteria are also beneficial as bioremediation agents because they produce IAA (Indole-3-Acetic Acid) and dissolve phosphate. In the research by Khulillah et al. (2019), it was reported that Coryneform sp. bacteria could grow on mancozeb 50 ppm test media with a percentage of 41-80%. Coryneform sp. bacteria are gram-positive bacteria from the Actinobacteria group and are widely used in various industries. According to Fatuhrahman (2019), the Actinobacteria group of bacteria can produce various bioactive compounds that can be useful for bioremediation.

ra (García-Peñalvo et al., 2021). Changes in the form of learning require teachers to change their methods and evaluation tools (Sutarto et al., 2020). However, even though it is difficult, students must be evaluated continuously with various online assessment activities (García-Peñalvo et al., 2021).

Characterization of Symbiotic Bacteria Degrading Chlorpyrifos Insecticide

The Bacteria isolates capable of degrading chlorpyrifos insecticide in contaminated media were further identified up to the genus level. Bacterial characterization was performed to identify bacteria at the genus level through steps such as morphological characterization and physiological and biochemical characterization. Morphological characterization was conducted by identifying macroscopically, including colony shape, elevation, colony color, edge, and cell shape observed under a microscope. Additionally, physiological, and biochemical characterization was carried out following the identification methods of Bergey's Manual of Determinative Bacteriology (Holt et al., 1994) and Schaad et al. (2001), which include gram staining, KOH 3% test, catalase test, endospore test, Oxidative-Fermentative test, and testing on YDC media.

Morphological Characterization

Morphological characterization of bacterial isolates was conducted by macroscopically identifying individual colonies on NA media. The observations included colony shape, elevation, colony color, and colony edge. In the morphological characterization, the results showed that the 10 identified bacterial isolates had a round shape, convex elevation, and a smooth edge. Isolates LC1, LC8 had a brownish-white color, isolate LC12 had a yellowish-white color, isolate GD7 had a slightly creamy white color, isolates LC13, DR3, DR7, and GD3 had a colony color of milky white, while isolates DR12 and DR13 had a colony color of yellow (Figure 3).

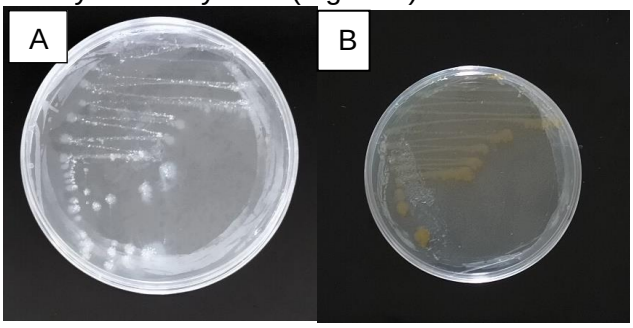

Figure 3. Single Bacterial Colonies on NA Media: (a) Single bacterial colony with yellow color, (b) Single bacterial colony with white color.

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Physiological and Biochemical Characterization

Physiological and biochemical characterization was conducted based on the identification methods outlined in Bergey's Manual of Determinative Bacteriology (Holt et al., 1994) and Schaad et al. (2001). The initial stages involved gram testing and 3% KOH testing. Isolates showing a red color in the gram staining test and the presence of mucus in the 3% KOH test were classified as gram-negative bacteria. If the gram staining test showed a bluish-purple color and no mucus was present in the 3% KOH test, the bacteria were classified as gram-positive. Subsequently, gram-negative bacteria underwent Oxidative-Fermentative testing and growth testing on YDC media. Conversely, gram-positive bacteria underwent endospore staining and catalase testing (Table 4).

Table 4. Results of Morphological, Physiological, and Biochemical Characterization of Symbiotic Bacteria

Morphological Characterization						Physiological, and Biochemical Characterization						
Isolates Code	Shape	Elevation	Color	Edge	Cell shape	Gram Staining	Uji KOH 3%	Katalase Test	Endospore	OF Test	YDC Test	Genus
LC1	Round	Convex	brownish white	Flat	Rod-Shaped	Red (-)	slimy (-)	NT	NT	Fermentative	White	*Erwinia* sp.
LC12	Round	Convex	yellowish white	Flat	Rod-Shaped	Red (-)	slimy (-)	NT	NT	Fermentative	White	*Erwinia* sp.
LC13	Round	Convex	milky white	Flat	Rod-Shaped	Red (-)	slimy (-)	NT	NT	Fermentative	White	*Erwinia* sp.
DR3	Round	Convex	milky white	Flat	Rod-Shaped	Red (-)	slimy (-)	NT	NT	Fermentative	White	*Erwinia* sp.
DR7	Round	Convex	milky white	Flat	Rod-Shaped	Red (-)	slimy (-)	NT	NT	Fermentative	White	*Erwinia* sp.
DR12	Round	Convex	Yellow	Flat	Rod-Shaped	Red (-)	slimy (-)	NT	NT	Fermentative	Yellow	*Pantoea* sp.
DR13	Round	Convex	Yellow	Flat	Rod-Shaped	Red (-)	slimy (-)	NT	NT	Fermentative	Yellow	*Pantoea* sp.
GD3	Round	Convex	milky white	Flat	Rod-Shaped	Red (-)	slimy (-)	NT	NT	Fermentative	White	*Erwinia* sp.
GD7	Round	Flat	Creamy white	Flat	Rod-Shaped	Blue (+)	not slimy (+)	Positive	Negative	NT	NT	*Corynef orm* sp.
GD8	Round	Convex	brownish white	Flat	Rod-Shaped	Red (-)	slimy (-)	NT	NT	Fermentative	White	*Erwinia* sp.

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Conclusion

The results of the research conducted to obtain symbiotic bacteria in S. exigua larvae can be summarized as follows:

1. Exploration of symbiotic bacteria in the midgut of S. exigua in shallot fields in Leces Subdistrict, Dringu Subdistrict, and Gending Subdistrict, Probolinggo Regency, yielded 41 bacterial isolates capable of surviving on NA media contaminated with the insecticide chlorpyrifos.
2. Testing the biodegradation of chlorpyrifos insecticide on 41 bacterial isolates resulted in 10 isolates capable of producing clear zones, namely LC1, LC12, LC13, DR3, DR7, DR12, DR13, GD3, GD7, and GD8.
3. The identification of the 10 bacterial isolates with the ability to degrade chlorpyrifos insecticide revealed that 7 isolates belong to the genus Erwinia sp., 2 isolates to the genus Pantoea sp., and 1 isolate to the genus Coryneform sp.

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