ARTICLE

Evaluation of antibacterial activity of black soldier fly (*Hermetia illucens* L.) larval gut extracts

Jaymark Orapa, Nephriteri Generale, Avvy Sheen Uy, Danielle Dalman, Mariel Rose Villarubia, Fleurdeliz Maglangit*

Department of Biology and Environmental Science, College of Science, University of the Philippines Cebu, Lahug, Cebu City, 6000, Philippines

ABSTRACT

The larvae of the black soldier fly (BSF), *Hermetia illucens* L., feed on various organic wastes, such as animal manures and food waste, which are often abundant in pathogenic microorganisms. BSF can thrive in such harsh environments, indicating that their gut microflora may possess a potent immune system that can produce a variety of bioactive metabolites, which may repress the growth of harmful pathogens. Nevertheless, the bioactivity potential of the gut microbiota of BSF larvae (BSFL) remains underinvestigated. In this work, we characterized the gut portions of the BSFL (*i.e.*, foregut, midgut, and hindgut) and evaluated the bioactivity of microbial gut extracts against *Escherichia coli* and *Bacillus subtilis*. Ten strains were isolated: three from the foregut (JN1, JN2, JN3), three from the midgut (JA4, JA5, JA6), and four from the hindgut (JB7, JB8, JB9, JB10). All gut isolates showed zones of inhibition (ZOI) against *B. subtilis* (6.27–7.80 mm) and *E. coli* (6.20–7.33 mm), suggesting the presence of broad-spectrum antimicrobial compounds. Based on the 16S RNA sequence similarity, the isolates JN2 and JA4, with the largest ZOIs, are likely new members of the genus *Bacillus*, while JA4 belongs to the genus *Providencia*. The results highlight the potential of BSFL gut as a promising source of antimicrobial compounds, contributing to sustainable waste management and advancements in biotechnology.

*Corresponding author
Email Address: ffmaglangit@up.edu.ph
Date received: 25 August 2024
Dates revised: 09 October 2024; 20 December 2024; 15 June 2025
Date accepted: 06 July 2025
DOI: https://doi.org/10.54645/202518SupHMZ-36

INTRODUCTION

Black Soldier Fly larvae (BSFL; *Hermetia illucens* L.) have garnered growing attention for their remarkable ability to convert low-value organic waste into valuable insect biomass (da Silva and Hesselberg 2020). Rich in proteins, micronutrients, energy, and essential fatty acids, BSF larvae have been regarded as innovative feed alternatives for poultry, pets, and fish (Maglangit et al 2024). BSF insects are neither pests nor disease vectors, making BSF larval production a safe, cost-effective, and environmentally friendly solution for managing organic waste and producing sustainable feed. As such, numerous research efforts have focused on optimizing BSF rearing conditions, feeding substrates, and conversion efficiency to produce high-quality, nutrient-rich biomass in optimal yields (Makkar et al. 2014; Scala et al. 2020).

BSF larvae feed voraciously on different types of organic waste, including animal manure, fruits and vegetable peels, and food waste inhabited by various pathogenic bacteria (Diola et al. 2024; Wang and Shelomi 2017). Several studies have demonstrated a notable influence of the rearing substrate on the gut microbiota of BSFL, and these symbionts may play crucial roles in waste bioconversion, nutrition, and immunity (Bruno et al. 2019; Shumo et al. 2021; Tanga et al. 2021). Furthermore, the gut microflora of BSFL is thought to possess a potent immune system that can repress the growth of harmful and undesired pathogens in contaminated substrates (Bruno et al. 2021). Hence, BSFL gut microbiota represents a rich repertoire of druggable molecules with remarkable bioactivities.

KEYWORDS

BSFL, BSFL gut, gut microbiota, gut extracts, zone of inhibition, antibiotics

BSFL reared on substrates known to carry a high microbial burden, such as manure, could produce more biologically and pharmacologically active substances. However, natural products bioprospecting from BSFL microbiota remains underinvestigated. Earlier studies have focused on BSFL hemolymph extracts, which have been shown to harbor antimicrobial peptides (AMPs), including cecropins and defensins, that exhibit potent antibacterial activities against various pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA) (Park and Yoe 2017a, 2017b). Meanwhile, evaluating the bioactivity of the three regions of the BSFL gut (*i.e.*, foregut, midgut, and hindgut) remains largely elusive.

Herein, we characterized the morphology of bacterial microbes from the three regions of the gut of BSFL using culture-dependent methods and reported, for the first time, the antibacterial activity of the microbial isolates from these three gut portions. Further research on the characterization and purification of gut extracts may significantly contribute to controlling the spread of harmful pathogens in waste and bioconversion systems, as well as to the discovery of antimicrobial compounds with the potential for developing therapeutic agents.

MATERIALS AND METHODS

Black Soldier Fly Rearing

The black soldier fly larvae (5th instar) were provided by Chesed, a local quail farm in Liloan, Cebu, Philippines. The farm rears BSFL on quail manure in bioponds (6 × 3 × 0.5 ft). Three hundred five-day-old larvae (5DOL) were supplied with 15–20 kg of waste daily until they turned into 5th instar (~12 days). Random samples of 5th instar BSFL (~50 larvae) weighing 180−200 mg were collected and placed on a tray containing manure (0.5 g) mixed with 5 mL of bromothymol blue indicator. The larvae were supplied with a substrate comprising a colorimetric indicator for 24 hours before dissection to facilitate the determination of the three sections (*i.e.*, foregut, midgut, hindgut) of the BSFL.

Determination of the Different Sections of the Larval Gut

Bromothymol blue changes color to yellow when the pH value of the gut is ≤ 3.0, yellow-green when the pH is 7, and blue or bluish-green when the pH is ≥ 8. After 24 hours of feeding, the larvae were rinsed with sterile distilled water to remove visible debris and then subjected to cold shock (-20 °C). Subsequently, they were briefly rinsed in sterile distilled water and immersed in 70% ethanol for one to two minutes to kill surface microbes and remove contaminants. Finally, they were rinsed with sterile distilled water (3×) to remove all traces of ethanol. The final rinse was inoculated onto nutrient agar to verify the effectiveness of the sterilization process. The surface sterilization and dissection were performed in a sterile environment (*i.e.*, biosafety cabinet). Sterile tweezers and scissors were used to dissect the gut, and the three regions (foregut, midgut, and hindgut) were determined based on the color changes of the indicator. Five BSFL were dissected, and each gut region was pooled and placed in a new, sterile Petri dish. The pH of each gut region was also verified using pH paper. A small amount of gut content was transferred onto the pH paper using sterile forceps for analysis.

Microbial Isolation from BSF Larval Gut

Microorganisms were isolated from BSF larvae using protocols described by Bruno et al. (2019) and Gorrens et al. (2021), with certain modifications. This study utilized three media: Luria-Bertani (M1), Actinomycete Isolation Agar (AIA), and Nutrient Agar (NA). Their pH values were adjusted using 6 M NaOH and 6 M HCl solution to correspond to each section of the BSFL gut (foregut: pH ~7; midgut: pH ~3; hindgut: pH ~8). Each gut portion was mixed thoroughly with 1 mL of autoclaved distilled water, and 100 µL was inoculated onto different culture media. All plates were incubated at 30 °C for 3–5 days. The bacterial strains obtained were purified by streaking and subculturing on selective agar plates until pure cultures were obtained. Isolates were obtained by selecting various morphological colonies from M1, NA, or AIA plates and subculturing them onto new plates to obtain pure cultures (24–48 h, 30 °C). The pure extracts were stored in 20% glycerol at -80 °C (Maglangit et al. 2022, 2020).

Morphological and Biochemical Characterization

The morphological characteristics of isolated bacteria (JN1, JN2, JN3, JA4, JA5, JA6, JB7, JB8, JB9, JB10) were determined according to Bergey's Manual of Systematic Bacteriology (Goodfellow et al. 2012). The codes "N", "A", and "B" represent neutral, acidic, and basic conditions, respectively. The strains were then subjected to a series of biochemical tests, including Gram staining to classify them as Gram-positive or Gram-negative and salt tolerance tests to assess osmotolerance. Furthermore, the enzyme production of the isolated microbes was evaluated, including catalase activity for hydrogen peroxide decomposition, oxidase activity for cytochrome oxidase production, and amylase activity for starch hydrolysis (Li et al. 2016).

Fermentation and Extraction

The seed cultures of each strain were prepared by inoculating 50 µL of the glycerol stocks in 50 mL of the nutrient broth (NB) and incubating for three days (120 rpm, 30 °C). Then, 500 mL NB was inoculated with the 3-day seed cultures (1:100) and incubated for seven days (120 rpm, 30 °C). The cultures were then extracted with ethyl acetate (500 mL) by thoroughly mixing the culture broth and the solvent in a separatory funnel to ensure contact between the two phases (*i.e.*, organic and aqueous layers). The mixture was then allowed to settle, and the ethyl acetate layer was carefully separated and collected. This process was repeated twice to maximize the recovery of bioactive compounds. The ethyl acetate extracts were combined and concentrated under reduced pressure using a rotary evaporator (40 °C, 200 rpm) to remove the solvent. The weight of the crude extract was then measured.

Antibacterial Activity

The crude extracts were tested against Gram-positive bacteria (*Bacillus subtilis* ATCC 6632) and Gram-negative bacteria (*Escherichia coli* ATCC 25922) using an agar disk diffusion assay, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI). The inoculum of the test microorganisms was standardized to 0.5 McFarland and then spread uniformly onto Mueller-Hinton (MH) agar plates using a sterile swab, rotating at a 60-degree angle each time to ensure even distribution. Susceptibility discs (6 mm) were then placed equally spaced onto the surface of the agar. Crude extracts (1 mg), dissolved in sterile dimethyl sulfoxide (DMSO; 5%v/v), were applied to discs at a final concentration of 1 mg per disc. The positive control consisted of a gentamicin susceptibility disc (10 µg, Oxoid^{TM}), and the negative control was sterile DMSO. The plates were sealed using parafilm and incubated at 37 °C for 18 hours. The bioassay tests were performed in triplicate. The zones of inhibition (ZOI) were measured using a calibrated ruler.

Phylogeny

The genomic DNA of the strains with the highest ZOIs (*i.e.*, JN2, JA4, and JB10) was extracted using the HiPurA™ Bacterial Genomic DNA Kit (HiMedia) according to the manufacturer's protocol. The genomic DNA of each strain was sent for PCR amplification and sequencing at the Philippine Genome Center. The results were processed using ApE (A Plasmid Editor) version 3.1.3.

The 16S rRNA gene sequences (>1000 bp) from both forward and reverse strands were assembled and cleaned using BioEdit to obtain high-quality consensus sequences. These sequences were analyzed using the Nucleotide Basic Local Alignment Search Tool (BLASTn) (NCBI) to identify closely related bacterial species based on sequence similarity. The top ten isolates with E-values less than 10^{-3} and a query coverage of at least 60% were selected and downloaded in FASTA format for inclusion in the phylogenetic analysis. Isolates meeting these criteria were imported into Molecular Evolutionary Genetics Analysis (MEGA) software version 12.0.11 (Tamura et al., 2021) and aligned using the Multiple Sequence Comparison by Log-Expectation (MUSCLE) algorithm.

Phylogenetic trees were constructed using the Maximum Likelihood method with 1000 bootstrap replications. The heuristic search began with automatically generated initial trees using the Neighbor-Join and BioNJ algorithms applied to a pairwise distance matrix estimated with the Maximum Composite Likelihood (MCL) method.

Data Analysis

One-way ANOVA tests were performed to compare the zone of inhibitions of ten extracts against *B. subtilis* and *E. coli*. Data analysis was performed using the R version 4.2.1.

RESULTS AND DISCUSSION

Determination of BSFL gut sections via the colorimetric indicator

Each gut portion of BSFL portrayed varying colors with the bromothymol blue indicator – the foregut was green, the midgut was yellow, and the hindgut was bluish green (Figure 1A, 1B, 1C). Different gut regions have varying pH (Figure 1D). The foregut had a neutral pH, as indicated by a yellow-green color, corresponding to a pH range of 7. The midgut had a slightly acidic pH, evidenced by a yellow color, corresponding to a pH range of 5–6. Finally, the hindgut had a dark green color corresponding to a slightly alkaline pH of 8–9.

The BSFL gut is comprised of three regions with different pH conditions, which play a role in the digestion and nutrient absorption processes (Meneguz et al. 2018). The initial tract, the foregut, initiates the breakdown and digestion of food. The midgut is acidic, allowing for the breakdown of ingested organic matter and the absorption of nutrients, water, and ions from the food material. The acidic environment serves as an initial barrier against microbial colonization, limiting the presence of microbes. Thus, the primary microbial inhabitants in the midgut are transient and mainly involved in secreting enzymes to break down complex organic compounds (Bruno et al. 2019). The hindgut is longer and more alkaline, which favors the colonization of a diverse microbial community. The hindgut's alkaliphilic environment enables beneficial bacteria to thrive and break down organic materials, releasing nutrients that the larvae can absorb. The hindgut is responsible for water reabsorption, fermentation, and excretion of undigested material (Seyedalmoosavi et al. 2022). The pH gradient in the gut of BSFL is crucial for its function, from food breakdown to nutrient release and absorption. Therefore, understanding the pH changes in different gut regions can provide valuable insight into digestion processes.

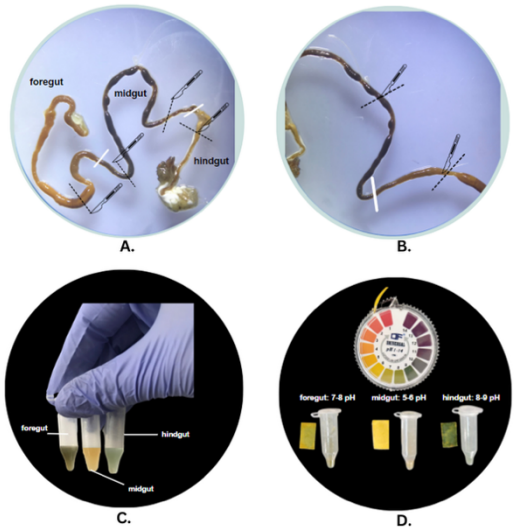

Figure 1: Determination of BSFL gut regions. A. BSFL gut with bromothymol blue indicator after 24 hours under a stereomicroscope; B. The scalpel icon (2 mm) shows the positions of the cuts for each gut region for microbial isolation; C. Color changes of the BSFL foregut, midgut, and hindgut with bromothymol blue indicator; and D. Color changes of the gut regions when tested with pH paper.

Morphological and biochemical characterization of gut microbial strains

Ten isolates were obtained from the different sections of the BSFL gut: three from the foregut (JN1, JN2, JN3), three from the midgut (JA4, JA5, JA6), and four from the hindgut (JB7, JB8, JB9, JB10) (Table 1, Figure S1).

The isolates varied in characteristics, with Gram-positive bacteria (JN1, JN2, JA4, JA6, JB7, JB9) appearing blue or purple and mostly showing round, smooth, and small forms. In contrast, Gram-negative bacteria (JN3, JA5, JB8, JB10) appeared pink or red, with diverse shapes and textures (Table 1). Spiral shapes were observed in JN1 and JN3, while cocci (spherical) forms appeared in JA5, JA6, JB8, JB9, and JB10, and bacillus (rod) shapes were seen in JN2, JA4, and JB7. White-colored colonies were mostly small, while the yellow colonies (JN3, JB7) were larger and had either a viscid or smooth texture. Irregularly shaped colonies (JA5, JB8, JB9) displayed varied margins, elevations, and colors.

Table 1: Morphological and biochemical characteristics of JN1-JN3, JA4-JA6, and JB7-JB10

Strain	Colony morphology	Gram stain	NaCl%	Catalase	Starch Hydrolysis	Oxidase
JN1	Round, Smooth, Entire, Raised, Small, White	+	0-5	+	-	-
JN2	Round, Smooth, Entire, Convex, Small, Yellow	+	0-5	+	-	+
JN3	Round, Viscid, Lobate, Flat, Moderate, Yellow	-	0-5	+	-	-
JA4	Round, Smooth, Entire, Convex, Large, White	+	0-5	+	+	-
JA5	Rhizoid, Mucoid, Entire, Umbonate, Large, Yellow	-	0-5	+	+	-
JA6	Round, Smooth, Entire, Convex, Small, White	+	0-5	+	+	+
JB7	Round, Viscid, Entire, Convex, Large, Yellow	+	0-5	+	-	-
JB8	Filamentous, Dry, Lobate, Raised, Medium, White	-	0-5	+	-	-
JB9	Rhizoid, Smooth, Undulate, Raised, Moderate, White	+	0-5	+	-	-

Strain	Colony morphology	Gram stain	NaCl%	Catalase	Starch Hydrolysis	Oxidase
JB10	Round, Smooth, Entire, Convex, Small, Yellow	-	0-5	+	+	-

The distinct pH conditions in the foregut, midgut, and hindgut create a niche for specific microbial populations that are adapted to survive in these environments—the foregut has a neutral pH, the midgut is acidic, and the hindgut is alkaline.

All isolates can withstand a maximum NaCl concentration of 5% (Table 1). Five isolates (JN1, JN3, JB7, JB8, and JB9) are catalase-positive but do not hydrolyze starch or produce oxidase enzymes. Three isolates (JA4, JA5, and JB10) produce catalase and amylase enzymes but lack oxidase enzyme activity. JN2 exhibits both catalase and oxidase activities but cannot hydrolyze starch. Only JA6 displays catalase and amylase activities and is also capable of aerobic metabolism.

The positive biochemical characteristics of the isolates suggest that they produce enzymes associated with their ability to survive in the gut environment of *H. illucens* L. larvae. This capability likely contributes to their overall bioactivity. Additionally, it can be inferred that catalase activity may help protect the bacteria from immune responses involving oxidative stress (Lushchak 2011). Studies on *Lactobacillus* species from insect microbiomes highlight the significance of enzymatic activities, such as catalase and amylase, in bacterial adaptation to their environments (Lee et al. 2015). These findings indicate that isolates with these enzymatic properties may be promising sources of novel bioactive compounds with antibacterial potential.

Antibacterial activity of BSFL gut microbial extracts

All bacterial gut extracts inhibited the growth of both Gram-positive bacteria, *B. subtilis* ATCC 6632, and Gram-negative bacteria, *E. coli* ATCC 25922, with inhibition zones ranging from 6.27 to 7.80 mm and 6.20 to 7.50 mm, respectively (Figure 2), indicating antibacterial activity. However, this bioactivity is significantly lower than that of the positive control, gentamicin, which displayed a ZOI of 20.08 ± 0.26 mm and 19.58 ± 0.12 mm against *B. subtilis* and *E. coli*, respectively.

The observed ZOI sizes varied among the isolates, which may reflect differences in their metabolic capabilities, the nature and quantity of antimicrobial compounds produced, or their mode of action. However, comparing the zone of inhibition of the ten extracts between *B. subtilis* and *E. coli*, the one-way ANOVA test did not indicate a significant difference ($p > 0.05$).

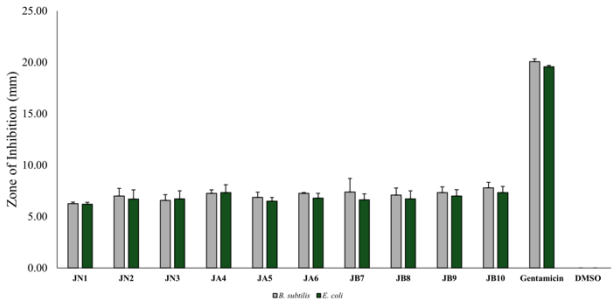

Figure 2: In vitro antibacterial activity of BSFL gut microbial extracts against Bacillus subtilis and Escherichia coli. All values are reported as mean ± SD (n=3); Gentamicin – positive control; DMSO – negative control.

Gram-negative bacteria are generally more challenging to target than Gram-positive bacteria due to their outer membrane, which acts as an additional barrier (Breijyeh et al. 2024). Therefore, antibacterial compounds from these extracts that can disrupt both types of bacterial cell walls could be valuable for treating a wide range of infections. The results underscore the potential of the BSFL gut as a promising source of antimicrobial compounds, which could play a pivotal role in sustainable waste management and biotechnological advancements. The gut microbiota of BSFL may play a role in defensive symbiosis, protecting the larvae from harmful microbes in their decomposing organic substrates. Integrating BSFL into bioconversion systems could potentially reduce the presence of harmful pathogens in organic waste, resulting in safer and more hygienic waste management practices (Diola et al. 2024).

To the best of our knowledge, this is the first report on the bioactivity of BSFL bacterial methanolic extracts derived from distinct sections of the gut. In a previous study, bacterial isolates were obtained from the whole gut of BSFL. Cultures of these isolates were screened for antibacterial activity and demonstrated inhibition against various pathogens, including *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa* (Tegtmeier et al. 2021). Earlier investigations also focused on the BSFL hemolymph extracts, which are rich in AMPs, including cecropins and defensins, that exhibit potent antimicrobial activity against various pathogens, including MRSA (Park and Yoe 2017a, 2017b), *Salmonella enterica* serovar Enteritidis (DMST 15679), and *E. coli* O157:H7 (DMST 12743) (Pimchan et al., 2024). Other studies have also focused on whole-larva or BSFL meals extracted with methanol, attributing the observed antibacterial activity to AMPs, fatty acids, or associated microbial metabolites (Auza et al. 2020; Geronda et al. 2024). These extracts inhibited the growth of *E. coli*, *Salmonella typhimurium*, and *P. aeruginosa* (Auza et al. 2020), with the 5th instar exhibiting the highest bioactivity (Geronda et al. 2024).

Further research in this area could lead to the development of novel natural antimicrobials derived from BSFL, which could be applied in agriculture to control bacterial diseases in crops or livestock. As the use of antibiotics in agriculture faces growing restrictions due to the risk of promoting antibiotic resistance, natural alternatives derived from BSFL could address this gap (Thanner et al. 2016).

The extracts obtained from the foregut, midgut, and hindgut (JN2, JA4, and JB10), which showed the highest ZOIs from each region, were further characterized by 16S rRNA sequencing.

Phylogeny of BSFL gut-isolated strains

The 16S rRNA gene sequencing and phylogenetic analysis revealed the identity of JN2, JA4, and JB10 strains (Figure 3).

JN2 and JA4 clustered closely with members of the genus *Bacillus*, forming a distinct clade with high bootstrap support (100%), indicating a strong evolutionary relationship (Figures 3A and 3B). The morphology of strains JN2 and JA4, characterized by rod-shaped cells (Table 1), aligns with the typical profile of *Bacillus* species, supporting their classification within this genus. Both strains are catalase-positive, which is consistent with the general characteristics of *Bacillus* (Uy et al. 2025). However, only JA4 is capable of hydrolyzing starch, while JN2 tested negative for this trait. In contrast, JN2 exhibited positive oxidase activity, whereas JA4 was oxidase-negative, suggesting physiological differences between the two isolates despite their shared genus. *Bacillus* species have also been previously identified in the gut of BSFL (Tegtmeier et al. 2021), further supporting the insect gut as a reservoir of bioactive microorganisms. Several *Bacillus* species are well-documented for their metabolic diversity and notable antibacterial properties, producing a wide range of antimicrobial peptides, enzymes, and

secondary metabolites effective against various microbial pathogens. The identification of bioactive *Bacillus* isolates makes them promising candidates for further exploration in antimicrobial research (Tran et al. 2022; Uy et al. 2025).

JB10 formed a clade with a bootstrap value of 100% with *Providencia vermicola* sp (Figure 3C). The morphology and biochemical characteristics of JB10 coincide with the results from a previous study on *Providencia*, a Gram-negative, catalase-positive, and oxidase-negative bacterium (Ayyal Al-Gbur 2020). Earlier reports have documented that *Providencia* sp. was the most dominant bacterial species detected from the guts of BSFL reared on chicken manure and kitchen waste (Shumo et al. 2021). *Providencia* species have also been identified in hemolymph-producing *Drosophila melanogaster* fruit flies (Juneja and Lazzaro 2009).

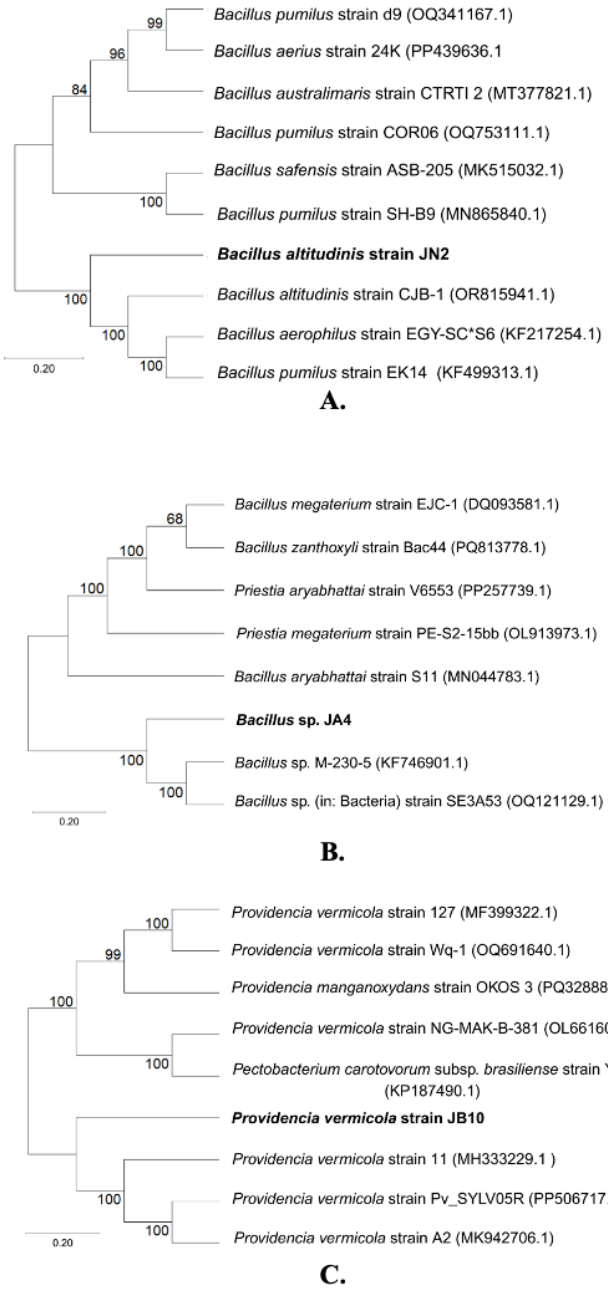

Figure 3: Phylogenetic trees for A. *Bacillus altitudinis* strain JN2; B. *Bacillus* sp. JA4; and *Providencia vermicola* strain JB10 based on high-quality 16S rRNA sequences. The phylogenetic tree was constructed using a maximum-likelihood approach based on similar sequences (hits) identified by BLAST from the NCBI GenBank database. Percentage bootstrap values from 1000 resamplings are indicated at the nodes.

Taken together, the isolates JN2 and JA4 are likely new members of the genus *Bacillus*, while JB10 belongs to the genus *Providencia*. These microbes may play vital roles in the degradation and conversion of organic wastes and the reduction of harmful pathogens in the substrates.

CONCLUSION

The gut of *H. illucens* larvae is characterized by distinct regions with varying pH, each potentially playing a unique role in digestion and microbial activity. Our results demonstrate that all gut extracts exhibited bioactivity against both Gram-positive and Gram-negative bacteria, highlighting their potential as promising sources of biologically active compounds. Based on 16S rRNA gene sequence analysis, JN2 (from the foregut) and JA4 (from the midgut) are likely new species of the genus

Bacillus, while JB10 (from the hindgut) belongs to the genus *Providencia*. Future studies should focus on isolating and characterizing the specific bioactive compounds produced by these microbes, with the goal of developing new therapeutic agents to address antimicrobial resistance.

ACKNOWLEDGEMENT

This study is funded by the Department of Science and Technology (DOST) through the Science for Change Program (S4CP) Collaborative Research and Development to Leverage Philippine Economy (CRADLE), and is monitored by DOST Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD) with Project No. 8647. The authors also thank the Department of Agriculture – Bureau of Agricultural Research (DA BAR) and the DOST - National Research Council of the Philippines (NRCP). The authors are grateful to Chesed Farm for providing the larvae needed for the study.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

Conceptualization and methodology – J.O., N.G., A.S.U., D.D., M.R.V., F.M.; Data analysis – J.O., N.G., A.S.U., D.D., M.R.V., FM; Writing original draft preparation – J.O., N.G., F.M.; Review and editing; F.M.; Supervision and funding acquisition – FM

REFERENCES

Auza FA, Purwanti S, Syamsu JA, Natsir A. Antibacterial activities of black soldier flies (*Hermetia illucens* L) extract towards the growth of *Salmonella typhimurium*, *E. coli* and *Pseudomonas aeruginosa*. IOP Conf. Ser.: Earth and Environmental Science 2020; 492 012024. https://doi.org/10.1088/1755-1315/492/1/012024.

Ayyal Al-Gbur NM. Isolation and molecular identification and antimicrobial susceptibility of *Providencia* spp. from raw cow's milk in Baghdad, Iraq. Veterinary Medicine International 2020, 2020, 1–6. https://doi.org/10.1155/2020/8874747.

Breijyeh Z, Jubeh B, Karaman R. Resistance of Gram-negative bacteria to current antibacterial agents and approaches to resolve it. Molecules 2020; 25(6): 1340. https://doi.org/10.3390/molecules25061340

Brenner DJ, Krieg NR, Staley JT, Garrity GM, Boone DR, Vos P, Goodfellow M, Rainey FA, Schleifer KH, eds. Bergey's Manual® of Systematic Bacteriology Volume 2: The Proteobacteria, Part B: The Gammaproteobacteria. Springer New York, NY, 2005: 1106. https://doi.org/10.1007/0-387-28022-7

Bruno D, Bonelli M, Filippis FD, Di Lelio I, Tettamanti G, Casartelli M, Ercolini D, Caccia S. The intestinal microbiota of *Hermetia illucens* larvae is affected by diet and shows a diverse composition in the different midgut regions. Applied and Environmental Microbiology 2019; 85: e01864-18. https://doi.org/10.1128/AEM.01864-18

Bruno D, Montali A, Mastore M, Brivio MF, Mohamed A, Tian L, Grimaldi A, Casartelli M, Tettamanti G. Insights into the immune response of the black soldier fly larvae to bacteria. Front. Immunol. 2021; 12, 745160. https://doi.org/10.3389/fimmu.2021.745160.

CLSI. Clinical and Laboratory Standards Institute: Performance Standards for Antimicrobial Susceptibility Testing Supplement M100S. 2016.

da Silva GDP, Hesselberg T. A review of the use of black soldier fly larvae, *Hermetia illucens* (diptera: stratiomyidae), to compost organic waste in tropical regions. Neotropical Entomology 2020; 49: 151–162. https://doi.org/10.1007/s13744-019-00719-z

Diola CS, Nacilla EJ, Pardillo CA, Alosbaños RS, Evacitas F, Maglangit F. Waste reduction and bioconversion of quail, chicken, and pig manure by black soldier fly (*Hermetia illucens* L.). Philippine Journal of Science 2024; 153(2): 609–618. https://doi.org/10.56899/153.02.11

Geronda CM, Choresca CH, Pedroso FL, Caipang CM, Repamonte JW, Fagutao FF. Nutritional Profile and Antibacterial Activity of the Black Soldier Fly (*Hermetia illucens*) Larvae Meal as a Potential Protein Source for Aquafeeds. Applied Biochemistry and Microbiology 2024; 60, 1375–1387. https://doi.org/10.1134/S0003683824604773.

Gorrens E, Moll LV, Frooninckx L, Smet JD, Campenhout LV. Isolation and identification of dominant bacteria from black soldier fly larvae (*Hermetia illucens*) envisaging practical applications. Frontiers in Microbiology 2021; 12: 665546. https://doi.org/10.3389/fmicb.2021.665546

Juneja P, Lazzaro BP. *Providencia sneebia* sp. nov. and *Providencia burhodogranariea* sp. nov., isolated from wild *Drosophila melanogaster*. International Journal of Systematic and Evolutionary Microbiology 2009; 59: 1108–1111. https://doi.org/10.1099/ijs.0.000117-0

Lee FJ, Rusch DB, Stewart FJ, Mattila HR, Newton ILG. Saccharide breakdown and fermentation by the honeybee gut microbiome. Environmental Microbiology 2015; 17: 796–815. https://doi.org/10.1111/1462-2920.12526

Li Q, Chen X, Jiang Y, Jiang C, Li Q, Chen X, Jiang Y, Jiang C. Cultural, physiological, and biochemical identification of actinobacteria, in actinobacteria - basics and biotechnological applications. IntechOpen 2016. https://doi.org/10.5772/61462

Lushchak VI. Adaptive response to oxidative stress: bacteria, fungi, plants and animals. comparative biochemistry and physiology part c. Toxicology & Pharmacology 2011; 153: 175–190. https://doi.org/10.1016/j.cbpc.2010.10.004

Maglangit F, Alosbaños RS, Lozano LMB, Evacitas FC. Guide to Rearing, Harvesting, and Processing Black Soldier Fly Larvae. Quezon City: UP Press. 2024: 57-65

Maglangit F, Kyeremeh K, Deng H. Deletion of the accramycin pathway-specific regulatory gene accJ activates the production of unrelated polyketide metabolites. Natural Product Research 2022; 37: 2753–2758. https://doi.org/10.1080/14786419.2022.2126466

Maglangit F, Zhang Y, Kyeremeh K, Deng H. Discovery of new antibacterial accramycins from a genetic variant of the soil bacterium, *Streptomyces* sp. MA37. Biomolecules 2020; 10: 1464. https://doi.org/10.3390/biom10101464

Makkar HPS, Tran G, Heuzé V, Ankers P. State-of-the-art on use of insects as animal feed. Animal Feed Science and Technology 2014; 197: 1–33. https://doi.org/10.1016/j.anifeedsci.2014.07.008

Meneguz M, Gasco L, Tomberlin JK. Impact of pH and feeding system on black soldier fly (*Hermetia illucens*, L; Diptera: Stratiomyidae) larval development. PLoS ONE 2018; 13: e0202591. https://doi.org/10.1371/journal.pone.0202591

Park SI, Yoe SM. A novel cecropin-like peptide from black soldier fly, *Hermetia illucens*: isolation, structural and functional characterization. Entomological Research 2017a; 47: 115–124. https://doi.org/10.1111/1748-5967.12226

Park SI, Yoe SM. Defensin-like peptide3 from black solder fly: identification, characterization, and key amino acids for anti-gram-negative bacteria. Entomological Research 2017b; 47: 41–47. https://doi.org/10.1111/1748-5967.12214

Pimchan T, Hamzeh A, Siringan P, Thumanu K, Hanboonsong Y, Yongsawatdigul J. Antibacterial peptides from black soldier fly (*Hermetia illucens*) larvae: mode of action and characterization. Scientific Reports 2024; 14, 26469. https://doi.org/10.1038/s41598-024-73766-1G

Scala A, Cammack JA, Salvia R, Scieuzo C, Franco A, Bufo SA, Tomberlin JK, Falabella P. Rearing substrate impacts growth and macronutrient composition of *Hermetia illucens* (L.) (Diptera: Stratiomyidae) larvae produced at an industrial scale. Scientific Reports 2020; 10: 1–8. https://doi.org/10.1038/s41598-020-76571-8

Seyedalmoosavi MM, Mielenz M, Veldkamp T, Da G, Metges CC. Growth efficiency, intestinal biology, and nutrient utilization and requirements of black soldier fly (*Hermetia illucens*) larvae compared to monogastric livestock species: a review. Journal of Animal Science and Biotechnology 2022; 13, 31. https://doi.org/10.1186/s40104-022-00682-7

Shumo M, Khamis FM, Ombura FL, Tanga CM, Fiaboe KKM, Subramanian S, Ekesi S, Schlüter OK. A Molecular Survey of Bacterial Species in the Guts of Black Soldier Fly Larvae (Hermetia illucens) Reared on Two Urban Organic Waste Streams in Kenya. Frontiers in Microbiology 2021; 12: 687103. https://doi.org/10.3389/fmicb.2021.687103

Tamura K, Stecher G, Kumar S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution 2021; 38 (7): 3022–3027. https://doi.org/10.1093/molbev/msab120.

Tanga CM, Waweru JW, Tola, YH, Onyoni AA, Khamis FM, Ekesi S, Paredes JC. Organic waste substrates induce important shifts in gut microbiota of black soldier fly (*Hermetia illucens l.*): coexistence of conserved, variable, and potential pathogenic microbes. Frontiers in Microbiology 2021; 12: 635881. https://doi.org/10.3389/fmicb.2021.635881

Tegtmeier D, Hurka S, Mihajlovic S, Bodenschatz M, Schlimbach S, Vilcinskas A. 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 2021; 9(8): 1642. https://doi.org/10.3390/microorganisms9081642

Thanner S, Drissner D, Walsh F. Antimicrobial resistance in agriculture. *mBio* 2016; 7(2): e02227-15. https://doi.org/10.1128/mBio.02227-15

Tran C, Cock IE, Chen X, Feng Y. Antimicrobial Bacillus: Metabolites and Their Mode of Action. Antibiotics 2022; 11(1):88. https://doi.org/10.3390/antibiotics11010088

Uy ASB, Anoos LM, Generale NG, Petalcorin MIR, Maglangit F. Evaluation of *in vitro* antibacterial activity and *in vivo* *Caenorhabditis elegans* assays of *Bacillus velezensis* AU1 and AU2. SciEnggJ 2025; 18 009-020. https://doi.org/10.54645/202518SupIKG-59

Vos P, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer KH, Whitman WB, eds. Bergey's Manual® of Systematic Bacteriology Volume 3: The Firmicutes. Springer New York, NY, 2009: 1450. https://doi.org/10.1007/978-0-387-68489-5

Wang YS, Shelomi M. Review of black soldier fly (*Hermetia illucens*) as animal feed and human food. Foods 2017; 6: 91. https://doi.org/10.3390/foods6100091