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ANTIFUNGAL, HYDROLYTIC ENZYME ACTIVITY, AND IDENTIFICATION OF GUT BACTERIAL IN FECES OF BLACK SOLDIER FLY (*Hermetia illucens*) LARVAE

AKTIVITAS ANTIFUNGI, ENZIM HIDROLITIK DAN IDENTIFIKASI BAKTERI PENCERNAAN DARI FESES LARVA BLACK SOLDIER FLY (*Hermentia illucens*)

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Abstract

Black soldier fly (BSF) (*Hermetia illucens*) is a type of fly that has larvae called maggots with potential as a waste bioremediation agent. Maggot from BSF has unique digestive characteristics with a diversity of bacteria in it which helps maggot digest organic materials by producing various hydrolytic enzymes. Fecal bacteria in maggots also have potential antifungal activity. This research aimed to identify the hydrolytic and antifungal enzyme activity of maggot fecal bacteria (*Hermetia illucencs*). Hydrolytic enzyme activity is carried out by measuring the hydrolytic zone in the test medium. The antifungal antagonist test was carried out on *Phytophthora* sp. using the dual agar culture method. The results of the enzyme activity test showed that isolate MNM 001 had proteolytic enzyme activity, MNM 002 had amylolytic, and proteolytic enzyme activity, and was able to dissolve P elements. MNM 003 had cellulolytic, amylolytic, and proteolytic enzyme activity. From the results of the antagonist test, MNM 001, MNM 002, and MNM 003 have antifungal activity against *Phytophthora* sp. of the three isolates, isolates MNM 002 and MNM 003 had the best hydrolytic enzyme activity and were identified using the 16S rRNA gene. The results of amplification of the 16S rRNA gene from MNM 002 and MNM 003 indicated that the two isolates were close to the genus Brevibacterium.

Keywords: Antifungi; Gut bacterial; Hydolitics enzyme; Maggot

Abstrak

Black soldier fly (BSF) (Hermetia illucens) adalah jenis lalat yang memiliki larva disebut maggot dengan potensi sebagai agen bioremediasi sampah. Maggot dari BSF memiliki karakterisitik pencernaan unik dengan keragaman bakteri di dalamnya yang membantu maggot mencerna bahan organik dengan menghasilkan beragam enzim hidrolitik. Bakteri pada feses maggot juga memiliki potensi aktivitas antifungi. Tujuan dari penelitian ini adalah untuk mengidentifikasi aktivitas enzim hidrolitik dan antifungi dari bakteri feses maggot (Hermetia illucens). Aktivitas enzim hidrolitik dilakukan dengan mengukur zona hidrolitik pada medium uji. Uji antagonis antifungi dilakukan terhadap Phytophthora sp. dengan metode dual culture agar. Hasil uji aktivitas enzyme menunjukkan isolat MNM 001 memiliki aktivitas enzim proteolitik, MNM 002 memiliki aktivitas enzim amilolitik, proteolitik, dan mampu melarutkan unsur P. MNM 003 memiliki aktivitas enzim selulolitik, amilolitik, dan proteolitik. Hasil uji antagonis MNM 001, MNM 002, dan MNM 003 memiliki aktivitas antifungi terhadap Phytophthora sp. Ketiga isolat tersebut, isolate MNM 001, MNM 002, dan MNM 003 memiliki aktivitas enzim hidrolitik terbaik dan diidentifikasi menggunakan gen 16S rRNA. Hasil amplifikasi gen 16S rRNA dari MNM 002 dan MNM 003 mengindikasikan bahwa kedua isolat tersebut memiliki kedekatan dengan genus Brevibacterium.

***Kata Kunci**: Antifungi; Enzim hidrolitik; Gut Bacterial; Maggot*

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INTRODUCTION

The black soldier fly (*Hermetia illucens*) is a saprophytic insect in the family *Stratiomyidae* and Ordo *Diptera*. Popa and Green (2012) mentioned that the Black Soldier Fly (BSF) had 4 phases of the lifecycle, starting with egg, larvae, pupa, and mature insect phase. BSF larvae, known as maggots, had a 1.8–20 mm length (Liu et al., 2019). Maggots will eat everything in front of them in their living media after hatching (Park et al., 2015). Maggot could digest organic waste and loosen it into smaller parts of particles (Pendyurin et al., 2021). Faizin et al. (2021) mentioned that maggots had a unique digestion characteristic to be able to absorb the solution, digest organic waste, and high tolerance to heat and acid.

The ability of maggots to digest organic waste is powered by the role of gut bacteria living in the maggot's digestion system (Zhineng et al., 2021). Several studies verified that gut bacteria living in maggot's digestion system help them to digest the organic waste (De Smet et al., 2018; Nguyen et al., 2015). A study by Tegtmeier et al. (2021) found 26 isolates of gut bacteria within the digestion system of maggots that had the potential as antimicrobials. Gut bacteria in maggots also had the potential in bioremediation to reduce antibiotic pollution from cattle waste (Yang et al., 2022). Maggot is utilized as a bioremediation agent based on their antimicrobial peptides ability by gut bacteria inside them that have antagonistic activity against zoonotic pathogens (Elhag et al., 2022). Besides being antibacterial, gut bacterial maggots also had the potential to be antifungal like other gut bacteria from several insects (Zhang et al., 2018)

One of the fungi pathogens that often attack plants is *Phytophthora* sp. which belongs to *Oomycota* and ordo *Peronosporales* as the genera have become interesting topics among researchers of their harmful impact on their host plants (Scott et al., 2019). Based on an analysis by Brasier et al. (2022) regarding *Phytophthora* sp. adaptability, *Phytophthora* sp. had good adaptability, which makes it a high-risk pathogen and remains difficult decease to control. *Phytophthora* sp. caused late blight disease in potatoes and tomatoes (Liswani et al., 2018). The species that causes late blight on tomatoes and potatoes is *P. infestans*. Meanwhile, the species that causes the disease in pepper is *P.capsica* (Švecová et al., 2017) Due to their excellent adaptability, *Phytophthora* sp. often has resistance towards fungicides.

The alternative against the activity of *Phytophthora* sp. is using bacteria with antifungal activity. The antifungal activity of bacteria is based on their bioactive metabolites such as bioactive compounds, lactic acid, acetic acid, fatty acid, hydrogen peroxide, and other metabolites produced by bacteria (Alaoui et al., 2021). That metabolite will actively inhibit the mycelium growth of *Phytophthora* sp.; according to a study by Diniz et al. (2022), bacteria with antifungal activity identified as bacteria with the capability to produce hydrolytic enzymes such as cellulose, protease, and chitinase. Masrukhin et al. (2021), found that bacteria with the highest antifungal activity were identified as bacteria that can produce the metabolite Bis(2-ethylexyl). Bacteria with chitinolitic activity also had an antifungal activity. This is proven by the study of the chitinase enzyme produced by *Streptomyces alfalfa* could inhibit the growth of fungal pathogens (Lv et al., 2021). The chitinase enzyme inhibits the growth of fungal pathogens by breaking glycosidic bonds, which are the primary components of fungal cell walls (Alaoui et al., 2021)

Several bacteria found to have antifungal activity produce lactic acid and have bioactive metabolite activity. A study by Zhang et al. (2018) succeeds in identifying gut bacteria with antifungal activity against *Phytophthora* sp. isolated from the cockroach digestion system. There are few studies about the antifungal activity of gut bacteria against *Phytophthora* sp. Meanwhile, *Phytophthora* sp. has become a severe trait to tomato and potato farmers in Indonesia. Therefore, this study intends to identify bacteria from BSF larvae feces that have antifungal activity against *Phytophthora* sp. that have a potency as biofungicide to overcome *Phytophthora* sp. infection on tomato and potato plants.

MATERIALS AND METHODS

Gut Bacterial Isolation

The sample was from maggot cultivation waste or maggot feces from Biotek Cipta Kreasi. Samples were kept in sterile plastic bags tightly closed for further analysis. Gut Bacterial was isolated

from maggot *H.illucens* feces by pour plate method using serial dilution. At 10^{-3}, 10^{-4}, and 10^{-5} dilutions, the samples were heated at 80 °C for 15 minutes. Then 100 µL samples from 10^{-3}, 10^{-4}, and 10^{-5} dilutions were spread into nutrient agar and incubated at 37 °C for 48 hours. Each dilution level was spread into a plate of nutrient agar.

The result of the isolation was purified and transferred to a new plate of nutrient agar. Determination of pure isolates using characteristic determination. The colony characterization was determined with the same color of the colony, the same shape, and the same morphological appearance. The results of purification were incubated at 37 °C for 48 hours.

Antagonistic Assay Towards *Phytophthora* sp.

Antifungal activity was determined using dual culture antagonistic assay. Bacterial isolates and *Phytophthora* sp. inoculated in the same plate of potato dextrose agar (PDA). The observation was made after 48 hours of incubation, and a clear zone formed around the colony to indicate the inhibition of *Phytophthora* sp. growth. The inhibition index of gut bacteria indicates the result of the antagonistic assay isolates towards *Phytophthora* sp. The inhibition index was measured using the formula IP $= \frac{R1-R2}{R1} \times 100\%$. IP = Inhibition index (%); R1= = colony diameter of fungi pathogen in control; R2= colony diameter of fungi pathogen in assay. The criteria of the inhibition index is 70–100 (++), the inhibition index is 40–59 (+), and the inhibition index is 0–39 (-) (Utami & Mujahidin, 2020).

Characterization of Hydrolytic Enzyme Activity

Cellulolytic Assay

The cellulolytic assay was used to identify the enzymatic activity of isolates to produce cellulose enzyme cellulose degrader (Murtiyaningsih & Hazmi, 2017) A qualitative assay of the enzymatic assay was determined after inoculating isolates to carboxymethyl cellulose (CMC) and detecting the clear zone using Congo red solution (Ferbiyanto et al., 2015). Isolates were incubated in CMC agar for 48 hours and dripped with Congo red to detect the clear zone. The enzymatic activity index was calculated using the formula from Dewiyanti et al. (2022), $\text{hydrolytic index} = \frac{\text{clear zone diameter} - \text{colony diameter}}{\text{colony diameter}}$.

Chitinolytics Assay

Chitinolytics assay was used to identify the enzymatic activity of isolates that produce the chitinase enzyme chitin degrader (Setia et al., 2015). The substrate used for the chitinolytic assay was chitin agar (0.4 g chitin, 0.07 g K_2HPO_4, 0.03 g KH_2PO_4, 0.05 g $MgSO_4$, $7H_2O$, 2 g agar, 1 mL/L trace element) dissolved in 100 mL distilled water. In the chitinolytic assay, the pH of the media must be considered and be at pH 6.7–8 so that the enzymatic activity can occur optimally (Nafisah et al., 2017). Enzymatic activity was detected after a clear zone formed for 7–10 days of incubation (Paulsen et al., 2016).

Amylolytics Assay

Amylase activity was conducted using the plug agar method in amylum agar (Wulandari et al., 2019.) Isolates were inoculated to amylum agar and incubated for 24–48 hours until a clear zone formed, detection of clear zone formation using Lugol solution. According to a study by Setyati et al. (2016) and Wulandari et al. (2019), a clear zone was formed due to amylase enzyme activity from isolates bacteria that hydrolyze amylum in agar.

Lipolytics Assay

An assay of lipolytic enzyme activity was inoculated on Tween agar media and incubated for 48 hours (Pham et al., 2021). Tween agar media was made from 0.5 g Tween-80, 0.05 g $CaCl_2$, 0.05 g K_2HPO_4, 0.05 g $NaNO_3$, 0.025 g $MgSO_4$, 0.025 g KCl, 0.01 g peptone, 0.75 g agar, and 50 mLaquades. Lipolytic enzyme activity is detected by whitish salt deposits around a colony of bacteria (Pham et al., 2021).

Proteolytic Assay

The proteolytic assay is used to identify the enzymatic activity of isolates to produce protease enzymes (Setiawan et al., 2016.).The assay was conducted using skim milk agar as substrate. Skim milk agar was used to hydrolyze proteins into amino (Fitriana et al., 2022). Isolates were incubated for 24–48 hours in skim milk agar. The proteolytic enzyme activity was detected by a clear zone formed due to hydrolysis of casein substrate activity in skim milk agar. The result of hydrolysis was used as a source of metabolism by the bacteria (Fitriana et al., 2022).

Phosphate and Potassium (Kalium) Solubility Assay

Phosphate and Potassium (kalium) are nutrients that play an essential role in plant growth. Potassium is absorbed by plants in the form of K^+ ions and has a role as a transport medium of nutrients from the soil into the plant (Nurmaliatik et al., 2021). A potassium solubility assay for bacteria was conducted using Alexandrov media agar (Sukmadewi et al., 2017) Potassium solubility activity was detected in the form of a clear zone around the bacteria colony. Phosphate solubility assay was conducted using plug agar method in Pikovskaya agar (0.05 g yeast extract, 1 g dextrose, 0.5 g $Ca_3 (PO_4)_2$, 0.05 g $(NH_4)_2 SO_4$, 0.02 g KCl, 0.01 g $MgSO_4$, 0.00001 g $MnSO_4$, 0.5 g $FeSO_4$, 1.5 g agar in 100 mL distilled water. Isolates were incubated for seven days at a temperature of 37 °C and detected by clear zone form. Index solubility was calculated using the formula from Sukmadewi et al. (2017), $\text{solubility index} = \frac{\text{diameter of clear zone} - \text{diameter of colony}}{\text{Diameter of colony}}$.

Identification of Gram Bacteria and Identification Using Gen 16S rRNA

Identification of Gram bacteria was conducted using the KOH method. Isolate bacteria were dripped with KOH 3% and withdrawn with a sterile loop wire. If the isolates formed mucus, the bacteria was a gram-negative bacteria. Otherwise, if the isolates didn't form mucus, the bacteria was a gram-positive bacteria.

Identification of gut bacteria isolates using gen 16S rRNA analysis. Gen 16S rRNA is the most comprehensive gen marker because it has the best resolution of phylogeny and has the most complete database (Yang et al., 2016). Isolates with high antagonistic index were identified using isolation kit bacteria. Isolates were going through the PCR process using forward primer 63f (5'-CAGGCCTAACACATGCAAGTC-3') and reverse primer 1387r (5'-GGGCGGWGTGTACAAGGC-3') (Marchesi et al., 1998). Mix PCR used were 20µL consisting of 10µL DNA Polymerase, 1µL from each primer, 6µL NFW, and 2µL template. Isolates went through a PCR process with setting pre-denaturation (95 °C for 4 minutes), denaturation (95 °C for 30 seconds), annealing (57 °C for 30 seconds), elongation (72 °C for 1 minute) and post-PCR (72 °C for 7 minutes) in 30 cycles. The product was visualized using agarose gel electrophoresis.

The results of PCR are sequence data, then analyzed using Seq-Trace until sequence data that ready to analyze with BLAST from NCBI. The BLAST results were then aligned using MEGA 11 to find out the genetic relationship and phylogeny of isolates towards another bacteria from the National Center for Biotechnology Information (NCBI) database.

RESULTS

Isolates Purification

A total of 7 isolates collected from maggot feces with different morphology were successfully purified on the medium nutrient agar. The macroscopic morphology of 7 isolates is shown in Table 1.

Table 1. Macroscopic morphology of gut bacterial isolated from maggot feces from maggot cultivation waste

Isolates	Macroscopic morphology of colonies			
	Shape	Margin	Color	Elevation
MNM 001	Irregular	Entire	Whitish	Flat
MNM 002	Irregular	Entire	Cream and whitish	Flat
MNM 003	Circular	Entire	White	Flat

Isolates	Macroscopic morphology of colonies			
	Shape	Margin	Color	Elevation
MNM 004	Circular	Entire	Whitish	Flat
MNM 005	Circular	Lobate	Whitish	Flat
MNM 006	Circular	Entire	Cream	Flat
MNM 007	Irregular	Lobate	White with the presence of yellow coloration from the surface	Raised

Antagonistic Activity of Isolates Gut Bacterial Towards *Phytophthora* sp.

The agar had been inoculated with *Phytophthora* sp. in the middle of the agar plate. After incubated for 24 hours, isolates were inoculated and incubated for the next 24 hours. Antifungal activity results were observed after a total of 48 hours of incubation by measuring the mycelium diameter of *Phytophthora* sp. in the control agar plate and agar plate inoculated with isolates. Based on an antagonistic test towards *Phytophthora* sp., 3 of 7 isolates were found to be able to inhibit *Phytophthora* sp. Isolates with antifungal activity were isolates from 10^{-3} dilutions (MNM 001) with R2 2.4 cm, isolates from 10^{-4} dilutions (MNM 002) with R2 2 cm; isolates from 10^{-5} dilutions (MNM 003) with R2 2.6 cm. The antifungal activities of the three isolates are shown in Table 2.

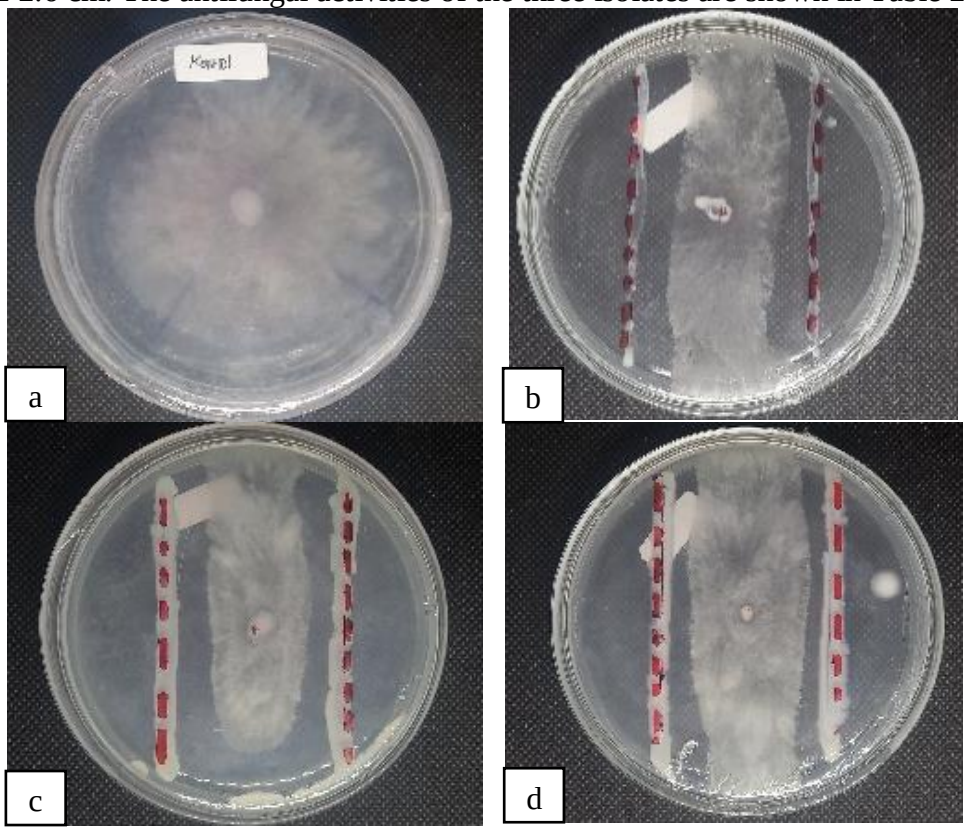

Figure 1. Control antagonistic assay, antifungal activity towards *Phytophthora* sp. (a), MNM 001 isolates (b), MNM 002 isolates (c), and MNM 003 isolates (d)

Based on the antagonistic test of isolates towards *Phytophthora* sp., the highest level of antifungal activity was shown by MNM 002 isolate with an inhibition index of 0%, as indicated by the shortest diameter of *Phytophthora* sp. growth. The highest antifungal activity of isolates is shown in Figure 1.

Table 2. Antifungal activity of gut bacterial isolated from maggot feces from maggot cultivation waste

Isolates	The diameter of *Phytophthora* sp. (cm)		Inhibition index (IP) (%)
	R1 (control)	R2 (treatment)	
NM 001	4	2.4	40
MNM 002	4	2	50
MNM 003	4	2.6	35

Isolates	The diameter of *Phytophthora* sp. (cm)		Inhibition index (IP) (%)
	R1 (control)	R2 (treatment)	
MNM 004	4	4	0
MNM 005	4	4	0
MNM 006	4	4	0
MNM 007	4	4	0

Extracellular Enzyme Production of Gut Bacterial Isolates

After the antagonistic assay, isolates with antifungal activity are referred to as MNM 001, MNM 002, and MNM 003. Identification of extracellular enzyme production was done to determine the ability of the isolates to produce hydrolytic cellulase, chitinase, amylase, protease, and lipase enzymes. Screening was also done to determine the ability of the isolates to dissolve phosphate and potassium ions. The results of the hydrolytic enzyme assay are shown in Figure 2.

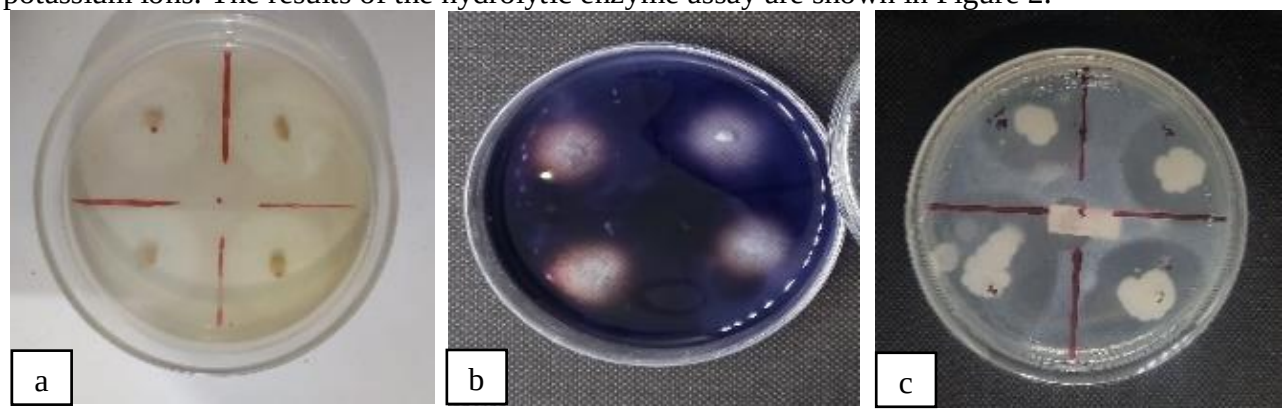

Figure 2. Hydrolytic enzyme assay of gut bacterial isolated from maggot feces on cultivation waste cellulolytic assay (a), amylolytic assay (b), and proteolytic assay (c)

From the cellulolytic enzyme assay, MNM 003 tested positive to had the ability to hydrolyze cellulose. It is indicated by the clear zone formed after the drip congo red solution to the CMC medium (Figure 2a). There were no isolates with the ability to hydrolyze chitin, as indicated by the absence of a clear zone after 7 days of incubation. MNM 002 and MNM 003 were tested with the ability to hydrolyze the starch in the medium used for amylolytic assay (Figure 2b). Meanwhile, in the lypolytic assay, there were no isolates tested positive to hydrolyze lypose in tween medium. All of the isolates MNM 001, MNM 002, and MNM 003, tested positive to hydrolyze the skim with the highest hydrolytic index of 1,41 from MNM 003 (Table 2, Figure 2c). It can be detected in the form of a clear zone after dripping lugol solution into the skim milk agar medium. MNM 002 was the only isolate with the ability to dissolve phosphate in the Pikovskaya medium, as indicated by a clear zone formed after 6 days of incubation. Meanwhile, no isolates formed a clear zone in the Alexandrov medium after 7 days of incubation. The results of hydrolytic enzyme and phosphate & and potassium solubility are presented in Table 3.

Table 3. Hydrolytic activity enzyme produced by gut bacterial isolates from maggot feces in maggot cultivation waste

Isolates	Hydrolytic index						
	Cellulolytic activity	Chitinolytic activity	Amylolytic activity	Lipolytic activity	Proteolytic activity	Phosphate solvent activity	Potassium solvent activity
MNM 001	-	-	-	-	0.89	-	-
MNM 002	-	-	4.03	-	0.82	0.75	-
MNM 003	4.97	-	2.5	-	1.41	-	-

Molecular and Gram Identification of Fecal Bacterial Isolates

After the hydrolytic enzyme assay, two isolates showed the best hydrolytic enzyme activity. The isolates, MNM 002 and MNM 003 were tested further for molecular and gram identification. Based on the gram identification, MNM 002 and MNM 003 both identified as gram-positive bacteria.

Molecular identification was performed using the amplification of gen 16S rRNA. The alignment results of sequence MNM 002 and MNM 003 aligned with BLAST showed that the isolates MNM 002 and MNM 003 had a resemblance to the *Brevibacterium* sp. The BLAST results are shown in Table 4. Furthermore, the BLAST results were aligned using MEGA 11 and the phylogenetic relationship of MNM 002 and MNM 003 with recognized members of *Brevibacterium* sp. shown in Figure 3.

Table 4. Similarity presentation gen 16S rRNA sequence of isolate MNM 002 and MNM 003

Isolates	Species affiliation (GenBank)	Query Cover	E value	Similarity	Accession number
MNM 002	*Brevibacterium linens*	97%	0.0	86.37%	KR140272.1
MNM 003	*Brevibacterium epidermidis*	100%	0.0	100%	KY992553.1

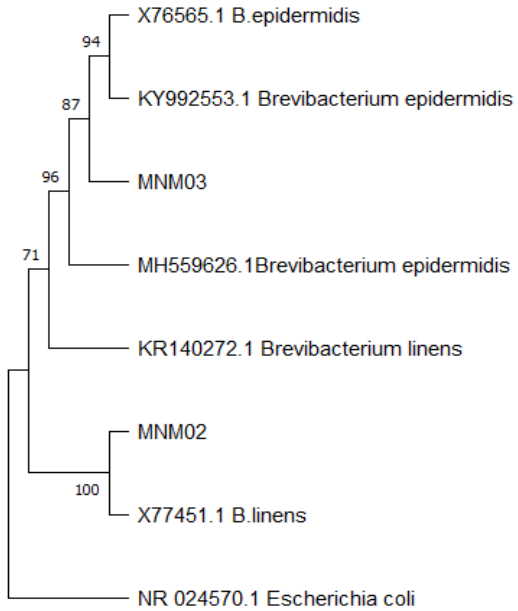

Figure 3. Phylogenetic tree based on 16S rRNA gene sequence, showing the phylogenetic relationship of isolate MNM 002 and MNM 003 with a recognized member of *Brevibacterium* sp.

DISCUSSION

A total of 7 isolates with different macroscopic morphology were collected from maggot feces and were tested for their antifungal activity towards *Phytophthora* sp. (Table 1). The result of the antagonistic assay was found 3 isolates have antifungal activity towards *Phytophthora* sp. (Figure 1 and Table 2). This result is under the study by Kim and Kim, (2020) that evidence of antifungal activity towards *Phytophthora* sp. by gut bacteria from *Allomyrina dichotoma* larvae. The 3 isolates are referred to as MNM 001, MNM 002, and MNM 003.

The isolation was done from maggot cultivation waste with some gut bacteria left in there. Sampling from maggot feces in maggot cultivation waste was based on a study by Pendyurin et al. (2021) which showed that maggot cultivation waste includes zoo compost. To become an ideal zoo compost, the cultivation waste needs the activity of several microbes from the maggot digestion system taken out with maggot feces (Insam & de Bertoldi, 2007). This was also supported by a study by Wang et al. (2022) stated that earthworms found bacteria left in their feces and it could modify the soil composition. So, therefore, sampling and isolation were done to identify antifungal and hydrolytic enzyme activity in gut bacteria from maggot feces in maggot cultivation waste.

From the cellulolytic assay, MNM 003 tested positive to have the ability to produce cellulose hydrolytic enzyme that works to inhibit the performance of cellulose as a specific biomolecule cell wall of *Phytophthora* sp. (Figure 2a). A study by de Lourenço et al. (2020) found that *Phytophthora* sp. has a special genome consisting of specific biomolecules that can degrade cellulose of plant cell walls.

Based on a study done by Zhineng et al. (2021), found that fecal bacteria of a maggot (*H. illucens)* played a role in digesting organic material in a maggot. Fecal bacteria from maggot *H. illucens* have the potency to produce the enzyme cellulose degrader (Dantur et al., 2015) With that ability, gut bacteria from maggot *H. illucens* have a big potential to become antifungal agents, especially for *Phytophthora* sp. Since, *Phytophthora* sp. has characteristics such as its cell walls being composed of cellulose just like the other oomycetes (Wu et al., 2019). So, gut bacterial from maggot *H.illucens* has big potential to become an antifungal agent for *Phytophthora* sp. by degrading its cell walls using cellulose hydrolytic enzyme.

MNM 001, MNM 002, and MNM 003 all tested positive for proteolytic assay, as indicates to have the ability to hydrolyze skim in skim agar medium (Table 3). It is under the study of Jing et al. (2020) that gut bacteria from insects had the enzymatic activity as protein degrader. This result is related to the attack mechanism of *Phytophthora* sp. towards its host plant using protein genome elicitins to degrade the plant cell wall (Hardham, 2005). Bacteria with proteolytic enzyme activity inhibit the growth of *Phytophthora* sp. by degrading those genome proteins in *Phytophthora* sp. from the amylolytic assay, MNM 002, and MNM 003 tested positive for amylolytic enzyme activity (Figure 2b). A study done by Gandotra et al. (2018) evidence that the amylolytic enzyme activity of gut bacteria played a role in the digestion system and carbohydrate metabolism in insects. MNM 002 is the only isolate with Phosphate solubility activity (Table 3). The result is supported by a study done by Callegari et al. (2020) also identified the ability of gut bacteria from maggot *H.illucens* as phosphate phosphate solvent and recycle the element of phosphorus as the marker of phosphate ion.

Molecular identification was performed on the isolates with the best result of hydrolytic enzyme activity, MNM 002 and MNM 003. The isolates were identified using 16S rRNA gene analysis. The result of the aligned 16S rRNA gene sequence from BLAST had a resemblance to the *Brevibacterium* sp. MNM 002 had a resemblance to the *Brevibacterium linens* while MNM 003 had a resemblance to the *Brevibacterium epidermidis* (Table 4). The resemblance was convinced by the neighbor-joining tree result from MEGA that suggests the genetic closeness and phylogenetic relationship between MNM 002 with *Brevibacterium linens* and MNM 003 with *Brevibacterium epidermidis* (Figure 3)

Based on a study of Forquin-Gomez et al. (2014) succeeded in isolating *Brevibacterium linens* from cheese and isolating *Brevibacterium epidermidis* from mammals' epidermis. Mounier et al. (2009) state that *B. linens* are found in a habitat with a high salinity. Maggots consume food waste and can resist high salinity, so it makes *B. linens* able to live within the digestion system of maggots. Hanim et al. (2021) also support the possibility of *B.linens* living in a maggot digestion system. According to Bonomo et al. (2015), *B. linens* have high proteolytic enzyme activity same as high proteolytic enzyme activity in MNM 002. *B.linens* also have the potential to increase plant's salinity tolerance (Chatterjee et al., 2018). A study by Esikova et al. (2023) isolated *B. epidermidis* from soil and identified *B. epidermidis* as gram-positive bacteria, same as the MNM 003. Esikova et al. (2023) also mentioned that *B. epidermidis* has potential as a bioremediation agent, and under-gut bacterial maggot potential as a bioremediation agent.

With the antifungal activity of MNM 001, MNM 002, and MNM 003 towards *Phytophthora* sp., it can be developed as biofungiside bacteria-based. The ability of MNM 002 as a phosphate solvent and its phylogenetic relationship with *B. linens* can increase a plant's salinity tolerance making it a potential bacteria for organic fertilizer and increasing the plant's tolerance to extreme conditions (Chatterjee et al., 2018). The ability of MNM 003 to produce cellulose, amylase, and protease enzymes and its phylogenetic relationship with *B.epidermidis*, make it a bioremediation agent with the ability to degrade organic waste and degrade toxic chemicals in soils (Esikova et al., 2023; Handayani et al., 2021).

CONCLUSION

Based on the results, 7 gut bacteria were isolated from maggot feces. Three of 7 gut bacterial isolates from maggot feces in maggot *H.illucens* cultivation waste, MNM 001, MNM 002, and MNM 003 isolates showed antifungal activity towards *Phytophthora* sp. and have some hydrolytic enzyme activity such as cellulolytic, amylolytic, proteolytic, and phosphate solubility. MNM 002 is the isolate

with the highest antifungal activity and has also been shown to have amylolytic and proteolytic enzyme activity. MNM 002 is the only isolate with the ability as a phosphate solvent. MNM 001 is an isolate with proteolytic enzyme activity. The best hydrolytic enzyme activity was shown in MNM 003 with cellulolytic, amylolytic, and proteolytic enzyme activity. MNM 003 was the isolate with the highest ability to hydrolyze cellulose and skim, and MNM 002 was the isolate with the highest ability to hydrolyze starch. As the isolates with the most hydrolytic enzyme activity, MNM 002 and MNM 003 were tested for molecular identification using the 16S rRNA gene. The amplification of 16S rRNA shows the genetic closeness between MNM 002 and MNM 003 with the *Brevibacterium* genus.

In this study, the antifungal activity was tested antagonistically on one species of fungus, namely *Phytophthora infestant*. Still, it needs to be tested antagonistically against several types of phytopathogenic fungi from other taxa. This is because the antifungal bioactive compound content produced by isolates MNM 02 and MNM 03 has not been identified. And it is not yet known whether they have the same antifungal activity.

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REFERENCES

Alaoui, K., Chafik, Z., Arabi, M., Abouloifa, H., Asehraou, A., Chaoui, J., & Kharmach, E. Z. (2021). *In vitro* antifungal activity of *Lactobacillus* against potato Late blight *Phytophthora infestans*. *Materials Today: Proceedings*, *45*, 7725-7733. doi: 10.1016/j.matpr.2021.03.338.

Bonomo, M. G., Cafaro, C., & Salzano, G. (2015). Genotypic and technological diversity of *Brevibacterium linens* strains for use as adjunct starter cultures in 'Pecorino di Filiano' cheese ripened in two different environments. *Folia Microbiologica*, *60*(1), 61-67. doi: 10.1007/s12223-014-0341-3.

Brasier, C., Scanu, B., Cooke, D., & Jung, T. (2022). Phytophthora: an ancient, historic, biologically and structurally cohesive, and evolutionarily successful generic concept in need of preservation. *IMA Fungus*, *13*(1). doi: 10.1186/s43008-022-00097-z.

Callegari, M., Jucker, C., Fusi, M., Leonardi, M. G., Daffonchio, D., Borin, S., … Crotti, E. (2020). Hydrolytic profile of the culturable gut bacterial community associated with *Hermetia illucens*. *Frontiers in Microbiology*, *11*. doi: 10.3389/fmicb.2020.01965.

Chatterjee, P., Samaddar, S., Niinemets, Ü., & Sa, T. M. (2018). *Brevibacterium linens* RS16 confers salt tolerance to *Oryza sativa* genotypes by regulating antioxidant defense and H+ ATPase activity. *Microbiological Research*, *215*, 89-101. doi: 10.1016/j.micres.2018.06.007.

Dantur, K. I., Enrique, R., Welin, B., & Castagnaro, A. P. (2015). Isolation of cellulolytic bacteria from the intestine of *Diatraea saccharalis* larvae and evaluation of their capacity to degrade sugarcane biomass. *AMB Express*, *5*(1). doi: 10.1186/s13568-015-0101-z.

Diniz, G. de-F. D., Cota, L. V., Figueiredo, J. E. F., Aguiar, F. M., da Silva, D. D., Lana, U. G. de. P., … de Oliveira-Paiva, C. A. (2022). Antifungal activity of bacterial strains from maize silks against *Fusarium verticillioides*. *Archives of Microbiology*, *204*(89), 1-13. doi: 10.1007/s00203-021-02726-4.

De Smet, J., Wynants, E., Cos, P., Leen, C., & Campenhout, V. (2018). Microbial community dynamics during the rearing of black soldier fly larvae (*Hermetia illucens*) and impact on exploitation potential. *Applied and Environmental Microbiology*, *84*(9), e02722-17. doi: 10.1128/AEM.

De Lourenço, D. A, Branco, I., & Choupina, A. (2020). Phytopathogenic oomycetes: A review focusing on *Phytophthora cinnamomi* and biotechnological approaches. *Molecular Biology Reports,47*(11), 9179-9188. doi: 10.1007/s11033-020-05911-8.

Dewiyanti, I., Darmawi, D., Muchlisin, Z. A., Helmi, T. Z., Arisa, I. I., Rahmiati, R., & Destri, E. (2022). Cellulase enzyme activity of the bacteria isolated from mangrove ecosystem in Aceh Besar and Banda Aceh. *IOP Conference Series: Earth and Environmental Science*, *951*(1). doi: 10.1088/1755-1315/951/1/012113.

Elhag, O., Zhang, Y., Xiao, X., Cai, M., Zheng, L., Jordan, H. R., … Zhang, J. (2022). Inhibition of zoonotic pathogens naturally found in pig manure by black soldier fly larvae and their intestine bacteria. *Insects*, *13*(1). doi: 10.3390/insects13010066.

Esikova, T. Z., Akatova, E. V., & Solyanikova, I. P. (2023). Epsilon-caprolactam- and nylon oligomer-degrading bacterium *Brevibacterium epidermidis* BS3: characterization and potential use in bioremediation. *Microorganisms*, *11*(2). doi: 10.3390/microorganisms11020373.

Faizin, R., Athaillah, T., & Munawarah, N. (2021). *The prospect of cultivating maggot (black soldier fly larvae) to build the village economy and reduce household waste.* Paper presented at the International Conference on Science, Technology, and Modern Society (ICSTMS 2020). Retrieved from https://www.researchgate.net/publication/354685852_The_Prospect_of_Cultivating_Maggot_Black_Soldier_Fly_Larvae_to_Build_the_Village_Economy_and_Reduce_Household_Waste

Ferbiyanto, A., Rusmana, I., & Raffiudin, R. (2015). Characterization and identification of cellulolytic bacteria from the gut of worker *Macrotermes gilvus*. *HAYATI Journal of Biosciences*, *22*(4), 197-200. doi: 10.1016/j.hjb.2015.07.001.

Fitriana, N., & Asri, M. T., (2022). Aktivitas proteolitik pada enzim protease dari bakteri rhizosphere tanaman kedelai (*Glycine max* L.) di Trenggalek. *LenteraBio: Berkala Ilmiah Biologi*, *11*(1), 144-152.

Forquin-Gomez, M. P., Weimer, B. C., Sorieul, L., Kalinowski, J., & Vallaeys, T. (2014). The family *Brevibacteriaceae*. In E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, & F. Thompson (Eds.), *The prokaryotes: Actinobacteria* (pp. 141-153). Berlin-Heidelberg: Springer-Verlag.

Gandotra, S., Bhuyan, P. M., Gogoi, D. K., Kumar, A., & Subramanian, S. (2018). Screening of nutritionally important gut bacteria from the Lepidopteran insects through qualitative enzyme assays. *Proceedings of the National Academy of Sciences India Section B - Biological Sciences*, *88*(1), 329-337. doi: 10.1007/s40011-016-0762-7.

Handayani, D., Naldi, A., Larasati, R. R. N. P., Khaerunnisa, N., & Budiatmaka, D. D. (2021). Management of increasing economic value of organic waste with maggot cultivation. *IOP Conference Series: Earth and Environmental Science*, *716*(1). doi: 10.1088/1755-1315/716/1/012026.

Hanim, A. M., Purnamasari, W., Prasnowo, A., Wulandari, R., Aziz, S., & Indrasari, M. (2021). Maggot BSF cultivation development strategy as economic resilience during a pandemic. *SINERGI: Jurnal Ilmiah Ilmu Managemen*, *11*(2), 8-21. doi: 10.25139/sng.v11i2.4153.

Hardham, A. R. (2005). *Phytophthora cinnamomi*. *Molecular Plant Pathology*, *6*(6), 589-604. doi: 10.1111/j.1364-3703.2005.00308.x.

Insam, H., & de Bertoldi. (2007). Microbiology of the composting process. In L. Z. Diaz, M. de Bertoldi, W. Bidlingmaier, & E. Stentiford (Eds.), *Waste management series* (pp. 25-48). Elsevier.

Nurmaliatik, N., Inti, M., Nurhidayat, E., Anggraini, D. J., Hidayat, N., Nurhuda, M., ... Maryani, Y. (2021). Studi pengaruh pemberian pupuk kandang dan guano fosfat terhadap serapan kalium tanaman kacang hijau (*Vigna radiata* L.). *Jurnal Pertanian Agros*, *23*(1), 44-52.

Jing, T. Z., Qi, F. H., & Wang, Z. Y. (2020). Most dominant roles of insect gut bacteria: Digestion, detoxification, or essential nutrient provision? *Microbiome*, *8*(1). doi: 10.1186/s40168-020-00823-y.

Kim, J. Y., & Kim, B. S. (2020). Plant growth promotion and biocontrol potential of various phytopathogenic fungi using gut microbes of *Allomyrina dichotoma* larva. *Research in Plant Disease*, *26*(4), 210-221. doi: 10.5423/RPD.2020.26.4.210.

Liswani, Y., Nurbailis., & Busniah,M. (2018). Eksplorasi cendawan endofit dan potensinya untuk pengendalian *Phytophthora palmivora* penyebab penyakit busuk buah kakao. *Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia*, *4*(2). doi: 10.13057/psnmbi/m040223.

Liu, C., Wang, C., & Yao, H. (2019). Comprehensive resource utilization of waste using the black soldier fly (*Hermetia illucens* (L.)) (*Diptera: Stratiomyidae*). *Animals, 9*(349). doi: 10.3390/ani9060349.

Lv, C., Gu, T., Ma, R., Yao, W., Huang, Y., Gu, J., & Zhao, G. (2021). Biochemical characterization of a GH19 chitinase from *Streptomyces alfalfa* and its applications in crystalline chitin conversion and biocontrol. *International Journal of Biological Macromolecules*, *167*, 193-201. doi: 10.1016/j.ijbiomac.2020.11.178.

Marchesi, J. R., Sato, T., Weightman, A. J., Martin, T. A., Fry, J. C., Hiom, S. J., & Wade, W. G. (1998). Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. *Applied and Environmental Microbiology*, *64*(2), 795-799.

Masrukhin., Putri, A. L., Sulistiyani, T. R., Ilyas, M., Purnaningsih, I., Saskiawan, I., & Niam, M. Y. (2021). Antifungal activity of bacterial isolates from straw mushroom cultivation medium against phytopathogenic fungi. *Journal of Tropical Biodiversity and Biotechnology*, *6*(1). doi: 10.22146/jtbb.59235.

Mounier, J., Monnet, C., Jacques, N., Antoinette, A., & Irlinger, F. (2009). Assessment of the microbial diversity at the surface of Livarot cheese using culture-dependent and independent approaches. *International Journal of Food Microbiology*, *133*(1-2), 31-37. doi: 10.1016/j.ijfoodmicro.2009.04.020.

Murtiyaningsih, H., & Hazmi, M. (2017). Isolation and cellulase enzyme activities assays in cellulolytic bacteria origin from soil waste. *135 Agritrop*, *15*(2).

Nafisah, H., Pujiyanto, S., & Raharjo, B. (2017). Isolasi dan uji aktivitas kitinase isolat bakteri dari kawasan geotermal Dieng. *Bioma: Berkala Ilmiah Biologi*, *19*(1), 22-29.

Nguyen, T. T. X., Tomberlin, J. K., & Vanlaerhoven, S. (2015). The ability of black soldier fly (*Diptera: Stratiomyidae*) larvae to recycle food waste. *Environmental Entomology*, *44*(2), 406-410. doi: 10.1093/ee/nvv002.

Park, S. I., Kim, J. W., & Yoe, S. M. (2015). Purification and characterization of a novel antibacterial peptide from black soldier fly (*Hermetia illucens*) larvae. *Developmental and Comparative Immunology*, *52*(1), 98-106. doi: 10.1016/j.dci.2015.04.018.

Paulsen, S. S., Andersen, B., Gram, L., & MacHado, H. (2016). Biological potential of chitinolytic marine bacteria. *Marine Drugs*, *14*(12). doi: 10.3390/md14120230.

Pendyurin, E. A., Rybina, S. Y., & Smolenskaya, L. M. (2021). Research of black soldier flies (*Hermetia illucens*) maggots zoo compost's influence on soil fertility. In S. V. Klyuev, & A.V. Klyuev (Eds.), Proceedings of the International Conference Industrial and Civil Construction, (pp. 42-49).

Pham, V. H. T., Kim, J., Chang, S., & Chung, W. (2021). Investigation of lipolytic-secreting bacteria from an artificially polluted soil using a modified culture method and optimization of their lipase production. *Microorganisms*, *9*(12). doi: 10.3390/microorganisms9122590.

Popa, R., & Green, T. (2012). *Biology and ecology of the black soldier fly*. Lake Oswego, Oregon: DipTerra LCC.

Scott, P., Badder, M. K. F., Burgess, T., Hardy, G., & Williams, N. (2019). Global biogeography and invasion risk of the plant pathogen genus Phytophthora. *Environmental Science and Policy*, *101*, 175-182. doi: 10.1016/j.envsci.2019.08.020.

Setia, I. N., & Suharjono . (2015). Diversitas dan uji potensi bakteri kitinolitik dari limbah udang. *Jurnal Biotropika*, 3(2), 95-98

Setiawan, A., Arimurti, S., Senjarini, K., & Biologi, S. J. (2016). Aktivitas proteolitik dan fibrinolitik isolat bakteri dari perairan pantai papuma Kabupaten Jember. *Berkala Sainstek*, *4*(1), 1-4. doi: 10.19184/bst.v4i1.4457.

Setyati, W. A., Habibi, A. S., Subagiyo, A., Ridlo, N. S., & Pramesti, R. (2016). Skrining dan seleksi bakteri simbion spons penghasil enzim ekstraseluler sebagai agen bioremediasi bahan organik dan biokontrol Vibriosis pada budidaya udang. *Jurnal Kelautan Tropis Maret, 19*(1), 11-20.

Sukmadewi, D. K. T., Anas, I., Widyastuti, R., & Cintaresmini, A. (2017). Uji fitopatogenitas, hemolisis serta kemampuan mikrob dalam melarutkan fosfat dan kalium. *Jurnal Ilmu Tanah dan Lingkungan, 19*(2), 68-73. doi 10.29244/jitl.19.2.68-73.

Švecová, E., Colla, G., & Crinò, P. (2017). Antifungal activity of *Boerhavia diffusa* L. extracts against *Phytophthora* spp. in tomato and pepper. *European Journal of Plant Pathology, 148*(1), 27-34. doi: 10.1007/s10658-016-1065-9.

Tegtmeier, D., Hurka, S., Mihajlovic, S., Bodenschatz, M., Schlimbach, S., & Vilcinskas, A. (2021). Culture-independent and culture-dependent characterization of the black soldier fly gut microbiome reveals a large proportion of culturable bacteria with potential for industrial applications. *Microorganisms, 9*(8). doi: 10.3390/microorganisms9081642.

Utami, U., & Mujahidin, A. (2020). Uji antagonisme beberapa fungi endofit pada tanaman kentang terhadap *Fusarium oxysporum* secara in vitro. *Jurnal Riset Biologi Dan Aplikasinya, 2*(1), 18-25.

Wang, Y., Wang, Z. J., Huang, J. C., Chachar, A., Zhou, C., & He, S. (2022). Bioremediation of selenium-contaminated soil using earthworm *Eisenia fetida*: Effects of gut bacteria in feces on the soil microbiome. *Chemosphere, 300*. doi 10.1016/j.chemosphere.2022.134544.

Wu, E. J., Wang, Y. P., Shen, L. L., Yahuza, L., Tian, J. C., Yang, L. N., … Zhan, J. (2019). Strategies of *Phytophthora infestans* adaptation to local UV radiation conditions. *Evolutionary Applications, 12*(3), 415-424. doi: 10.1111/eva.12722.

Wulandari, D., & Purwaningsih D., (2019). Morphological, biochemical, and molecular identification and characterization of amylolytic bacteria in tubers of *Colocasia esculenta*. *Bioteknologi & Biosains Indonesia, 6*(2), 247-258.

Yang, B., Wang, Y., & Qian, P. Y. (2016). Sensitivity and correlation of hypervariable regions in 16S rRNA genes in phylogenetic analysis. *BMC Bioinformatics, 17*(1). doi: 10.1186/s12859-016-0992-y.

Yang, C., Ma, S., Li, F., Zheng, L., Tomberlin, J. K., Yu, Z., … Cai, M. (2022). Characteristics and mechanisms of ciprofloxacin degradation by black soldier fly larvae combined with associated intestinal microorganisms. *Science of the Total Environment, 811*. doi: 10.1016/j.scitotenv.2021.151371.

Zhang, F., Sun, X. X., Zhang, X. C., Zhang, S., Lu, J., Xia, Y. M., … Wang, X. J. (2018). The interactions between gut microbiota and entomopathogenic fungi: a potential approach for biological control of *Blattella germanica* (L.). *Pest Management Science, 74*(2), 438-447. doi: 10.1002/ps.4726.

Zhineng, Y., Ying, M., Bingjie, T., Rouxian, Z., & Qiang, Z. (2021). Intestinal microbiota and functional characteristics of black soldier fly larvae (*Hermetia illucens*). *Annals of Microbiology, 71*(1). doi: 10.1186/s13213-021-01626-8.