THE POTENTIAL OF THERMOPHILIC CHITINOLYTIC BACTERIA IE JUE, ACEH, INDONESIA AS LARVICIDE OF *Helicoverpa armigera* (HÜBNER)

Lenni Fitri*, Deby Liestia Anggi &Yekki Yasmin
Department of Biology,
Faculty of Mathematics and Natural Sciences,
Universitas Syiah Kuala, Banda Aceh,
Aceh province, Indonesia
*Corresponding author: *lennifitri@unsyiah.ac.id*

ABSTRACT

This study aims to determine the potential of local isolate thermophilic chitinolytic bacteria as larvicide of *Helicoverpa armigera* (Hübner). The bacteria that have been isolated from hot springs Ie Jue, Aceh Besar, were then characterized morphologically and tested for their larvicidal potential against the larvae of *H. armigera*. The parameters observed include the number of chitinolytic bacterial isolates as well as their morphological characteristics, the number of dead larvae, the number of larvae developing into pupae, and morphological characteristics of the larvae before and after the treatment with chitinolytic bacteria. The results of morphological characterization showed that the isolates obtained were seven Gram-positive isolates and four Gram-negative isolates, as well as nine isolates in the form of bacilli, while one isolate of diplococci and diplobasil were each. The highest ability of isolate in causing larval mortality was shown by IBK1, which killed approximately 2.67 (67%) individuals of the larvae, while IBK6 and IBK9 showed the lowest ability in causing larval mortality with average larval mortality of 0.33 (8%) individuals. On the other hand, the number of larvae that are able to develop into pupae is mostly found in IBK 6 and IBK 9, while the fewest are found in IBK 1. After the treatment with the bacteria, the morphology of the larvae body was found to be brown to black, soft, runny, crushed, and smelly. The potential use as larvicides to control *H. armigera* larvae has been proven in this study that isolated from thermophilic chitinolytic bacteria.

Keywords: chitinolytic bacteria, *Helicoverpa armigera* (Hübner), thermophiles, Ie Jue

ABSTRAK

Kajian ini bertujuan untuk menentukan potensi pemencilan bakteria kitinolitik termofil tempatan sebagai larvisid terhadap *Helicoverpa armigera* (Hübner). Bakteria yang telah dipencilkan dari Kolam Air Panas, Ie Jue, Aceh Besar, Indonesia kemudian dicirikan secara morfologi dan telah diuji potensi larvasid terhadap larva *H. armigera*. Parameter yang dikaji adalah jumlah pencilan bakteria kitinolitik dan ciri morfologi, jumlah larva mati, jumlah larva yang mampu menjadi pupa dan morfologi larva sebelum dan selepas rawatan. Hasil pencirian morfologi menunjukkan bahawa pencilan yang diperoleh adalah tujuh pencilan Gram-positif dan empat pencilan Gram-negatif, serta sembilan pencilan dalam bentuk bacilli, sementara

masing-masing satu pencilan diplococcus dan diplobasil. Keupayaan tertinggi pencilan untuk menyebabkan kematian larva ditunjukkan oleh IBK1 yang membunuh sekitar 2.67 (67%) individu, sementara kemampuan terendah untuk menyebabkan kematian larva ditunjukkan oleh IBK6 dan IBK9 dengan rata-rata kematian larva 0.33 (8%) individu. Morfologi larva setelah rawatan menunjukkan bahawa badan berwarna coklat hingga hitam, badannya lembut, berair dan hancur serta berbau busuk. Potensi larvisid untuk mengawal larva *H. armigera* telah dibuktikan dalam kajian ini yang dipencilkan dari bakteria kitinolitik termofil.

Kata kunci: bakteria kitinolitik, *Helicoverpa armigera* (Hübner)*,* termofil, Ie Jue

INTRODUCTION

Helicoverpa armigera (Hübner) is a polyphagous insect. During the larval stage, this insect is a pest of more than 60 species of cultivated plants and wild plants (Czepak et al. 2013). Plants that host *H. armigera* larvae include tobacco, maize, sorghum (wheat-maize), cotton, hemp, potato, jatropha, nuts, vegetables, and ornamental plants (Kalshoven 1981). The attack of *H. armigera* larvae can cause damage to the host plant. The damage caused by *H. armigera* attacks on tomato plants reached 80% and soybean pods could reach 35.50% (Herlinda 2005).

The nature of the polyphagous possessed by *H. armigera* and the unavailability of varieties that are resistant to these pests causes the control practices carried out to date are still dependent on the use of insecticides (Adnan & Handayani 2010). Using insecticides for a long time to deal with pest attacks can cause resistance and environmental pollution. To anticipate the negative impact of these insecticides, it is necessary to utilize the natural enemies of insect pests that are available in nature.

Biological control is a control strategy that is considered the most appropriate and effective in suppressing the growth of insect pests (Priyatno et al. 2011). One way of biological control is to use pathogenic microbes which are agents of natural enemies, including chitinolytic bacteria. Chitinolytic bacteria produce the chitinase enzyme to break down chitin substances (Yuniarti & Blondine 2005). Examples of chitinolytic bacteria that have been identified are *Bacillus circulans, Streptomyces lividans, Aeromonas* sp. and *Serratia marcescens* (Liu et al. 2015). Research from Thamthiankul et al. (2001) stated that chitinase (chiA71) from *B. thuringiensis* identified as exochitinase was reported to cause the mortality of *Aedes aegypti* larvae. Other studies reported that the chitinolytic activity produced by *S. marcescens* caused mortality in larvae of *Spodoptera litura* (Krishanti et al. 2017). Ardani et al. (2012) succeeded in isolating chitinolytic bacteria from hot springs Ie Seuum and having the ability to cause the mortality of *Aedes aegypti* L. chitinolytic as larvicides against *H. armigera* (Hübner) larvae. Thus, the aim of this study is to obtain chitinolytic bacterial isolates from hot springs Ie Jue, Aceh Besar, and to determine the potential of chitinolytic bacteria as larvicides against *H. armigera* larvae.

MATERIALS AND METHODS

Materials

The materials used in this study were water from Ie Jue hot springs, Aceh Besar, Indonesia as a source of isolates, *Helicoverpa armigera* (Hübner) larvae, corn, honey, plastic cups (4 cm x 5 cm), plastic containers (18 cm x 18 cm), 70% alcohol, 96% alcohol, methylene blue, safranin, Lugol, immersion oil, sterile gauze, physiological NaCl solution, shrimp shells, Nutrient Broth, $MgSO_4.7H_2O$, K_2HPO_4, yeast extract, agar, 2M HCl, and 10M NaOH.

Sampling Area and Water Sampling

The water sampling was carried out using Ginting (2009) method. Before the water samples were taken, the physical and chemical parameters were measured in the field. The first parameter to be measured is the water temperature at each sampling point using a thermometer immersed for 3 minutes into each sampling source. The second parameter is the pH of the water at each sampling point which is measured by a universal pH indicator by dipping it into the surface of the water. Then, the color obtained is matched with the pH table listed on the universal pH box. Water samples were taken from the hot spring pool as much as 200 ml from each point of collection and then put into a sterilized thermos so that the water temperature could be maintained and labeled. The samples were then taken to the Microbiology Laboratory, Department of Mathematics and Natural Sciences, Syiah Kuala University.

Chitinolytic bacteria were isolated from hot spring Ie Jue in the Lam Teuba area, Aceh Besar, Indonesia. Samples were taken from hot springs at four different points (Figure 1). Each sample point was repeated for three times. Water samples are taken using a long dipper that has been sterilized using 70% alcohol, then put into a hot water flask and tightly closed. There are four sampling locations, point 1 at 5º30.246 N and 95º37.442E. Point 2 at 5º30.243 N and 95º37.445 E, point 3 at 5º30.240 N and 95º37.452 E, point 4 at 5º30.246 N and 95º37.442 E. Seulawah Agam is one of Indonesia's geothermal fields which has several manifestation zones, including the northern zone of Ie Jue. Manifestations in the Ie Ju area include warm soil, steaming earth, hot springs, fumaroles, and puddles mud (Wangsa & Ismail 2018).

Preparation of Colloidal Chitin

The sample used for chitin preparation was shrimp (*Litopenaeus vannamei*) shell waste. The stacked shrimp shells are clean and dried in the sun for one day. The dried shrimp skin is then ground into a fine powder. Twenty grams of chitin were dissolved in 400 mL of concentrated HCl and stored in a refrigerator at 4ºC for 24 hours. The solution was filtered later 200 mL of cold distilled water was added to the precipitate formed. The residue is taken, and cold distilled water is added. The solution was then neutralized by adding 10 M NaOH to reach pH 7. The solution was then centrifuged at a speed of 4000 rpm for 15 minutes. The supernatant was removed and added back to cold distilled water as much as 200 mL to remove NaCl. The solution was stirred to dissolve the remaining salt and then centrifuged at 4000 rpm for 15 minutes. The colloid kit obtained is stored in a refrigerator at 4ºC (Pujiyanto et al. 2008).

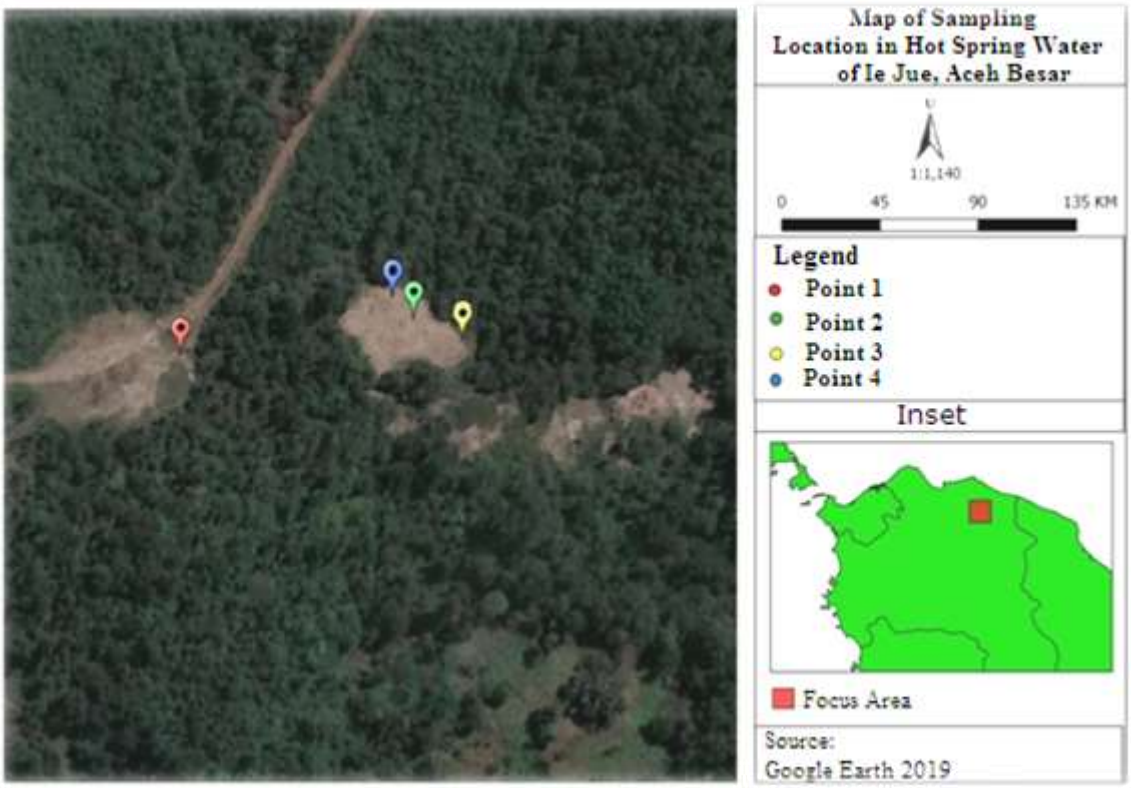


Figure 1. Map of sampling location in Ie Jue Hot springs, Lam Teuba, Aceh Besar, Aceh, Indonesia

Preparation of Chitin Agar Medium

Chitin agar is a selective agar medium (Nufus & Tresnani 2016) consisting of 0.1% $MgSO_4.7H_2O$, 0.02% K_2HPO_4, 0.1% yeast extract, and 1.5% agar. Media materials for chitin agar and 0.5% chitin colloid were sterilized using an autoclave for 15 minutes at a temperature of 121ºC. After cooling, the chitin colloid is mixed with the media to sterile the chitin. The medium for sterile chitin is poured into a petri dish. The media is then stored in an incubator upside down before use (Pujiyanto et al. 2008).

Isolation and Selection of Chitinolytic Bacteria

Chitinase-producing microorganisms can be obtained from the source by growing in media containing chitin (Herdyastuti et al. 2009). Chitinolytic bacteria were isolated from hot water Ie Jue in Lam Teuba area, Aceh Besar. Samples were taken in hot springs at four different points then stored in sterilized sample bottles. The sample obtained as much as 1 mL and then poured on chitin agar medium and incubated for 48-72 hours at 60ºC. Colonies that grow on the agar surface are chitinolytic bacteria isolates. The isolates obtained were then stored at 4ºC for maintenance (Pujiyanto et al. 2008). Chitinolytic bacteria were grown back on chitin agar medium to obtain a pure culture. Pure isolates were incubated at 60ºC for 1-5 days.

Characterization of Chitinolytic Bacteria

Characterization of chitinolytic bacteria was carried out through macroscopic and microscopic observations. Macroscopic observations were the colony shape, edges, elevation, color, and surface of the bacterial colony. In addition, microscopic observations were made on the shape of bacterial cells and Gram stain (Hadioetomo 1993).

Rearing of *Helicoverpa armigera* (Hübner) larvae

Helicoverpa armigera larvae were obtained from infested fruit plant from the market. Propagation and maintenance of *H. armigera* larvae following the method of Javar et al. (2013) modified. *Helicoverpa armigera* was fed with corn and placed in a plastic container. The larvae are kept in a plastic container (4 cm high x 5 cm in diameter) with an air cavity in the lid. Each plastic container contains one larva to prevent larvae from eating each other. The larvae that have become pupae are transferred to a plastic container (18 cm high x 18 cm diameter) covered with sterile gauze as a place for laying eggs and the bottom of the container is covered with sterile tissue.

The imago that appeared was given 10% honey. 10% honey is prepared by diluting 10 mL of honey with 90 mL of distilled water. 10% honey is put in a plastic container (4 cm high x 5 cm in diameter) with the lid of the container that has been perforated. The hole in the container's lid is inserted into a cotton roll. Cotton roll functions to absorb honey to make it easy for imago to consume. The eggs from imago will stick to the gauze so that the gauze must be changed every two days. The gauze that has been taken is put in a plastic that has been given an air cavity, then placed in a container to be incubated until the eggs hatch into larvae. The larvae are then collected and maintained until the 3rd instar, where the 3rd instar larvae will be given a test treatment (Figure 2).

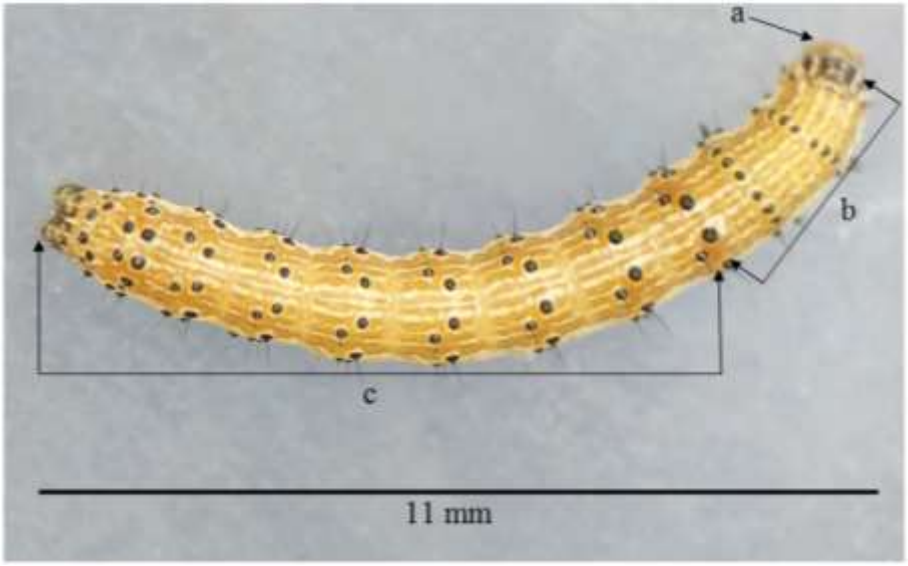


Figure 2. Morphology of *H. armigera* larvae before treatment, information: a) Caput; b) thorax; c) abdomen

Biological Test

Tests were carried out using the modified dipping method (Balfas & Wilis 2009). The bacterial cultures that have been obtained are grown on 200 mL of Nutrient Broth media until they reach a density of 108 CFU/mL. The corn kernels were dipped in each bacterial isolate until they were completely immersed, then dried for 1 minute. This process was repeated three times. Only seeds soaked with Nutrient Broth were used as a control. The feed is replaced with fresh fruit every day for ten days. Larval viability was observed daily.

Data Analysis
The data on the amount of mortality of *H. armigera* larvae were processed by the Kruskal-Wallis test using SPSS software. If there is a significantly different among treatments, then the means were further tested using Mann Whitney U Test carried out with a 95% confidence interval, while for larvae morphology is analyzed in descriptive form and displayed in the form of images.

RESULTS AND DISCUSSION

Thermophilic Bacteria Isolate from Ie Jue Hot Spring, Aceh Besar.
The chitinolytic bacteria obtained were 11 pure bacterial isolates. The results obtained were different from other studies, where Ardani et al. (2012) succeeded in isolating four isolates of chitinolytic bacteria from hot springs Ie Seuum, Aceh Besar. Nafisah et al. (2017) isolated four chitinolytic bacteria from the Dieng geothermal area.

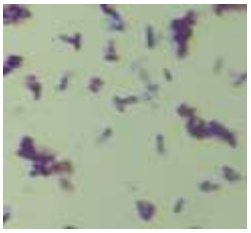
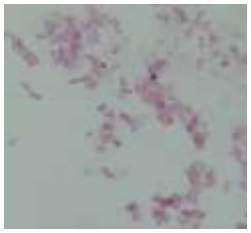
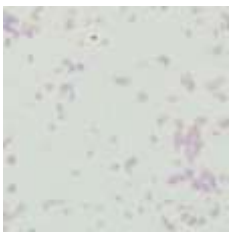
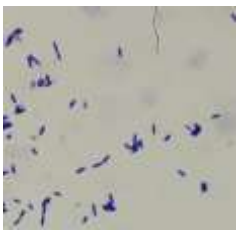
The various chitinolytic bacteria were obtained at four different temperatures. Five isolates were obtained from a temperature of 87ºC, two isolates at a temperature of 90ºC, one isolate at a temperature of 92ºC, and three isolates at a temperature of 94ºC. Abiotic factors can influence the difference in the number of isolates obtained. One of the abiotic factors that affect bacterial life is temperature. Temperature is one factor that influences the growth of microorganisms, the speed of enzyme synthesis, and the rate of enzyme inactivation (Nasran & Ariyani 2003). The environmental conditions found at the sampling location in Ie Jue are warm ground, steaming ground, hot springs, fumaroles, mud puddles and moss. The temperature range at the hot water sampling location was 87-94ºC with a neutral water pH of 7. Pitri et al. (2015) succeeded in isolating bacteria from the hot springs of the Medang River which has a temperature range of 45-88ºC. Fitri and Yasmin (2013) succeeded in isolating chitinolytic bacteria from hot springs Ie Seuum with a temperature range of 85-88ºC. The results of this study succeeded in obtaining the most isolates at a temperature of 87ºC, not much different from the two studies which obtained the most isolates at 88ºC.

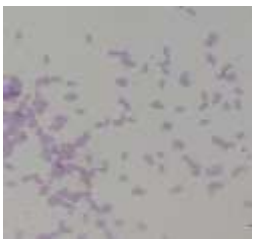
Morphological Characteristics of Bacteria Chitinolytic Isolate Thermophile
The results of colony characterization showed that the colony shape obtained varied (Table 1), starting from an irregular shape and spreading, round with extending edges and threaded. The edges of the colony are irregular, branched, notched, and silicate. The colony elevation is flat, like a crater, flat and grips into the media. Isolates are cream and white. The colony surface is rough and smooth.

Observations on the morphological characteristics of bacterial colonies need to be made to facilitate the identification of bacterial species. According to Lay (1994), based on the morphological characteristics of bacterial colonies, the identification process of the microorganism can be carried out, but to obtain a perfect identification result, it must be followed by a biochemical test.

Microscopic observations were carried out using the Gram stain method. According to Pelczar and Chan (2005), Gram stain can be used for microscopic characterization of bacteria by looking at the staining results and the shape of bacterial cells. The Gram staining results of each isolate showed variations, namely Gram-negative and positive, as well as the shape of bacterial cells, namely bacilli and cocci (Table 1). This follows Pitri et al. (2015) and Nafisah et al. (2017) who reported that the results of Gram staining of hot springs bacterial isolates varied.

Table 1. Morphological (macroscopic and microscopic) characterization of the thermophilic chitinolytic bacterial isolate Ie Jue, Aceh Besar

Macroscopic			
	IBK 1	IBK 2	IBK 3
Microscopic			
	Gram-Negative (1000x magnification)	Gram-Positive (1000x magnification)	Gram-Negative (1000x magnification)
Macroscopic			
	IBK 4	IBK 5	IBK 6
Microscopic			
	Gram-Negative (1000x magnification)	Gram-Positive (1000x magnification)	Gram-Positive (1000x magnification)
Macroscopic			
	IBK 7	IBK 8	IBK 9

Microscopic	 Gram-Positive (1000x magnification)	 Gram-Positive (1000x magnification)	 Gram-Positive (1000x magnification)
Macroscopic	 IBK 10	 IBK 11	
Microscopic	 Gram-Positive (1000x magnification)	 Gram-Negative (1000x magnification)	

Gram stain is used to determine bacterial cells' morphology and distinguish Gram-positive and Gram-negative bacteria. The determination of Gram-positive and Gram-negative is seen from the binding of the dye which is bound by the bacterial cell wall. Budin et al. (2012) state that Gram-positive bacteria have a thick peptidoglycan layer, whereas Gram-negative bacteria only have a thin peptidoglycan layer covered by lipopolysaccharides and lipoproteins. Upon decolorization with alcohol or acetone, only Gram-positive bacteria remain purple, while Gram-negative bacteria lose the purple color.

Gram-positive bacteria have a cell wall structure with a thick peptidoglycan content and a lower lipid content than Gram-negative bacteria, so the bacterial cell wall will be easier to dehydrate due to treatment with alcohol. Cappucino and Sherman (2002) stated that dehydrated cell walls cause the pores of cells to become small, and their permeability is reduced so that the crystal violet dye which is the main substance cannot leave the cells and the cells will remain purple. According to Purwoko (2007), Gram-negative bacteria have a higher lipid content in their cell walls and lipids generally dissolve in alcohol and acetone.

Mortality of *Helicoverpa armigera* (Hubner)

The results of the Kruskal Wallis test analysis obtained after ten days of treatment did not show any significant difference in a record of the mortality of *H. armigera* larvae ($P > 0.05$), among 11 isolates of chitinolytic bacteria used as larvicides. Similarly, Ardani et al. (2012) stated that chitinolytic bacterial isolates had no significant effect in causing the mortality of *Aedes aegypti*

L. This is presumably because each isolate had physical parameter values (temperature and pH) that were not much different.

Table 2 showed that the mortality of *H. armigera* larvae, when administered with chitinolytic bacteria, coded IBK 1 isolate had the highest effect compared to 10 other isolates, with the percentage of mortality of *H. armigera* larvae of 67%. It is assumed that IBK 1 has the highest enzyme activity. Meanwhile, IBK 6 and IBK 9 had the lowest percentage level of 8%, so the two isolates were thought to have the most insufficient enzyme activity. The difference in the effect of giving bacteria on larval mortality is thought to be due to differences in the chitinase enzyme activity in each isolate. According to Matsumoto (2006), chitinase can hydrolyze β, 4 glycosidic bonds in the structure of chitin (amino-glucose polysaccharide N-acetyl-β-D-glucosamine). Zulfiana et al. (2017) stated in their research that chitinase found in insect feed could inhibit insect growth. So that the higher the activity of the chitinase enzyme, the more it will affect the mortality rate of larvae.

Each treatment showed results that were not significantly different. However, the administration of chitin bacterial isolates affected suppressing the *H. armigera* larvae population. This is evidenced by the test larvae that were given IBK 1 treatment-experienced the mortality of 2.7 tails or were able to kill eight larvae of *H. armigera* from a total of 12 test larvae, while the control larvae of *H. armigera* did not experience mortality.

Larvae that Survive to Enter the Pupa Stage

Provision of chitinolytic bacteria with isolate codes IBK 6 and IBK 9 does not affect the process of changing the larval stage into a pupa. This can be seen from the formation of a complete pupa up to 92%, while IBK 1 isolate does not affect the process of changing the larval stage into a pupa because the larvae can become pupae only 33% (Table 3).

Table 2. Mortality rate of *H. armigera* larvae by administering chitinolytic bacterial isolate (IBK)

No.	Treatment	Number of Larvae	Average of Mortality	Percentage of mortality
1	Control	12	0	0%
2	IBK 1	12	2.67	67%
3	IBK 2	12	1.67	42%
4	IBK 3	12	0.67	17%
5	IBK 4	12	1	25%
6	IBK 5	12	1.67	42%
7	IBK 6	12	0.33	8%
8	IBK 7	12	1.67	42%
9	IBK 8	12	1.33	33%
10	IBK 9	12	0.33	8%
11	IBK 10	12	2	50%
12	IBK 11	12	1.33	33%

Table 3. Average ability of *H. armigera* larvae to become pupae by administering chitinolytic bacterial isolate (IBK)

No.	Treatment	Number of Larvae	The Larvae that Survive to Enter the Pupa Stage	
			Average	Percentage
1	Control	12	4.00	100%
2	IBK 1	12	1.33	33%
3	IBK 2	12	2.33	58%
4	IBK 3	12	3.33	83%
5	IBK 4	12	3.00	75%
6	IBK 5	12	2.33	58%
7	IBK 6	12	3.67	92%
8	IBK 7	12	2.33	58%
9	IBK 8	12	2.67	67%
10	IBK 9	12	3.67	92%
11	IBK 10	12	2.00	50%
12	IBK 11	12	2.67	67%

After the end of the observation time, the larvae that managed to become pupae were able to develop into a physically normal imago, of the same size as in normal imago, except for one larva treated with IBK 7 which was unable to become pupae completely and died during the pupation process. Fitri and Yasmin (2014) stated that the failure of pupa formation occurs because bacteria can hydrolyze chitin on the outside of the larva's exoskeleton which results in disruption of the metamorphosis process of larvae into pupae.

Larvae that can become pupae are thought to have a body defense system. Musser et al. (2002) stated that the larvae of *Helicoverpa* sp. have the enzyme glucose oxidase. According to Wineri et al. (2014), glucose oxidase breaks down sugar into D-glucono-1,5-lactone and hydrogen peroxide, which have anti-bacterial effects. Based on Glare et al. (2017) larvae have a defense system in the oral cavity in the form of reactive oxygen or anti-microbial compounds. Apart from the oral cavity, another defense system is a digestive system that can filter out incoming microbes. The physicochemical conditions in larvae are also able to inhibit bacterial growth.

Morphology of *Helicoverpa armigera* Larvae

Helicoverpa armigera larvae are like insects in general, consisting of the head, thorax, and abdomen (Figure 2). The larvae used in this study were III instar *H. armigera* larvae. Third instar larvae were chosen because they are more active in moving and foraging. The morphology of third instar *H. armigera* is easier to distinguish than other instars.

Symptoms of mortality by chitinolytic bacteria that occurred in *H. armigera* larvae were seen through changes in larvae morphology from before and after treatment. Before treatment, the larvae were active and had high feeding activity. Microscopic observation showed that the larvae were bright and fresh, and the head, thorax, abdomen, and anus looked normal (Figure 2). After administering chitinolytic bacterial isolates, the morphological changes of the dead

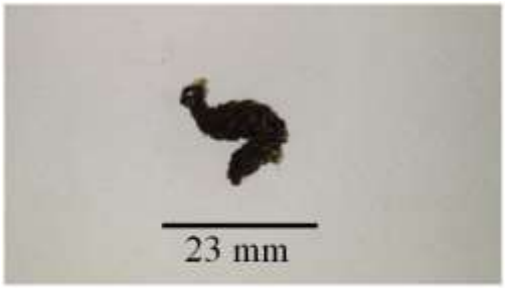
larvae had different specifications for each test isolate.

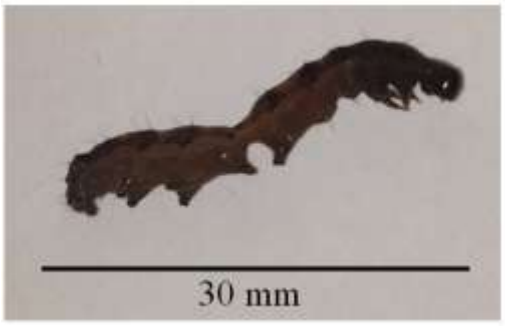
Generally, they showed the same pre-mortality symptoms, namely slowing down movement, reduced eating activity, and a tendency to remain silent which eventually dies. The carcasses of the test larvae in this study smelled foul and were getting smaller. According to Tampubolon et al. (2013), symptoms of bacterial attack in insects begin with signs of inactivity, decreased appetite, weakness, insect feces become liquid, and discharge from several parts of the body. Adamo et al. (2010) explained that some of the symptoms that indicate that larvae have been infected by bacteria are stopping eating, paralysis, diarrhea, or vomiting. Reduction or cessation of feeding activity is one of the larval defense mechanisms in dealing with exposure to pathogenic bacteria as well as increasing immune function (Adamo et al. 2007).

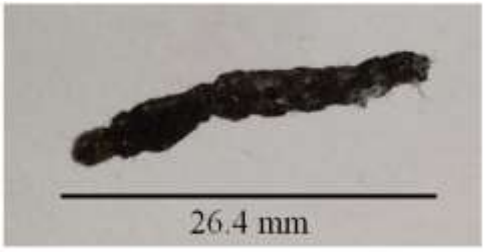
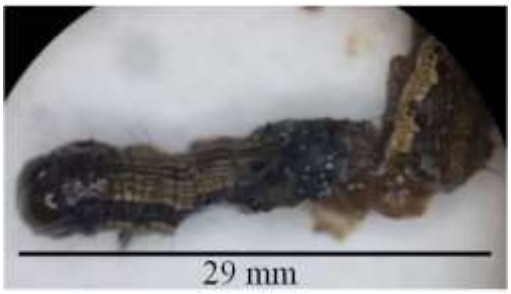
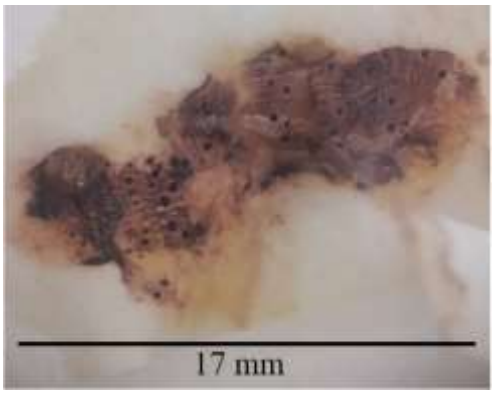
The physiological effect based on the toxicity of bacterial isolates cannot be ascertained, but through the symptoms of mortality caused to *H. armigera* larvae, it is known that there is an interaction between bacterial toxins and the symptoms they cause. Symptoms of the mortality of *H. armigera* larvae that were given treatment can be seen in Table 4. IBK 1 was the most effective isolate in suppressing the mortality of *H. armigera* larvae. Some of the characteristics of the mortality were not much different from the IBK 2 and IBK 11 treatments, namely, the body was soft, smelly, there was a molting formation, the color of the body turned dark brown, while the head and thorax turned black (Table 4.) This result complying with Fadhullah et al. (2015), who reported that after being treated with *Bacillus thuriengensis* isolates, there was a change in appetite, slow movement, and a change in body color in insects. The high number of bacterial cells causes the change in larval body color in the hemolymph, the activity of these bacteria causes bacteremia and necrosis, resulting in color changes (Götz 1972).

Symptoms of the mortality of larvae after being given IBK 4 treatment, the body is flaccid and watery, the head and spiracles are blackened, especially in the abdomen, there is a formation of molting (Table 4). Symptoms of mortality in this study are similar to the results of research by Tampubolon et al. (2013), namely, larvae begin to move slowly, appetite decreases, larvae start to soften, ooze, smell, and experience a change in color starting from brownish green then black, and dry.

Table 4. Characteristics of the mortality of *Helicoverpa armigera* larvae after being treated with chitin bacterial isolate (IBK)

No.	Isolate Code	Images	The Characteristic Features of Mortality
1.	IBK 1		• Body flabby • There is molting freeze • The body-color turns dark brown, while the head and thorax turn black • Smelling

2.	IBK 2		• Body flabby • Body-color changed to brown • Smelling
3.	IBK 3		• Normal body-color • Body swells and hardens • The abdomen is burnt like a burn
4.	IBK 4		• Body flabby and watery • The body-color turns brown • There is molting stock • Smelling
5.	IBK 5		• Body flabby and watery • All parts of the body are blackened • There is a base for molting

6.	IBK 6		• Normal body-color except for the red abdomen • Flabby body • Smelling
7.	IBK 7		• Larvae die during the pupation process • The abdomen has formed a pupa but the head has not succeeded in becoming a pupa
8	IBK 8		• Flabby body • Black body-color • Smelling
9.	IBK 9		• Normal body-color • Body flabby and watery • The body is easily destroyed • Smelling
10.	IBK 10		• The body is initially swollen and reddish in color • The body of the larva is crushed when moved • Red discharge • Smelling

11.	IBK 11		• Flabby body • Black body-color • There is a base for molting • Smelling

Symptoms of larval mortality caused by IBK 4 are similar to those caused by *Bacillus thuriengensis*. Adam et al. (2014), stated that larvae infected with *B. thuriengensis* had the characteristics of a shriveled, flabby body and blackened body color. Based on research by Bahagiawati (2002), there was an osmotic disturbance in the larva's body exposed to toxins from *B. thuriengensis* which caused the rupture of cells in the larva's intestine. The cells that break down and dry out will result in a black change in the larvae exposed to *B. thuriengensis* toxins.

The similarity of symptoms of mortality due to the influence of IBK 4 with *B. thuriengensis* is strongly suspected due to the presence of parasporal crystals. Yamashita et al. (2005) stated that *B. thuringiensis*, a Gram-positive bacterium, produces parasporal inclusions during sporulation. The parasporal inclusion contains δ-endotoxin that often exhibits insecticidal activity against several insect orders. The δ-endotoxin is currently classified into two families, Cry and Cyt proteins. Ginting et al. (2020) stated that the cry protein is very deadly to Lepidoptera species. The Cry proteins are solubilized and proteolytically activated by midgut proteases of the susceptible insects under alkaline conditions. According to Vachon et al. (2012) the inclusion bodies release protoxins, which digestive proteases in the larval gut fluid convert to active toxins. The activated Cry toxins cross the peritrophic membrane (PM) and bind to gut epithelial cells. It is generally accepted that the pore-forming Cry toxins then insert into gut epithelial cell membranes and rupture inflicted cells.

Symptoms of the mortality of *H. armigera* larvae treated with IBK 6, IBK 8, IBK 9, and IBK 10 isolates are the same as the results of research by Zulfiana et al. (2017) which have similar characteristics, where the initial body of the larva is swollen and reddish. The larva's body disintegrates upon transfer, giving off a red, smelly discharge. After being treated with bacterial chitin isolates, the larvae became unwilling to eat and the feces was more liquid than the feces on control larvae in the form of granules. The infected and dead larvae become flaccid and when the skin is touched, they will break, and the body fluids come out blackish-red (Table 4.)

This softening of the body can be caused by the thinning of the insect cuticles due to enzymatic processes by bacteria in the body of the test insects. Matsumoto (2006) states that chitinase is an enzyme that can hydrolyze the β-1,4-glycosidic bonds in the structure of chitin (amino-glucose polysaccharide N-acetyl-β-D-glucosamine). Based on (Terra & Ferreira 2005) , chitinase will induce damage to the peritrophic membrane in the digestive tract of insects and cause a significant reduction in nutrient absorption. According to Berini et al. (2016), chitinase cause perforations in the chitin containing cuticle region or peritrophic membrane of the insect pests leads to insect mortality. The damage in the insect gut might have resulted in nutritional imbalance due to numerous processes viz., water excretion or retention,

food acquisition, which further leads to slow growth and ultimately leads to insect mortality.

CONCLUSION

Based on the results obtained, the isolates of thermophilic chitinolytic bacteria can be potentially used as larvicides or biopesticdes to control *H. armigera* larvae. Further research should be conducted on identification, physiology and molecular tests of chitinolytic bacteria as well as the proper dosage of larvicides with variations of larval instars.

ACKNOWLEDGMENT

The author would like to thank the Microbiology division of the Department of Biology the Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Aceh, Indonesia for the facilities and support given in this research.

REFERENCES

Adam, T., Juliana, R., Nurhayati & Thalib, R. 2014. Bioessay of *Bacillus thuringiensis* Berliner bioinsecticide against *Spodoptera litura* Fabricius. *Prosiding Seminar Nasional Lahan Suboptimal* 74: 1-7.

Adamo, S.A., Fidler, T.L., & Forestell, C.A. 2007. Illness-induced anorexia and its possible function in the caterpillar, *Manduca sexta*. *Brain, behavior, and immunity* 21(3): 292-300.

Adamo, S.A., Bartlett, A., Le, J., Spencer, N. & Sullivan, K. 2010. Illness-induced anorexia may reduce trade-offs between digestion and immune function. *Animal Behaviour* 79(1): 3-10.

Adnan, A.M. & Handayani. 2010. Predation ability of earwigs (*Euborellia annulata* Fabricus) against the Corn Borer *Helicoverpa armigera* (Hübner). *Prosiding Pekan Serealia Nasiona,* pp. 380-388.

Ardani, F., Yasmin, Y. & Fitri, L. 2012. Potential of Isolate Chitinolitic Bacteria from Hot springs, as Biological Control of *Aedes aegypti* L larva. *Jurnal Biologi Edukasi* 4(2): 77-81.

Bahagiawati, A. 2002. *Bacillus thuringiensis* application as a Bioinsecticide. *Buletin Agrobio* 5(1): 21-28.

Balfas, R. & Willis, M. 2009. Effect of Medicinal Plants Extract on Mortality and Survival of *Spodoptera litura* F. (Lepidoptera, Noctuidae). *Buletin Penelitian Tanaman Rempah dan Obat* 20(2): 148-156.

Berini, F., Caccia, S., Franzetti, E., Congiu, T., Marinelli, F., Casartelli, M. & Tettamanti, G. 2016. Effects of *Trichoderma viride* chitinases on the peritrophic matrix of Lepidoptera. *Pest management science* 72(5): 980-989.

Budin, G., Chung, H.J., Lee, H. & Weissleder, R. 2012. A magnetic Gram stain for bacterial detection. *Angewandte Chemie* 124(31): 7872-7875.

Cappucino, J.G. & Sherman, N. 2002. *Microbiology: A Laboratory Manual.* San Fransisco: The Benjamin Cummings Publishing Company.

Czepak, C., Albernaz, K.C., Vivan, L.M., Guimarães, H.O. & Carvalhais, T. 2013. First reported occurrence of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in Brazil. *Pesquisa Agropecuária Tropical* 43: 110-113.

Fadhullah, A.A., Hoesain, M. &Haryadi, N.T. 2015. Application of bioinseticide for controlling pest *Spodoptera litura*, *Helicoverpa* spp., *Cyrtopeltis tenuis* on tobacco (in Indonesian). *Berkala Ilmiah Pertanian* 1(1): 1-6.

Fitri, L. & Yasmin, Y. Isolation of chitinolytic bacteria from Hot Springs Ie Suum Masjid Raya District, Aceh Besar District, Aceh. Prosiding Seminar Nasional Biologi, Medan, 13 April 2013.

Fitri, L. & Yasmin, Y. 2014. The Potential of local chitinolitic bacteria isolates as larvacide of *Aedesa egypti* L. *Makara Journal of Health Research* 18: 1-6.

Ginting, S., Zarkani, A., Wibowo, R.H. & Sipriyadi, S. 2020. New invasive pest, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) attacking corn in Bengkulu, Indonesia. *Serangga* 25 (1): 105-117.

Ginting, Y. 2009. Bacterial isolation and thermophile amylase activity test from the spirit of hot springs of North Sumatra Mountain. Thesis. USU, Medan.

Glare, T.R., Jurat-Fuentes, J.L. & O'Callaghan, M. 2017. Basic and applied research: Entomopathogenic bacteria. In Lacey L. A. (ed.). *Microbial Control of Insect And Mite Pests,* pp. 47-67. San Diego: Elsevier Academic Press Inc.

Götz, P. 1972. "*Rickettsiella chironomi*": An unusual bacterial pathogen which reproduces by multiple cell division. *Journal of Invertebrate Pathology* 20(1): 22-30.

Hadioetomo, R.S. 1993. *Basic Microbiology in Practice*. Jakarta: Gramedia. [in Indonesian].

Herdyastuti, N., Raharjo, T.J., Mudasir & Matsjeh. S. 2009. Chitinase and chitinolytic microorganism: Isolation, characterization and potential. *Indonesian Journal of Chemistry* 9(1): 37-47.

Herlinda, S. 2005. Bio-Ecology of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) on tomato. *Agria* 2(1): 32-36.

Javar, S., Sajap, A.S., Mohamed, R., & Hong, L.W. 2013. Suitability of *Centella asiatica* (Pegaga) as a food source for rearing *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) under laboratory conditions. *Journal of Plant Protection Research* 53(2): 184-189.

Kalshoven, L.G.E. 1981. *Pests of Crops in Indonesia*. Jakarta: PT Ichtiar Baru Van-Hoeve.

Krishanti, N.P.R.A., Wikantyoso, B., Zulfitri, A. & Zulfiana, D. 2017. Entomopathogenic bacteria as biocontrol agent against *Spodoptera litura* (F.) larvae. *Jurnal Ilmu-Ilmu Hayati,* 16(1): 1-110.

Lay, B.W. 1994. *Analisis Mikroba di Laboratorium*. Jakarta: PT Raja Grafindo Persada.

Liu, P., Cheng, D. & Miao, L. 2015. Characterization of thermotolerant chitinases encoded by a *Brevibacillus laterosporus* strain isolated from a suburban wetland. *Genes* 6(4): 1268-1282.

Matsumoto, K.S. 2006. Fungal chitinases. *Advancess in Agricultural and Food Biotechnology* 6: 289-304.

Musser, R.O., Hum-Musser, S.M., Eichenseer, H., Peiffer, M., Ervin, G., Murphy, J.B. & Felton, G.W. 2002. Caterpillar saliva beats plant defences. *Nature* 416 (6881): 599-600.

Nafisah, H., Pujiyanto, S. & Raharjo, B. 2017. Isolation and testing of chitinase activity of bacterial isolates from the Dieng geothermal area. *Bioma: Berkala Ilmiah Biologi*

19(1): 22-29.

Nasran, S. & Ariyani, F. 2003. Production of chitinase and chitin deacetylase from *Vibrio harveyi*. *Jurnal Penelitian Perikanan Indonesia* 9(5): 33-38.

Nufus, B.N. & Tresnani, G. 2016. Populations of normal bacteria and chitinolytic bacteria in the digestive tract of sand lobster (*Panulirus homarus* L.) given chitosan. *Jurnal Biologi Tropis* 16(1): 15-23.

Pelczar, M.J. & Chan, E.C.S. 2005. *Fundamentals of Microbiology 1*. Jakarta: Figures Universitas Indonesia Press.

Pitri, R.E., Agustien, A. & Febria, F.A. 2015. Isolation and characterization of amilothermophilic bacteria from Sungai Medang Hotspring. *Jurnal Biologi Universitas Andalas* 4(2): 119–122.

Priyatno, T.P., Dahliani, Y.A., Suryadi, Y., Samudera, I.M., Susilowati, D.N., Rusmana, I., Wibowo, B.S. & Irwan, C. 2011. Identification of red bacterial entomopathogens on brown planthopper (*Nilaparvata lugens* Stål). *Jurnal Agro Biogen* 7: 85-95.

Pujiyanto, S., Kusdiyantini, E., Hadi, M. 2008. Isolation and selection of local isolates of chitinolytic bacteria that have the potential to control *Aedes aegypti* L. mosquito larvae. *Biodiversitas* 9(1): 5-8.

Purwoko, T. 2007. *Fisiologi Mikroba*. Jakarta: Bumi Aksara.

Tampubolon, D.Y., Pangestiningsih, Y., Zahara, F. & Manik, F. 2013. Pathogenicity test of *Bacillus thuringiensis* and *Metarhizium anisopliae* against mortality of *Spodoptera litura* Fabr (Lepidoptera: Noctuidae) in laboratorium. *Jurnal Online Agroekoteknologi* 1: 783-793.

Terra, W.R. & Ferreira, C. 2005. Biochemistry of digestion. In Gilbert, L.I., Iatrou, K. & Gill, S.S. (eds.), pp. 171-224, *Comprehensive Molecular Insect Science Biochemistry and Molecular Biology*. Oxford, UK: Elsevier.

Thamthiankul, S., Suan-Ngay, S., Tantimavanich, S. & Panbangred, W. 2001. Chitinase from *Bacillus thuringiensis* subsp. Pakistani. *Applied Microbiology and Biotechnology* 56: 394-401.

Vachon, V., Laprade, R. & Schwartz, J. 2012. Current models of the mode of action of *Bacillus thuringiensis* insecticidal crystal proteins: A critical review. *Journal of Invertebrate Pathology* 111: 1–12.

Wangsa, A. & Ismail, N. 2018. Quantitative interpretation of gravity anomaly data in geothermal field Seulawah Agam, Aceh Besar. *Journal of Aceh Physics Society 7*(1): 6-12.

Wineri, E., Rasyid, R. & Alios, Y. 2014. Comparison of inhibitory power of natural honey and packaged honey in vitro against streptococcus beta hemolyticus group a as the cause of pharyngitis. *Jurnal Kesehatan Andalas 3*: 376-380.

Yamashita, S., Katayama, H., Saitoh, H., Akao, T., Park, Y. S., Mizuki, E. & Ito, A. 2005. Typical three-domain cry proteins of *Bacillus thuringiensis* strain A1462 exhibit cytocidal activity on limited human cancer cells. *Journal of biochemistry* 138(6): 663-672.

Yuniarti, R.A. & Blondine, C.P. 2005. The effectivity of vectobac and *misocyclops aspericornis* predator as a biological control agent of *Aedes aegypti* larvae in water jars. *Kedokteran Yarsi* 13(1): 102-110.

Zulfiana, D., Krishanti, N.P.R.A., Wikantyoso, B. & Zulfitri, A. 2017. Entomopathogenic bacteria as biocontrol agent against *Spodoptera litura* (F.) larvae. *Jurnal Ilmu-Ilmu Hayati* 16(1): 1-110.