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Exploration of Symbiotic Bacteria with Potential as Degraders of Cypermethrin Insecticide in the Digestive Tract of Onion Caterpillar *Spodoptera exigua* (Lepidoptera: Noctuidae) in Kediri Regency

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ABSTRAK

Ulat bawang (Spodoptera exigua Hubner) merupakan salah satu hama utama pada tanaman bawang merah yang menyebabkan kerusakan signifikan dengan memakan bagian dalam daun dan melubangi ujung daun. Di Kecamatan Badas, Plemahan, dan Kepung, pengendalian hama ini dilakukan secara intensif menggunakan insektisida sipermetrin, yang telah memicu resistensi pada S. exigua. Resistensi ini diduga dipengaruhi oleh keberadaan bakteri simbion dalam saluran pencernaan ulat yang mampu mendegradasi bahan aktif insektisida sipermetrin. Penelitian ini bertujuan untuk mengeksplorasi, mengidentifikasi, dan mengetahui potensi bakteri simbion dalam mendegradasi sipermetrin pada saluran pencernaan S. exigua di Kabupaten Kediri. Penelitian dilakukan di tiga kecamatan di Kabupaten Kediri dan Laboratorium Toksikologi Tanaman, Fakultas Pertanian Universitas Brawijaya, menggunakan metode Rancangan Acak Lengkap (RAL) untuk menguji kemampuan bakteri simbion dalam mendegradasi sipermetrin. Hasil penelitian menunjukkan bahwa terdapat 26 isolat bakteri dari saluran pencernaan S. exigua yang berhasil diisolasi, dengan 14 isolat menunjukkan kemampuan mendegradasi sipermetrin. Dua isolat terbaik, B7 dan P6, yang termasuk dalam genus Erwinia, diduga mampu mendegradasi insektisida sipermetrin, dengan masing-masing menghasilkan rerata zona bening 1,025 cm dan 1,2 cm. Identifikasi lebih lanjut mengungkapkan bahwa isolat tersebut terdiri dari 9 isolat genus Pantoea, 4 isolat genus Erwinia, dan 1 isolat genus Clostridium.

Kata kunci: *Spodoptera exigua*, Bakteri simbion, Sipermetrin, Resistensi, Degradasi insektisida

ABSTRACT

Onion caterpillar (*Spodoptera exigua* Hubner) is one of the major pests of onion plants, causing significant damage by consuming the inner parts of the leaves and perforating the leaf tips. In the Badas, Plemahan, and Kepung sub-districts, control of this pest is intensively carried out using the insecticide cypermethrin, which has led to resistance in *S. exigua*. This resistance is suspected to be influenced by the presence of symbiotic bacteria in the caterpillar's digestive tract that can degrade the active ingredient of the insecticide cypermethrin. This study aims to explore, identify, and determine the potential of symbiotic bacteria in degrading cypermethrin in the digestive tract of *S. exigua* in Kediri Regency. The research was conducted in three sub-districts in Kediri Regency and at the Plant Toxicology Laboratory, Faculty of Agriculture, Brawijaya University, using a Completely Randomized Design (CRD) method to test the ability of symbiotic bacteria to degrade cypermethrin. The results showed that 26 bacterial strains were successfully isolated from the digestive tract of *S. exigua*, of which 14 isolates showed the ability to degrade cypermethrin. The two best isolates, B7 and P6, belonging to the genus Erwinia, are suspected to be capable of degrading the insecticide cypermethrin, producing average clear zones of 1.025 cm and 1.2 cm, respectively. Further identification revealed that the isolates consisted of 9 isolates of the genus *Pantoea*, 4 isolates of the genus *Erwinia*, and 1 isolate of the genus *Clostridium*.

Keywords: *Spodoptera exigua*, symbiotic bacteria, cypermethrin, resistance, insecticide degradation

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1. INTRODUCTION

One of the main pests of shallots is the beet armyworm (*Spodoptera exigua* Hübner), which attacks plants quickly, simultaneously, and over a large area. This caterpillar is active at night and hides in the crevices of the leaf sheaths during the day. *S.*

exigua is known as a polyphagous pest that also attacks crops such as chili, cabbage, tomato, spinach, cotton, corn, tobacco, and soybeans (Y. M. Sari et al., 2017). A yield loss of up to 42% can occur if there are three to five larvae per cluster of shallot plants (Memah & Kaligis, 2016). The potential decline in shallot production in East Java during May – June 2022 reached 23.5% due to this pest (Puryantoro & Wardiyanto, 2022).

The control of *S. exigua* is generally carried out using synthetic insecticides because they are considered fast, efficient, and effective by farmers. In West Java, the cost of purchasing insecticides by tomato farmers reaches 50% of the total expenses incurred (Setiawati et al., 2001, in Endah Retnani & Oktaviasari, 2016). Dependence on insecticides triggers various negative impacts such as pest resistance, reduced biodiversity, and the death of natural enemies of the pests. Other impacts include resurgence, the death of non-target insects, secondary pest outbreaks, and pesticide residues (Jayanti & Suprapta, 2020). Therefore, a more sustainable approach to pest control is needed.

Kediri Regency is one of the largest shallot production centers in East Java, with Badas, Plemahan, and Kepung sub-districts being the highest production areas (BPS East Java, 2019; BPS Kediri Regency, 2021). Farmers in this area intensively use cypermethrin insecticides to control *S. exigua*. Cypermethrin is a pyrethroid insecticide that acts as a stomach poison and is effective in killing target pests (Furlong et al., 2008). This insecticide is applied every 2-3 days, almost daily during the rainy season. The excessive use of insecticides increases the risk of resistance in *S. exigua*.

Resistance of *S. exigua* to insecticides, such as cypermethrin, has been reported in several areas, including China (Che et al., 2013). Factors influencing resistance include selection pressure, type of insecticide, application frequency, and biological aspects such as pest behavior and symbiotic bacteria (Astuti et al., 2014). Symbiotic bacteria in the digestive tract of pests can degrade insecticide toxins, accelerating resistance development (Hadi et al., 2021). Several bacterial genera, including *Providencia* sp., *Pseudomonas* sp., and *Enterobacter* sp., have been reported to degrade certain insecticides (Kusiyanto et al., 2019). However, research specifically identifying and characterizing the role of symbiotic bacteria in *S. exigua* remains limited. The gut microbiota of *S. exigua* may harbor bacterial species that contribute to insecticide detoxification, yet their metabolic pathways and genetic mechanisms remain unclear. Given the geographical variations in insecticide resistance and the potential role of microbial communities, further investigation is needed to identify specific bacterial strains and their impact on resistance. Understanding this relationship could offer new insights into microbial-based resistance management strategies, contributing to more sustainable pest control approaches.

This research aims to explore symbiotic bacteria in the digestive tract of *S. exigua* that have the potential to degrade cypermethrin, assess their ability to break down the insecticide, and identify specific bacterial species that could contribute to sustainable pest management. By understanding the role of these bacteria in insecticide resistance, this study seeks to provide innovative solutions to mitigate resistance caused by excessive insecticide use. Ultimately, the findings are expected to support the development of more effective and environmentally friendly pest control strategies.

2. RESEARCH METHODOLOGY

2.1. Time and Location

This research was conducted in two phases from December 2022 to June 2023. The first phase involved field surveys and sampling in the sub-districts of Badas, Plemahan, and Kepung, Kediri Regency. The second phase was conducted at the Plant Toxicology Laboratory, Faculty of Agriculture, Brawijaya University, for resistant larvae selection, bacterial isolation, and in vitro bioassay.

Step 1: Sampling of *S. exigua* Larvae

Field surveys were conducted to collect *S. exigua* larvae. GPS devices were used to determine sample coordinates. Larvae were placed in perforated plastic boxes lined with gauze for air circulation.

Step 2: Resistant Larvae Selection

Collected larvae were fed with food soaked in cypermethrin insecticide. Larvae that survived were identified as resistant and selected for further bacterial isolation.

Step 3: Bacterial Isolation

The digestive tracts of resistant larvae were extracted to isolate symbiotic bacteria. The spread plate method was used on Nutrient Agar media to obtain single bacterial colonies.

Step 4: Cypermethrin Degradation Test

The cypermethrin degradation ability of isolated bacteria was tested using a modified method of Borkar (2017). Media mixed with cypermethrin and congo red were incubated, and bacterial degradation ability was determined by measuring clear zone diameters around the filter paper.

Step 5: Morphological and Physiological Characterization

Morphological and physiological tests, including morphological characteristics such as shape, color, edge, and elevation. Gram staining, oxidative-fermentative, YDC media test, endospore staining, and 3% KOH solubility tests, were conducted. The results were interpreted based on Schaad et al. (2001).

Step 6: Data Analysis

A Completely Randomized Design (CRD) was applied with multiple treatments and replications.

Data were analyzed using ANOVA, and if significant differences were found, Duncan's Multiple Range Test (DMRT) was performed at a 5% error level.

3. RESULTS AND DISCUSSION

3.1. Isolation and Purification of Symbiotic Bacteria in the Digestive Tract of *S. exigua*

The research began by isolating bacteria from the digestive tract of *S. exigua* collected from the Badas, Kepung, and Plemahan sub-districts in Kediri, East Java, using a 10^{-1} dilution method, followed by culturing on NA media (Figure 1). The isolated bacteria were purified based on distinct morphological characteristics such as shape, color, edge, and elevation (Figure 2), resulting in 26 bacterial isolates—10 from Badas, 5 from Kepung, and 11 from Plemahan (Table 1). Symbiotic bacteria, known for their beneficial interactions with their hosts, aid in digestion within the host's digestive tract. Cellulase-producing bacteria, like those found in the digestive systems of plant-eating insects, can enhance digestive processes, as seen in *Bombyx mori* larvae (Sari et al., 2013). In *S. exigua*, these symbiotic bacteria are believed to help degrade pesticide active ingredients, highlighting their potential role in pesticide degradation.

Figure 1. Bacterial Isolation Results

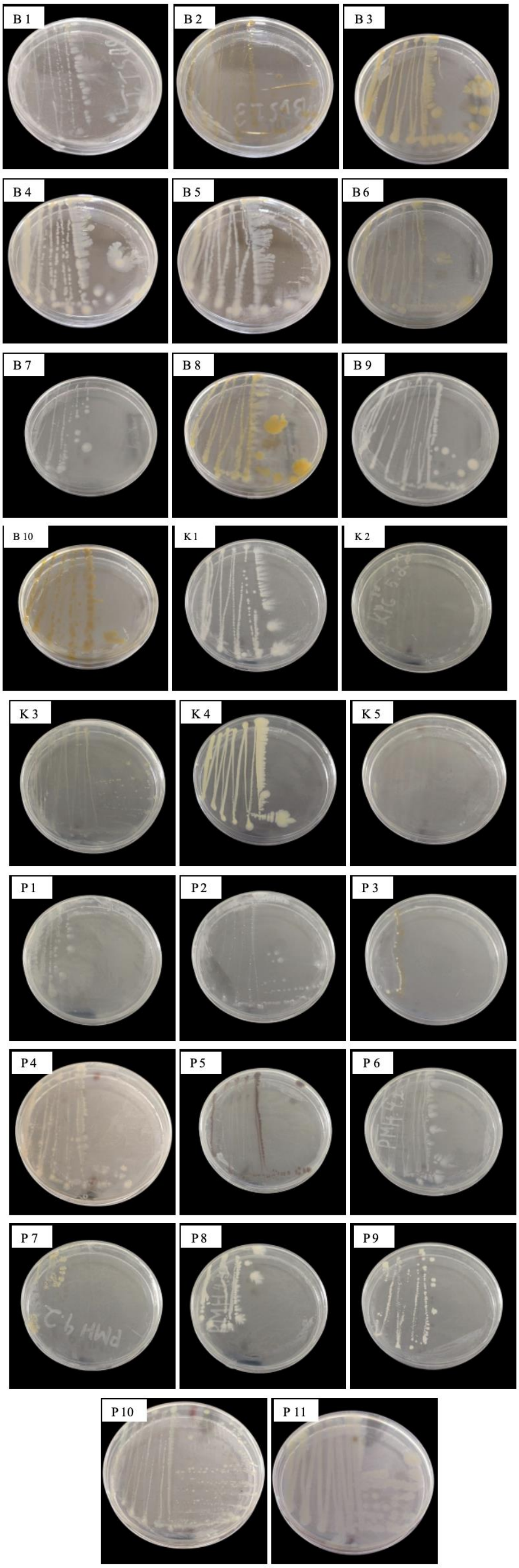

Figure 2. Bacterial Purification Results

3.2. Testing the Ability of Symbiotic Bacteria to Degrade Cypermethrin in the Digestive Tract of *S. exigua*

The results of testing the ability of symbiotic bacteria to degrade cypermethrin-based insecticides on 26 isolates, with four repetitions at the recommended cypermethrin dose of 1.5 ml/L, showed a highly significant effect according to variance analysis (ANOVA) with a 5% error rate. This indicates that the treatment significantly influenced the diameter of the clear zone produced by each bacterial isolate. Over the 96-hour observation period, all isolates were able to grow in cypermethrin-contaminated media. However, not all isolates were able to degrade the insecticide, although a clear zone was formed by most isolates (Table 2). During the 24 to 96-hour observations, isolate P 6 showed the highest results in several periods, while isolate P 11 showed the lowest clear zone diameters.

In addition to clear zone formation, the percentage of cypermethrin degradation also varied among the 14 bacterial isolates over the 24-96 hour observation period (Table 3). At 24 hours, isolate B 7 showed the highest degradation percentage at 47.83%, while P 6 had the lowest at 4%. By 96 hours, P 6 showed a significant increase, reaching a maximum degradation percentage of 66.67%, while P 3 recorded the lowest at 36.84%. Based on the test results, isolates B 7 and P 6 are suspected to have the best ability to degrade cypermethrin (Figure 3). Differences in the effectiveness of the bacteria in degrading cypermethrin are likely influenced by each isolate's ability to produce enzymes that break down the chemical bonds of cypermethrin into simpler compounds (Rori et al., 2020).

Table 1. Results of Bacterial Purification

Sample Origin	Isolate Code	Sample Origin	Isolate Code	Sample Origin	Isolate Code
Badas	B 1 B 2 B 3 B 4 B 5 B 6 B 7 B 8 B 9 B 10	Kepung	K 1 K 2 K 3 K 4 K 5	Plemahan	P 1 P 2 P 3 P 4 P 5 P 6 P 7 P 8 P 9 P 10 P 11

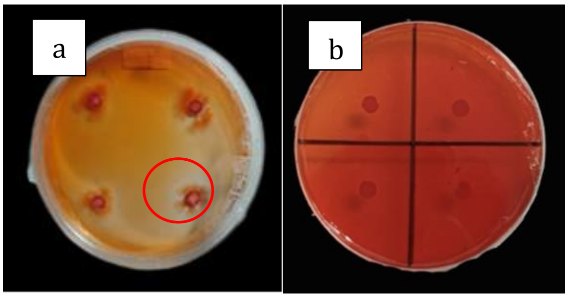

Figure 3. a) Bacterial Isolate with the Highest Average Clear Zone b) Control (Congo Red Media without Clear Zone)

Table 2. Average Diameter of the Clear Zone

Treatment	Average diameter of the clear zone at a cypermethrin dose of 1.5 ml/L (cm)			
	24 Hour	48 Hour	72 Hour	96 Hour
Control	0 a	0 a	0 a	0 a
B 1	0 a	0 ab	0 a	0,975 cde
B 2	0,475 cd	0,5 cd	0,675 bc	0,725 cde
B 3	0,45 bcd	0,6 d	0,7 bc	0,825 cde
B 5	0,475 cd	0,55 d	0,75 c	0,85 cde
B 7	0,55 d	0,65 d	0,95 c	1,025 de
B 8	0 a	0 a	0,85 c	0,925 cde
B 10	0,525 cd	0,625 d	0,7 bc	0,75 cde
K 1	0 a	0 ab	0 a	0,825 cde
K 2	0,25 abcd	0,325 bcd	0,55 bc	0,6 bcd
K 5	0,525 cd	0,675 d	0,75 c	0,825 cde
P 3	0,325 bcd	0,4 cd	0,5 bc	0,5 bc
P 4	0,225 abc	0,55 d	0,675 bc	0,75 cde
P 6	0,325 bcd	0,35 cd	0,5 bc	1,2 e
P 11	0,15 ab	0,175 abc	0,225 ab	0,225 ab

Note: Numbers followed by the same letter in the same column indicate no significant difference based on Duncan's test at a 5% error level

Table 3. Percentage of Cypermethrin Degradation

Treatment	Percentage of cypermethrin degradation (%)			
	24 Hour	48 Hour	72 Hour	96 Hour
Control	0	0	0	0
B 1	0	0	0	61.90
B 2	35.14	36.84	46.67	48.94
B 3	42.86	50.00	53.85	57.89
B 5	44.19	47.83	55.56	58.62
B 7	47.83	52.00	61.29	63.08
B 8	0	0	58.62	60.66
B 10	46.67	51.02	53.85	55.56
K 1	0	0	0	52.94
K 2	0	4.00	29.41	52.94
K 5	46.67	52.94	55.56	57.89
P 3	22.58	29.41	36.84	36.84
P 4	27.27	47.83	52.94	55.56
P 6	4.00	7.69	25.00	66.67
P 11	0	0	0	0

3.3. Characterization of Symbiotic Bacteria Degrading Cypermethrin in the Digestive Tract of *S. exigua*

A total of 14 bacterial isolates that produced clear zones on contaminated media have been identified to the genus level. The characterization process was conducted in two stages: morphological characterization and physiological and biochemical characterization. Morphological characterization involved observing the shape, edges, elevation, and color of single bacterial colonies (Table 4). The majority of the bacterial isolates were circular in shape, with 8 isolates, followed by irregular shapes with 5 isolates, and filamentous with 1 isolate. The edges of the bacterial isolates were mostly entire, with 10 isolates, while the others showed undulate, erose, and filamentous edges. After completing the morphological characterization, the bacterial isolates were further tested through physiological and biochemical characterization.

Physiological and biochemical characterization followed the methods outlined in Bergey's Manual of Determinative Bacteriology (1994) and Schaad et al (2001). The tests conducted included Gram staining, the 3% KOH test, endospore staining, oxidative-fermentative tests, and growth testing on YDC media. Of the 14 isolates, 13 were Gram-negative, and 1 isolated was Gram-positive (Table 5). The Gram-positive bacterial isolate showed the presence of spores after spore staining. All isolates exhibited fermentative characteristics in the oxidative-fermentative test. The next step was to further classify the bacteria based on the Gram staining test, which revealed fundamental differences in the structure of the bacterial cell walls.

The Gram staining test was conducted to classify the isolates as either Gram-positive or Gram-negative (Rahmatullah et al., 2021). Gram-negative bacteria appeared red under the microscope, while Gram-positive bacteria appeared blue (Figure 4). Additionally, the 3% KOH test was used to detect slime in Gram-negative bacteria, with 13 isolates showing slime presence (Figure 5). The P 4 isolate, which was Gram-positive, produced spores in the spore staining test (Figure 6). The results indicated that the Gram-positive bacteria belonged to the genus Clostridium (Holt et al., 1994 & Schaad et al., 2001). After determining the Gram status of each isolate, further testing was conducted using YDC media to identify the genus of the bacterial isolates.

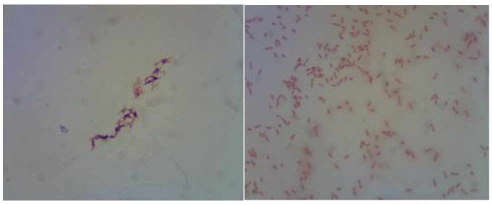

Figure 4. Gram Test Results: Gram-Positive Appears Blue in Isolate P 4 & Gram-Negative Appears Red in Isolate P 1

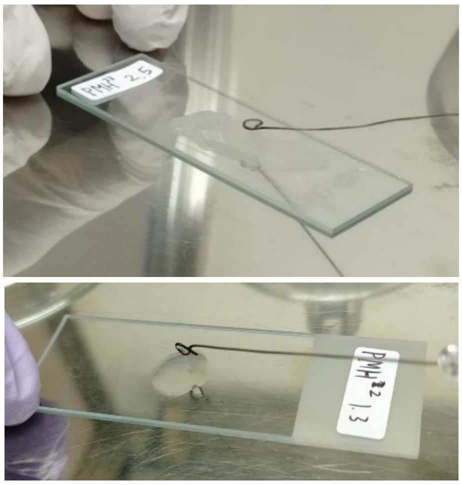

Figure 5. Gram Test Results: Gram-Positive Appears Blue in Isolate P 4 & Gram-Negative Appears Red in Isolate P 1

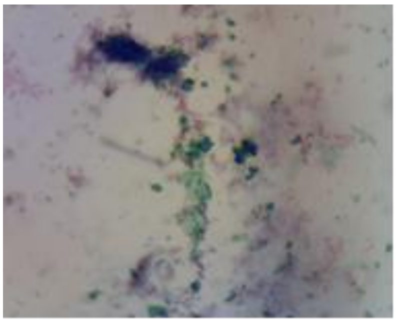

Figure 6. Positive Spore Staining Result in Isolate P

Table 4. The results of the Morphological Characterization of Cypermethrin-Degrading Bacteria

Isolate Code	Shape	Edge	Elevation	Color
B 1	Irregular	Undulate	Flat	Cloudy White
B 2	Circular	Flat	Convex	Yellow
B 3	Circular	Flat	Umbonate	Yellow
B 5	Irregular	Undulate	Raised	Milky White
B 7	Circular	Flat	Umbonate	Cloudy White
B 8	Circular	Flat	Umbonate	Yellow
B 10	Irregular	Flat	Convex	Yellow
K 1	Circular	Flat	Umbonate	Cloudy White
K 2	Circular	Flat	Raised	Yellow
K 5	Circular	Flat	Flat	Yellow
P 3	Irregular	Flat	Flat	Yellow
P 4	Irregular	Undulate	Flat	Milky White
P 6	Circular	Flat	Convex	Cloudy White
P 11	Circular	Flat	Convex	Yellow

Table 5. Physiological and Biochemical Characterization

Isolate Code	Cell Shape	Gram Staining	3% KOH Test	Endospore	OF Test	YDC Test
B 1	Rod	Red	Slimy	Not Tested	Fermentative	White
B 2	Rod	Red	Slimy	Not Tested	Fermentative	Yellow
B 3	Coccus	Red	Slimy	Not Tested	Fermentative	Yellow
B 5	Rod	Red	Slimy	Not Tested	Fermentative	Yellow
B 7	Rod	Red	Slimy	Not Tested	Fermentative	White
B 8	Rod	Red	Slimy	Not Tested	Fermentative	Yellow
B 10	Rod	Red	Slimy	Not Tested	Fermentative	Yellow
K 1	Rod	Red	Slimy	Not Tested	Fermentative	White
K 2	Coccus	Red	Slimy	Not Tested	Fermentative	Yellow
K 5	Rod	Red	Slimy	Not Tested	Fermentative	Yellow
P 3	Rod	Red	Slimy	Not Tested	Fermentative	Yellow
P 4	Rod	Blue	Non-slimy	Positive	Oxidative	Not Tested
P 6	Rod	Red	Slimy	Not Tested	Fermentative	White
P 11	Rod	Red	Slimy	Not Tested	Fermentative	Yellow

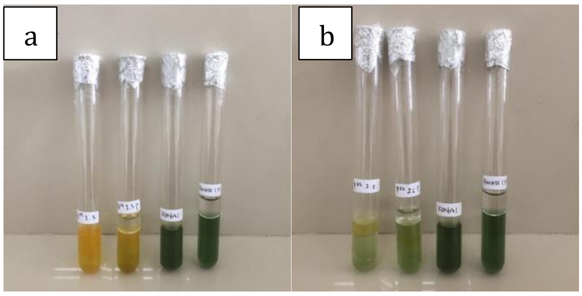

Figure 7. a) Fermentative Reaction on Isolate B 2, b) Oxidative Reaction on Isolate P 4

Based on the results of the oxidative-fermentative test, 13 test isolates were found to be anaerobic (fermentative), as indicated by a color change in the growth medium from its initial greenish blue to yellow in both the medium with added paraffin and the medium without paraffin (Figure 7). The test results showed that one isolate, P4, was aerobic (oxidative), as evidenced by the absence of a color change, indicating that the bacterium exhibited oxidative characteristics (Yuka *et al*., 2021).

The YDC media test was used to classify the bacteria into the genera Erwinia and Pantoea. Bacteria that grew with white colonies were categorized into the genus Erwinia, while yellow colonies were classified into the genus Pantoea Schaad et al. (2001). Four isolates showed white colony growth, while nine isolates produced yellow colonies. Isolates B 1, B 7, K 1, and P 6 were classified into the genus Erwinia, while the other isolates belonged to the genus Pantoea (Figure 8). The genus identification results obtained using YDC media paved the way for understanding the potential of these bacteria in pesticide degradation.

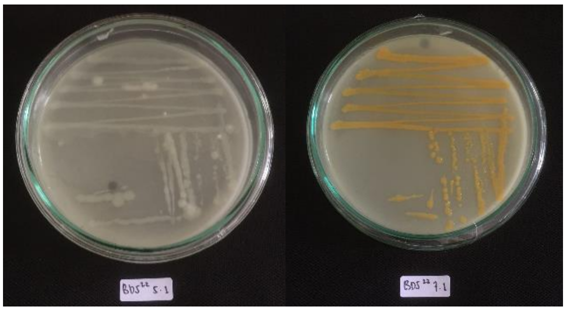

Figure 8. Isolate B 7 with White Colonies & Isolate B 10 with Yellow Colonies

Based on Bergey's Manual of Determinative Bacteriology (1994) and Schaad et al (2001) The identification results indicated that the 14 bacterial isolates belonged to three genera: Pantoea, Erwinia, and Clostridium (Table 6). The genus Pantoea has the ability to degrade insecticides with active ingredients such as phosphoric acid and pyrethroids. The genus Erwinia is also capable of degrading various complex pesticide molecules, including cypermethrin and other insecticides (Ramya et al., 2016). The genus Clostridium, which is anaerobic, is involved in the fermentation and decomposition of organic matter and has the potential to degrade organochlorine pesticides (Umadevi et al., 2017). These three genera were found in the digestive tract of *Spodoptera exigua* and have the potential to degrade pesticide active ingredients in contaminated environments.

Table 6. Identification Results of Cypermethrin-Degrading Symbiotic Bacteria

District	Genus	Number of Isolates	Isolate Code
Badas	*Pantoea*	5	B 2, B 3, B 5, B 8 & B 10
	Erwinia	2	B 1 & B 7
Kepung	*Pantoea*	2	K 2 & K 5
	Erwinia	1	K 1
Plemahan	Pantoea	2	P 3 & P 11
	Erwinia	1	P 6
	Clostridium	1	P 4

4. CONCLUSION

Research on symbiotic bacteria capable of degrading cypermethrin in the digestive tract of *S. exigua* resulted in 26 bacterial isolates that were able to survive in media contaminated with the insecticide containing cypermethrin as its active ingredient. In degradation capability tests, 14 bacterial isolates exhibited clear zones after 96 hours of observation. Further identification revealed that 9 bacterial isolates belonged to the genus Pantoea, 4 isolates to the genus Erwinia, and 1 isolate to the genus Clostridium These results confirm that these bacteria have potential as bioremediation agents for cypermethrin. This finding also provides valuable insights into the role of symbiotic bacteria in pesticide degradation within insect pests.

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