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Isolation and Screening Microorganisms from Black Soldier Fly Larvae (*Hermetia illucens*) that Producing Amylase, Protease and Cellulase

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Abstract: The Black Soldier Fly (BSF) has gained significant attention for its ability to decompose various types of organic waste and produce valuable enzymes related to its digestive system. These enzymes hold immense potential for applications across diverse industrial sectors, with promise in the livestock industry. This study aimed to extract and assess amylase, protease, and cellulase enzymes from microorganisms present in BSF larvae fermentation. The isolation technique employed was the spread plate method, followed by rigorous screening for the presence of these enzymes. The results showed 46 isolates of 26 bacterial isolates and 20 yeast isolates. In the amylase enzyme screening, a substantial 25 bacterial and 19 yeast isolates exhibited positive amylase activity. For cellulase, 20 bacterial and 14 yeast isolates displayed positive results. In the case of protease, 16 bacterial and 12 yeast isolates demonstrated protease enzyme activity. Notably, nine isolates exhibited the remarkable capability to produce multiple enzymes, including eight bacterial and one yeast isolate. These results showcase the rich enzymatic potential of BSF-associated microorganisms, offering exciting prospects for their application in various industrial sectors, especially in enhancing the efficiency and sustainability of livestock production.

Keywords: Amylase; Black soldier fly; Cellulase; Microorganisms; Protease

Introduction

The Black Soldier Fly (*Hermetia illucens*) is a fly species from the order Diptera with the Stratiomydae family and the genus *Hermentia* (Widyastuti, 2021). The percentage of BSF larvae's nutritional content is quite high, namely 44.26%, with a fat content of 29.65%. The value of amino acids, fatty acids and minerals in the larvae is also not inferior to other protein sources. Hence, BSF larvae are ideal raw materials that can be used as animal feed. *H. illucens* are insects that are not in the human environment, pollute the environment, spread disease, or damage plants. However, *H. illucens* is a saprophytic insect that can digest harmful bacteria in waste, which positively impacts the environment (Liu et al., 2019). In addition, the Black Soldier Fly is also known as a fly that can decompose various kinds of organic waste, such as vegetable and food scraps (Holmes et al., 2012).

Black Soldier Fly has various phases in its life. The Black Soldier Fly (*H. illucens*) larvae, better known as maggot (Rumondor et al., 2015), is in the longest phase of its life cycle. The larval phase lasts 3-4 weeks (Fahmi, 2015). This differs from domestic insects such as

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Calliphoridae and Muscidae, which have a shorter larval phase than the adult one (Hastutiek et al., 2013). This phenomenon is widely used to classify black soldier fly (maggot) larvae as bioconversion agents because most of their lives act as decomposers. The adult phase of the black soldier fly (*H. illucens*) is a fairly short phase of 6-8 days compared to the adult phase of domestic insects, which has an adult phase of 2 to 3 months. This phenomenon shows that black soldier flies' larvae are not indicated as agents of spreading disease (Hastutiek et al., 2013). During the larval phase, the black maggot soldier fly will continue to eat until it is close to the prepupa phase; during the prepupa phase, it does not eat and will leave food sources (Hastutiek et al., 2013). Next, the prepupa will look for a dry place to take shelter until it enters the pupal phase. The pupal phase will last for 6-7 days, and after that, the pupa will metamorphose into an adult black soldier fly (Fahmi, 2015).

The Black Soldier Fly produces beneficial enzymes. The enzymes contained in BSF are related to their digestive system. Enzymes that are capable of being produced by BSF, such as amylase, lipase, and protease, during their metabolism. In the gut of BSF larvae, there are *B. subitilis* bacteria which can produce amylase. In the digestive tract, BSF has high protease enzyme activity (Kim et al., 2021). In addition, the BSF digestive tract also produces cellulase enzymes (Nisa, 2018).

Enzyme production and trade are dominated by hydrolytic enzymes such as amylase, protease, cellulase, catalase, and lipase. So many studies worldwide began to seek information about these hydrolytic enzymes (Sedijani et al., 2022; Sukmawati, Saidah, et al., 2018; Sukmawati et al., 2019). Hydrolytic enzymes are one of the most important enzymes in the industrial field (Rahmiati et al., 2016; Sukmawati et al., 2021). One of the benefits of hydrolytic enzymes in the industrial field is its application in animal husbandry. One of the main things in livestock is feed processing in the form of the availability of nutrients for optimal livestock growth and development. Additional substances, including enzymes, are given to increase the availability of nutrients in feed (Vertygo, 2021). Therefore, this study was conducted on isolating and screening amylase, protease, and cellulase enzymes from microorganisms in fermented Black Soldier Fly larvae feed.

Method

Materials

The materials used were PCA media, Yeast Malt Agar (YMA media), Nutrient Agar (NA media), 70% alcohol, spiritus, distilled water, cellulase enzyme screening test media (CMC, Yeast Extract, peptone, agar and NaCl), amylase (Soluble starch, peptone, yeast extract, $MgSO_4$, CaCl, KH_2PO_4, and agar) and proteases (skim milk, peptone, yeast extract, glucose, and agar), iodine, Congo red and NaCl.

Isolation of Microorganisms from Black Soldier Fly (BSF) Larvae

The isolation of microorganisms from black soldier fly larvae contained 20 samples, each of which was used as much as 1 gram. The method used in sampling is random or random with five different points in one container with four repetitions each. The sample is then surface sterilized using an aseptic wipe (alcohol-wet tissue) repeatedly until there are no stains attached to the sample on the tissue. The aseptic wipe has an alcohol content of 70% and is usually used to disinfect small surfaces (Panousi et al., 2009). Next, the sample is immersed in sufficient distilled water until the sample is immersed for 5 to 10 minutes in a petri dish (height 18 mm × diameter 95 mm). The sample is transferred to 70% alcohol in a petri dish and then immersed in distilled water for 5 to 10 minutes. Before dilution, 60 falcons were prepared and filled with 9.9 ml of distilled water.

The clean Black Soldier Fly larvae were mashed using a sterile mortar and put into a falcon containing 0.1 grams of distilled water. Dilution was carried out 10^{-2} and 10^{-3} with 5 points, four times the treatment (KO+KP, KO, KP, Control) with the dilution method (multilevel dilution), which is a process to dissolve and release microbes from their substrate into distilled water so that they become easier to handle (Sukmawati, Wahyudi, et al., 2018; Widiasti et al., 2020). The dilution results in the falcon were poured using a micropipette into a petri dish containing PCA media. The petri dish is then glued with plastic wrap and stored at room temperature. Colonies will grow within 24 hours to 48 hours. Microorganisms that grow are then purified by inoculating several times on PCA media in a Petri dish, streaking, and incubating for 24 hours at 37°C until all the cultures are completely pure (Gorrens et al., 2021).

Preparation of YMA and NA (Colony library) Growth Medium

Preparation of YMA and NA media with 200 ml each. Then, the media was poured into sterile Petri dishes, as many as 12 YMA Petri dishes and 12 NA Petri dishes. After the media has frozen, the Petri dishes can be glued together using plastic wrap and given 1 cm x 1 cm line for the colony library. Next, numbering is done with a distance of 1 empty box. Next, colonies from PCA media were taken by aseptic loops and streaked into YMA and NA media according to the numbering performed. The YMA and NA Petri dishes were resealed and waited for the colonies to grow. YMA media takes 24 hours, while NA takes 48 hours.

Protease Assay

In the protease assay, the petri dish that has been poured with protease media and has frozen is divided into 5 to 6 quadrants. The composition of the media used in the protease enzyme activity test was skim milk (28 g/L), peptone (5 g/L), and yeast extract (25 g/L), glucose (1 g/L), and agar (15 g/L). So, based on these calculations, 4.2 g of skim milk is needed, 0.75 g of peptone, 3.75 g of yeast extract, 0.15 g of glucose and 2.25 g of agar for 150 ml of media. Furthermore, all isolates were incubated at 28°C for 48 hours. To prove that the isolate can produce protease enzymes can be done by looking at the presence of a clear zone. Furthermore, qualitative measurements were carried out with parameters (+++) for all clear zones in quadrants, (++) for half clear zones in quadrants, (+) for clear quarter zones in quadrants, and (-) which did not have clear zones (Sukmawati et al., 2021).

Amylase Assay

The selective media used for the amylase test are YPSA (Yeast Peptone Starch Agar) media with the composition (g/L): 10 g soluble starch; 5 g of peptones; 2 g yeast extract; 0.1 g MgSO4.7H2O; 0.1 g CaCl2.7H2O; 0.5 g KH2PO4, 20 g agar. Based on these calculations, this study required 150 ml of media, so 1.5 g of soluble starch was used; 0.75 g of peptones; 0.3 g yeast extract; 0.015 g MgSO4.7H2O; 0.02 g CaCl2.7H2O; 0.075 g KH2PO4 and 3 g agar. Incubation was carried out by inoculating isolates into YPSA media with media conditions divided into five quadrants. Furthermore, gram iodine is used to detect the clear zone, which identifies the activity of the amylase enzyme. Furthermore, qualitative measurements were carried out with the parameters (+++) for all clear zones in the quadrants, (++) for the half-clear zones in the quadrants, (+) for the quarter-clear zones in the quadrants, and (-) which had no clear zones (Arman et al., 2020).

Cellulose Assay

The selective media used for the amylase test were CMC (Carboxyl Methyl Cellulose) media with the composition (g/L): 10 g yeast extract, 5 g peptone, 25 g agar, and 18 g CMC. Based on these calculations, 150 ml of media is needed with a composition of 1.5 g yeast extract; 0.75 g of peptones; 3.75 g agar; 2.7 g of CMC and 8.766 g of NaCl. Incubation was carried out by inoculating yeast into CMC media with media divided into four quadrants. Furthermore, identifying cellulases by looking at the clear zones in the isolates was examined using Congo red as a detection agent. After letting it sit for about 15 minutes, it can be rinsed with 5.844 NaCl dissolved in 100 ml of distilled water, done three times. Furthermore, qualitative measurements were carried out with the parameters (+++) for all clear zones in the quadrants, (++) for the half-clear zones in the quadrants, (+) for the quarter-clear zones in the quadrants, and (-) which had no clear zones (Arman et al., 2020).

Result and Discussion

Black Soldier Fly larvae are used in waste management systems because they can degrade organic waste. Degrading organic waste using BSF larvae is a promising alternative in waste management because decomposing waste with BSF can reach 70% (Darmawan et al., 2017). The black soldier flies larvae intestine is the habitat of amylolytic bacteria, commonly used to decompose organic waste (Bogianto, 2019). Black Soldier Fly larvae can bio convert organic waste. In addition, larvae feed a source of nutritional needs for their metabolism. Microbial decomposition of organic waste so that it can involve a microbial consortium from the Black Soldier Fly larvae bioreactor. Microbes produce several enzymes, including hydrolytic enzymes, which hydrolyze macromolecules (carbohydrates, proteins and lipids) (Farooq et al., 2021).

Black Soldier Fly larvae samples from this study were taken from the fermentation of Black Soldier Fly larvae from Bekasi. Black Soldier Fly larvae fermentation was differentiated from various treatments, namely probiotic (KP), oily (KO), KO + KP (oleaginous + probiotic), and control (Figure 1). Isolation was carried out using the spread plate method, which was repeated three times, namely 10^{-1}, 10^{-2} and 10^{-3}. The isolation results from the fermentation of Black Soldier Fly larvae showed that 397 colonies had grown on PCA media for 24-48 hours and were incubated at room temperature. In the purification process, isolates were selected based on color and texture, so 46 isolates were obtained, with details of 20 yeast isolates and 26 bacterial isolates.

After isolation, screening or enzyme tests are carried out on bacteria and yeast. Three hydrolytic enzymes were tested, namely amylase, cellulase, and protease. Hydrolytic enzymes are one of the potential enzymes that can be widely applied in the industry (Novianti, 2023). In addition, enzymes produced by living things can help speed up the chemical reaction processes of these living things (Pricilia et al., 2018). From the enzyme test, a clear zone will form around the colony, indicating that the isolate can hydrolyze the compounds in the medium. The clear zone to identify the enzyme is examined using the media according to the enzyme to be tested as a detection agent. Furthermore, qualitative measurements were carried out with parameters (+++) for all clear zones in quadrants, (++) for half clear zones in quadrants, (+) for

quarter clear zones in quadrants, and (-) which had no clear zones.

Amylase Assay

Amylase is the dominant hydrolase enzyme that hydrolyzes the glycosidic bonds in starch molecules and produces dextrins and oligosaccharides (Sundarram et al., 2014). In biotechnology, amylase is an important enzyme, especially for industrial applications (Gopinath et al., 2017). In addition, the application can also be used in the laboratory. Most of the amylase enzymes are synthesized from microbes such as bacteria, fungi and yeast (Farooq et al., 2021).

In the amylase test, a clear zone will form. The clear zone around the colony indicated that the isolate could hydrolyze starch. Starch will form a deep blue complex with an iodine reagent. The iodine-starch reaction is caused by the presence of amylose and iodine helices forming I_3-, which fills the helix core. Active hydrolysis of starch by the amylase enzyme will cause the starch-iodine complex to decompose (Wulandari et al., 2018).

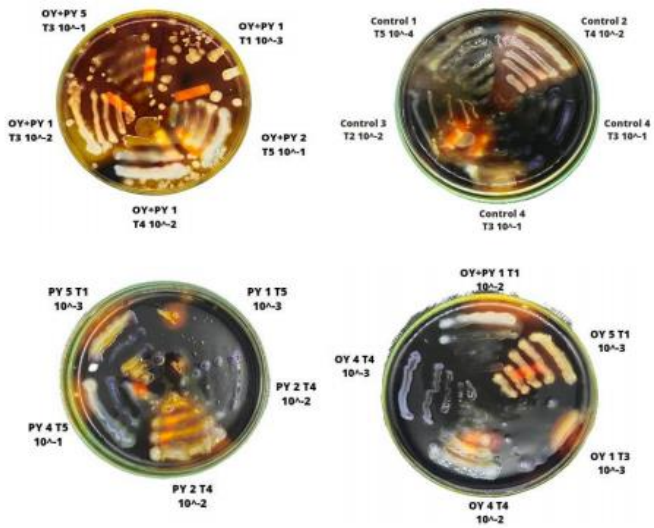

Figure 1. Amylase enzyme assay in yeast

Figure 2. Amylase enzyme assay in bacteria

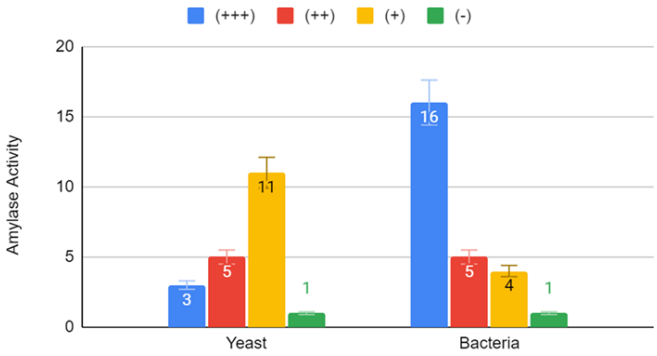

Figure 3. Results of amylase activity assay in microorganisms obtained from Black Soldier Fly larvae. Error bars in the figures represent standard deviation

The results of the study can be seen in Table 1 enzyme test results from Black Soldier Fly yeast larvae showed that from 20 isolates, only one isolate did not produce the enzyme amylase. At the same time, 19 other isolates produced amylase enzymes, with details of 11 isolates (+), five isolates (++), and three isolates (+++). Isolate (+++) is an isolate that produces all clear zones in its quadrant, namely isolate KP 2. T4 $10^{-(-2)}$), KO 5. T1 $10^{-(-3)}$), and Control 2. T4 $10^{-(-2)}$). According to Wulandari (2017), the main source of the amylase enzyme is yeast microorganisms.

From the research results, it can be seen in Table 1. Enzyme Test Results from Black Soldier Fly Larvae Bacteria showed that from 26 isolates, only one isolate did not produce the enzyme amylase, namely isolate KO+KP 1 T4 $10^{-(-1)}$) (Figure 2 and Figure 3). In comparison, the other 25 isolates produced amylase enzymes, with details of 4 isolates (+), five isolates (++), and 16 isolates (+++). The (+++) isolate is an isolate that produces all clear zones in its quadrant, namely isolate KP 2. T2 $10^{-(-1)}$), KP 3. T2 $10^{-(-3)}$), KP 2. T510$^{-(-2)}$), KP 3 T5 $10^{-(-2)}$), KP 5. T5 $10^{-(-2)}$), KP 5. T5 $10^{-(-3)}$), KO 2. T1 $10^{-(-2)}$), KO 6. T1 $10^{-(-2)}$), KO + KP 2. T5 $10^{-(-1)}$), KO+KP 3 T5 $10^{-(-2)}$), KO+KP 5 T5 $10^{-(-2)}$), Control 4. T2 $10^{-(-2)}$), Control 2. T4 $10^{-(-1)}$), Control 3. T4 $10^{-(-1)}$), Control 7. T4 $10^{-(-3)}$).

The results showed that each colony had quite a high amylase activity, especially in bacterial isolates. According to research (Muharram, 2021), bacteria have the highest activity value taken from the crude extract of Black Soldier Fly larvae. The activity of the crude extract is very high because all components of the body of the Black Soldier Fly larvae include the microbial consortium. Differences in the activity of amylase production depend on the microorganism's genus, species, and strain. In addition, sources of microbial origin and environmental factors such as pH, temperature, and feed sources will affect the production of amylase enzymes from Black Soldier Fly larvae microorganisms.

Table 1. Enzyme Assay Results of Yeast Isolated from Black Soldier Fly Larvae

Isolate	Amylase Test	Cellulase Test	Protease Test
OY 5. T1(10--3)	+++	++	++
PY 2.T4(10--2)	+++	+	+
Control 2. T4(10--2)	+++	+	+
OY+PY 2.T5 (10--1)	++	+++	-
OY+ PY 1.T4 (10--2)	++	++	-
OY 4.T4 (10--2)	++	+	+
OY + PY 5.T3 (10--1)	++	-	+
OY + PY 1.T3 (10--2)	++	-	-
OY + PY 1.T1 (10--3)	+	+++	+
Control 4. T3(10--1)	+	+++	+
Control 3. T2 (10--2)	+	++	+
Control 3. T1 (10--2)	+	++	-
PY 5.T1(10--3)	+	++	-
OY + PY 1.T1 (10--2)	+	+	+
PY 1.T5(10--3)	+	+	-
PY 4. T5(10--1)	+	-	+
Control 1. T5(10--1)	+	-	+
OY 1.T3 (10--3)	+	-	+
PY 1. T2(10--1)	+	-	-
PY 4. T4(10--3)	-	+	-

Cellulase Assay

Cellulase enzymes can be synthesized by various living things, including bacteria, fungi, and plants (Madadi, 2017). In this experiment, cellulase tests were carried out on bacteria and yeast microorganisms in BSF larvae fermentation. It is done by growing bacteria and yeast on CMC (Carboxyl Methyl Cellulose) media. CMC is used as a selective medium to allow the growth of bacteria that can digest cellulose because it only contains cellulose as an energy source (Guder et al., 2019). After growing on CMC media, then tested using congo red for 15 minutes and then cleaned using NaCL with five rinses. The size of the cellulolytic activity is indicated by the value of the cellulolytic index, which is the value that expresses the quotient of the diameter of the clear zone formed by the diameter of the growing colony.

The results of the study can be seen in Table 1. Enzyme Test Results from Black Soldier Fly Yeast Larvae showed that from 20 isolates, there were 14 isolates positive for cellulase production, with details of 6 isolates (+), five isolates (++), and three isolates (+++) (Figure 5 and Figure 6). The (+++) isolate was an isolate that produced all clear zones in its quadrant. Namely, there were isolates KO+KP 1. T1 $10^{-(-3)}$, KO+KP 2. T5 $10^{-(-1)}$, and Control 4. T3 $10^{-(-1)}$.

From the research results, it can be seen from Table 2. Enzyme test results from Black Soldier Fly Larvae bacteria obtained results from 26 isolates, 20 isolates positively produced cellulase enzymes, with details of 6 isolates (+), six isolates (++), and eight isolates (+++). The (+++) isolate is an isolate that produces clear zones in all its quadrants, namely isolate Ko+Kp 4. T1 $10^{-(-1)}$, Ko+Kp 4. T1 $10^{-(-2)}$, Ko+Kp 3 . T3 $10^{-(-3)}$, Ko+Kp 1. T4 $10^{-(-2)}$, Ko+Kp 4. T4 $10^{-(-2)}$, Ko+Kp 2. T5 $10^{-(-1)}$, Ko+ Kp 3. T5 $10^{-(-2)}$, and Ko+Kp 5. T5 $10^{-(-2)}$.

Figure 4. Cellulase enzyme assay in yeast

Figure 5. Cellulase enzyme assay in bacteria

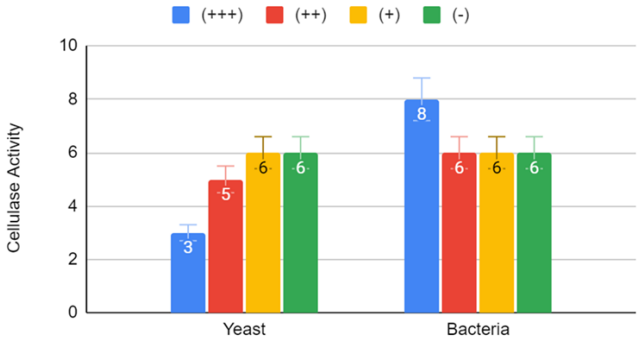

Figure 6. Results of cellulase activity assay in microorganisms obtained from Black Soldier Fly larvae. Error bars in the figures represent standard deviation

The results showed that each colony had quite a high cellulase enzyme activity. The cellulase enzyme is an enzyme that can hydrolyze β(1-4) bonds in cyclodextrin cellulose, cellobiose, and other cellulose derivatives into simple sugars or glucose (Idiawati,

2014). The presence of cellulose in a substrate can induce the formation of cellulase enzymes by cellulolytic microorganisms. Cellulase enzymes are obtained from a mixture of endoglucanase, exoglucanase, and β-glucosidase enzymes, which can be produced by microorganisms, both yeast and bacteria (Sulistyarsi et al., 2016). The system for breaking down cellulose into glucose consists of three types of cellulase enzymes, namely endo-β-1.4-glucanase, exo-β-1.4-glucanase, and β-glucosidase. Exo- β-1.4-D-glucanase which breaks down cellulose from reducing and non-reducing ends to produce cellulose and or glucose, and β-glucosidase (cellobiase), which breaks down cellobiose to produce glucose (Nababan et al., 2019).

Table 2. Enzyme Assay Results of Bacteria Isolated from Black Soldier Fly Larvae

Isolate	Amylase Test	Cellulase Test	Protease Test
OY + PY 3. T5(10--2)	+++	+++	+
OY + PY 2. T5(10--1)	+++	+++	-
OY + PY 5. T5(10--2)	+++	+++	-
Control 3. T4 (10--1)	+++	++	++
Control 7. T4 (10--3)	+++	++	+
Control 4. T5 (10--3)	+++	++	+
Control 4. T2 (10--2)	+++	++	-
OY 6.T1 (10--2)	+++	+	++
PY 2.T2 (10--1)	+++	+	+
PY 5.T5 (10--3)	+++	+	+
PY 5.T5 (10--2)	+++	+	-
Control 2. T4 (10--1)	+++	+	-
OY 2.T1 (10--2)	+++	-	++
PY 3.T5 (10--2)	+++	-	++
PY 3.T2 (10--3)	+++	-	-
PY 2.T5 (10--2)	+++	-	-
OY + PY 4. T1 (10--1)	++	+++	+
OY + PY 4. T1 (10--2)	++	+++	-
Control 1. T3 (10--1)	++	++	+
OY 3.T4 (10--2)	++	++	-
OY 2.T2 (10--1)	++	-	+
OY + PY 4. T4(10--2)	+	+++	++
OY + PY 3. T3 (10--3)	+	+++	-
OY 4.T3 (10--2)	+	+	+
OY 3.T4 (10--1)	+	-	+
OY + PY 1. T4(10--2)	-	+++	+

Protease Assay

Proteases are protease peptidases or proteolytic enzymes, which are a group of enzymes that catalyze the hydrolysis of peptide bonds (Aladdin et al., 2017). Protease enzymes are biocatalysts for protein breakdown reactions. This enzyme will catalyze hydrolysis reactions involving the element of water in substrate-specific bonds (Mahdiyah, 2015). Therefore, this enzyme is included in the main group of hydrolase enzymes. Proteases are very complex enzymes possessing various physicochemical and catalytic properties. Proteases can be produced extracellularly and intracellularly and have an important role in cell metabolism and the regularity of processes within cells.

Figure 7. Protease enzyme assay in yeast

Figure 8. Protease enzyme assay in bacteria

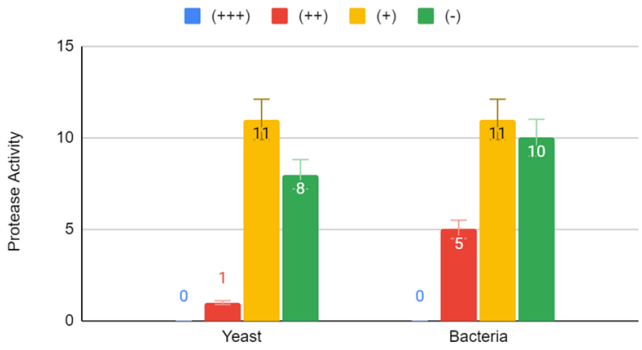

Figure 9. Results of protease activity assay in microorganisms obtained from Black Soldier Fly larvae. Error bars in the figures represent standard deviation

Proteases have been categorized based on several standards; proteases are classified according to the position of the peptide bond, which is split into two major groups exopeptidases and endopeptidases (Malek et al., 2016). They can also be classified as an acidic range (pH 2.0 to 6.0), neutral (pH 6.0 to 8.0) or alkaline (pH 8.0 to 13.0) proteases (Souza et al., 2015). In this experiment, a protease test was carried out on bacteria and yeast microorganisms on BSF larvae fermented feed. Growing bacteria and yeast do it on protease test media. Isolates were streaked with five lines on a petri dish containing media for the protease test. The isolate with the most cellulase enzyme activity was seen from the clear zone. The presence of a clear zone around the colony indicates a positive reaction. The size of the proteolytic activity is indicated by the value of the proteolytic index, which is the value that expresses the quotient of the diameter of the clear zone formed by the diameter of the growing colony.

From the results of the study, it can be seen in Table 1. Enzyme Test Results from Black Soldier Fly Yeast Larvae obtained results from 20 isolates; 12 isolates were positive for producing protease, with details of 11 isolates (+) and one isolate (++) (Figure 7, and Figure 8). Isolate (++) is an isolate that produces a clear zone in its half quadrant, found in isolate KO 5. T1 10-(-3). The results of the study can be seen in Table 2. Enzyme test results from Black Soldier Fly Larvae bacteria obtained results from 26 isolates; 16 were positive for producing protease enzymes, with details of 11 isolates (+) and five isolates (++). Isolate (++) is an isolate that produces a clear zone in its half quadrant: isolate KP 3. T5 $10^{-(-2)}$, KO 2. T1 $10^{-(-2)}$, KO 6. T1 $10^{-(-2)}$, KO + KP 4. T4 $10^{-(-2)}$, and Control 3. T4 $10^{-(-1)}$ (Figure 9).

The results showed that each colony had quite a high amylase activity, especially in bacterial isolates. Bacteria have high proteolytic activity in the logarithmic phase because the cells are in optimum conditions for metabolism and reproduction. The research results show that the five isolates that produce clear zones around the colonies grow optimally at 48 hours. The protease enzyme activity is influenced by many factors, namely temperature, pH, media concentration, and incubation time (Mahdiyah, 2015). The protease activity increases with increasing temperature until the optimum temperature is reached, after which a further increase will cause the protease activity to decrease. At temperatures lower than the optimum temperature, enzyme activity is also low due to the low available activation energy. This energy is needed to create conditions at the complex functional level from the enzyme and substrate molecules. In extreme changes in pH, enzymes can experience denaturation due to interference with various non-covalent interactions that maintain the stability of the 3-dimensional structure of enzymes (Mahdiyah, 2015).

The study results showed nine isolates, namely eight bacterial isolates and one yeast isolate, whose isolates had multiactivity or could produce several enzymes and clear zones in all quadrants (+++). From these observations, out of 20 yeast isolates, only one had the multiactivity ability. In contrast, for bacteria, out of 26 bacterial isolates, there were eight bacteria that had multiactivity. According to Wills et al. (2012), a bacterial isolate can adaptation environmental conditions, causing bacteria to try to adapt by producing several enzymes for survival.

Table 3. Protease, Amylase, and Cellulase Enzyme Assay Results on Black Soldier Fly Bacteria and Yeast Larvae

Enzyme Assay	Bacteria	Yeast
Amylase + Protease	PY 3. T5 (10--2) OY 2. T1(10--2) OY 6. T1(10--2)	OY 5. T1(10--3)
Amylase + Cellulase	OY + PY 2. T5(10--1) OY + PY 3. T5(10--2) OY + PY 5. T5(10--2)	
Cellulase + Protease	OY + PY 4. T4(10--2)	
Amylase+Protease+Cellulase	Control 3.T4(10--1)	

From the observations based on Table 3. Protease, Amylase, and Cellulase Enzyme Test Results on Bacteria and Yeast Larvae of Black Soldier Fly on bacteria for isolates that produce amylase + protease enzymes and clear zones in all quadrants. There are three isolates: KP 3 T5 (10^{-2}) and KO 2. T1 (10^{-2}), KO 6. T1 (10^{-2}). Meanwhile, there is one isolate for yeast isolates that produce amylase + protease enzymes and clear zones in all quadrants, namely KO 5. T1 (10^{-3}). In bacteria for isolates that produce amylase + cellulase enzymes and clear zones in all quadrants, namely isolates, there are three isolates, including isolates KO + KP 2. T5 (10^{-1}), KO + KP 3 T5 (10^{-2}), KO + KP 5 T5 (10-2). For bacteria for isolates that produce cellulase + protease enzymes and produce clear zones in all quadrants, there is one isolate, namely KO + KP 4. T4 (10^{-2}). In addition, bacteria also contain isolates that produce all enzymes, namely amylase + cellulase + protease and produce clear zones in all quadrants, namely Control isolate 3. T4 (10^{-1}).

Conclusion

Our investigation of enzymes in BSF larvae fermentation revealed the presence of amylase, cellulase, and protease enzymes. Isolation using the spread plate method yielded 46 isolates, comprising 26 bacterial and 20 yeast isolates. Notably, amylase screening identified

25 bacterial and 19 yeast isolates with positive amylase activity, while cellulase screening yielded 20 bacterial and 14 yeast isolates positive for cellulase enzymes. Additionally, protease screening identified 16 bacterial and 12 yeast isolates producing protease enzymes. Remarkably, nine isolates exhibited multiactivity, with eight bacterial isolates and one yeast isolate producing multiple enzymes. Among these, one isolate produced amylase, cellulase, and protease enzymes, while the remaining eight produced a combination of two enzymes, including amylase, cellulase, or protease. These findings highlight the diverse enzymatic potential within BSF-associated microorganisms, holding promise for various industrial applications.

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Conflict of Interest
All authors involved in this research declare that there is no conflict of interest.

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