JPPIPA 11(1) (2025)

Jurnal Penelitian Pendidikan IPA

Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

Metagenomic Exploration of the Black Soldier Fly *(Hermetia illucens)* as an Organic Waste Decomposer for Environmental Bioremediation Efforts

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Received: August 09, 2024
Revised: December 08, 2024
Accepted: January 25, 2025
Published: January 31, 2025

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DOI: 10.29303/jppipa.v11i1.9926

Abstract: The Black Soldier Fly (BSF) (Hermetia illucens Linnaeus, 1758) has long been recognized as an organism used in organic waste processing through bioconversion methods. H. illucens is known to digest organic materials into nutrient sources utilized for biomass formation with the assistance of decomposer microbiota. However, research on the structure and composition of its microbiota remains limited. This study aims to identify microbiota and their structural composition in both the larval and adult fly phases, based on organic waste feeding in tropical regions. Additionally, it seeks to provide recommendations for relevant stakeholders in identifying potential environmental bioremediation agents. The research method employed is a survey study with quantitative sample analysis. The amplification process in this study uses primers from the (V1-V9) regions of the 16S rRNA gene. Data analysis is conducted using the QIIME (Quantitative Insights into Microbial Ecology) method, utilizing high-throughput sequencing community data with QIIME2 software version 3.5.3. Microbiota from the families Lactobacillaceae and Morganellaceae have been identified as dominant in larvae, while Staphylococcaceae and Bacillaceae dominate in adult flies. Morganella morganii, Herbaspirillum piri, Dysgonomonas capnocytophagoides, and Clostridium intestinale are potential candidates for organic waste bioremediation from BSF larvae. Meanwhile, Sphingobacterium wenxiniae, Lachnoclostridium phytofermentans, Mammaliicoccus sciuri, and Corticicoccus populi are bioremediation candidates from BSF flies. The genera Enterococcus, Morganella, and Dysgonomonas are found in both temperate and tropical climate regions. However, Providencia, Klebsiella, Scrofimicrobium, and Actinomyces, which are found in the gut of BSF larvae in temperate regions, are absent in BSF larvae from tropical Indonesia. Conversely, Limosilactobacillus, Entomomonas, Lachnoclostridium, and Clostridium are not found in the gut of BSF larvae in temperate regions.

Keywords: Bioremediation agents; Hermetia Illucens; microbiota; Metamorphosis; BSF Larval metagenomics.

Introduction

Household waste management has always been an interesting topic of discussion due to the ongoing challenges ranging from small-scale to global issues. In Indonesia, waste management generally follows an outdated framework, employing the end-to-pipe approach (Darmawan et al., 2020). Final waste disposal is carried out in landfills (TPA), which still rely on open dumping systems, leading to the degradation of water, air, and soil quality in the surrounding areas (Prajnawita et al., 2020).

How to Cite:
irwanto, Rumanta, M., & Kunda, R. M. (2025). Metagenomic Exploration of the Black Soldier Fly (Hermetia illucens) as an Organic Waste Decomposer for Environmental Bioremediation Efforts. *Jurnal Penelitian Pendidikan IPA*, *11*(1), 1193–1205. https://doi.org/10.29303/jppipa.v11i1.9926

One method of household waste processing is through bioconversion using the Black Soldier Fly (BSF) (Hermetia illucens Linnaeus, 1758) (Yuwita & Fitria, 2022). BSF is a well-known insect that processes organic waste into a nutrient source used for larval metabolism during metamorphosis into adult flies. This ability makes BSF an effective organic waste degrader, capable of rapidly processing organic waste (Amelia & Rafidah, 2021) while also reducing methane gas emissions (Hanifah, 2022).

The degradation of organic waste by BSF larvae (H. illucens) is closely related to the role of gut microbiota. Gut microbiota is responsible for most metabolic processes associated with food intake and digestion, but it can also significantly influence health and behavior (Klammsteiner et al., 2020). A stable core microbial community in the gut provides general metabolic competence for substrate degradation and resilience against extrinsic disturbances such as dietary changes or pathogens (Klammsteiner et al., 2020).

The composition and diversity of microbiota in BSF larvae have been previously studied using metagenomic analysis. IJdema et al. (2022) reported that in Belgium, the microbiota composition in BSF larvae is dominated by the genera Enterococcus, Morganella, and Providencia, followed by Klebsiella and Scrofimicrobium. Meanwhile, Klammsteiner et al. (2020) reported that in Austria, the microbiota composition in BSF larvae is dominated by a simple group of highly abundant bacterial species, including Actinomyces sp., Dysgonomonas sp., Enterococcus sp., and Actinomycetales, which are known to play a key role in organic matter degradation.

Previous metagenomic studies have identified the composition of gut microbiota in BSF larvae when fed with chicken feed, fruits/vegetables, and grass clippings (IJdema et al., 2022), as well as agricultural by-products, livestock feed, household waste, fruit/vegetable waste, manure, and oily water runoff (Klammsteiner et al., 2020). These studies were conducted in Belgium and Austria, both located in Western Europe with a temperate climate. Further research is needed to examine the composition and structure of microbiota in BSF in tropical climates such as Indonesia.

This study aims to: 1. Analyze the structure, composition, and profile of microbiota in both larval and adult fly phases; 2. Examine microbial communities in BSF that have the potential to be used for waste bioremediation; 3. Provide recommendations for organic waste management through bacterial consortiums from BSF larvae; 4. Identify pathogenic and non-pathogenic bacteria present in BSF larvae and adult flies; 5. Compare the microbiota of BSF in temperate regions with those found in tropical regions.

Method

Research Method

This study employs a survey-based research approach by quantitatively analyzing samples. The amplification process in this study uses primers from the (V1-V9) regions of the 16S rRNA gene. Data analysis is conducted using QIIME (Quantitative Insights into Microbial Ecology) methodology, with high-throughput sequencing community data processed through QIIME2 software (version 3.5.3). The analysis of larvae and adult fly samples is carried out at the Genomic Laboratory of Solidaritas Indonesia (GSI) – Jakarta.

Research Design

Study Area

The research study is conducted in Ako Village, Pasangkayu District, Pasangkayu Regency, West Sulawesi Province, Indonesia (Figure 1).

Figure 1. Location of Ako Village, Showing the Sampling Site of H. illucens: Coordinate Points (1°9'6.08"S, 119°23'2.59"E)

Materials

The materials used for the larval rearing and sample preparation stages include: organic waste (leftover rice, vegetables, side dishes, and unused vegetable scraps), water, and 95% alcohol. For laboratory analysis, the materials used include: salt, and PhiX bacterial meta biome DNA.

Procedure

Sample Preparation

a. Preparation of BSF Breeding Media

The breeding media preparation is conducted as follows:

1) Prepare an egg-laying bait medium for adult flies by mixing approximately 100 grams of fish pellet

porridge in a plastic container covered with a cloth napkin. Place the medium in a room with a temperature of 27-30°C (Tomberlin et al., 2009).

2) Observe the bait medium until BSF flies approach the medium, indicating that the adult flies are attracted to lay eggs.

b. Preparation of BSF Larvae Rearing Media

1) Conduct daily observations to check for the presence of eggs in the bait medium. If eggs are present, transfer them to a hatching container (a plastic container lined with tissue paper). Wait a few days until the eggs hatch and larvae measuring 0.8 – 1 mm become visible. Feed them fish pellet porridge until they reach 4-5 days old.
2) Prepare organic waste porridge as larval feed by blending household organic waste with water until it reaches a porridge-like consistency.
3) Prepare a plastic container with a lid to be used as the larval rearing container.
4) Add one ladle of organic waste porridge to the sample container and record the initial feed weight.
5) Once the larvae are 5-7 days old, transfer them to the prepared feeding media. Place 30 larvae into each sample container.
6) Daily observations should be conducted to monitor larval development and feed condition. The recorded data includes: larval length, larval age, and larval color.
7) Transfer larvae that change color to yellowish-brown into a preparation container for laboratory sample shipment.

Sample Collection, Preparation, and DNA Extraction

1) Collection of Larvae Samples

A total of 12 larvae were randomly collected from the treatment sample during the third week of May 2024, leaving 18 larvae in the sample. Each larva was preserved in 95% alcohol in a Falcon tube labeled as Code 1 and was shipped to the GSI Laboratory under cold conditions. The remaining larvae continued their instar phases until they developed into adult BSF flies. Each larva was examined under a stereomicroscope (Olympus, Germany) at GSI Laboratory, using a dichotomous key and character matrix that included growth stages and sex identification, based on Anastos (1950). The larvae samples were then frozen at GSI Laboratory for further analysis.

2) Collection of BSF Fly Samples

The 18 larvae previously sent to the laboratory were transferred to a sealed transparent plastic container to continue their metamorphosis into adult BSF flies. Once the BSF flies emerged, 12 adult flies were collected from the sample container. Each fly was preserved in 95% alcohol in a Falcon tube labeled as Code 1, and the samples were shipped to GSI Laboratory under cold conditions.

3) DNA Preparation and Extraction

The pre-PCR (Polymerase Chain Reaction) and PCR procedures were conducted using sterile equipment and a sterile laboratory environment to prevent cross-contamination. Larvae and flies were cleaned for one minute in 95% ethanol to remove all microorganisms from their external surfaces. Next, homogenization was performed by grinding each larva and fly in PBS phosphate-buffered saline solution (without Ca^{2+} and Mg^{2+}, pH = 7.4). The samples were then centrifuged, and the supernatant (300 µL) was used for DNA extraction. DNA extraction was carried out using a spin-column kit (EURx, Poland) following the manufacturer's protocol with modifications. The quality and quantity of DNA were assessed using a WPA UV1101 spectrophotometer (WPA, The Old Station, Linton, Cambridge, UK) to ensure a minimum DNA concentration of 10 ng/µL. Finally, the extracted DNA samples (100 µL) were stored at -20°C for further analysis.

Molecular Analysis (PCR) for Metagenomic 16S rRNA Sequencing

Amplification of the 16S rRNA gene sequence was performed using Start-Warm HS-PCR Mix (A&A Biotechnology, Gdynia, Poland) and ddWater (aseptic, nuclease-free). The primers used for 16S rRNA gene amplification were based on full-length 16S primers (Nanopore). These primer sequences were the most suitable primer pairs for Next-Generation Sequencing (NGS): (27F): 5'- AGA GTT TGA TCM TGG CTC AG -3'; (1492R): 5'- GGT TAC CTT GTT ACG ACT T-3'). The PCR protocol used for NGS analysis was as follows: pre-denaturation: 95°C for 3 minutes, denaturation 25 cycles 95°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 30 seconds, post-extension: 72°C for 5 minutes, next the PCR amplicons were then subjected to electrophoresis in 2% agarose gel (Sigma-Aldrich, Germany), stained with Midori Green (Nippon Genetics Europe GmbH, Germany), and electrophoresed at 90 volts for 45 minutes. The PCR amplification results were visualized using UV light with the 100 Gel Logic System (Kodak Imaging System, Inc., USA). PCR amplification products (1,500 bp in size) were selected for further metagenomic investigation (Nanopore MySeq).

The NGS process was carried out following the Nanopore 16S metagenomic protocol (Nanopore MySeq, Inc., San Diego, California, USA). The final paired-end DNA library was prepared with an insert size of approximately 1,500 bp using a primer series covering variable regions V1 to V9 of the 16S rRNA gene. The quantity and quality of the metagenomic library were

evaluated through electrophoresis using the 2200 Agilent TapeStation Instrument with a Genomic DNA ScreenTape Assay (Agilent Technologies Inc., Santa Clara, CA, USA). Samples were pooled in equal proportions and sequenced for 600 cycles using the MiSeq Platform (Macrogen, Seoul, Korea) with v3 reagents (paired-end reads 2 × 300 bp). Additionally, 10% PhiX bacterial metabiome DNA was added to the samples as an internal control. The paired-end reads were recorded in FASTQ format, and the FASTQ data were automatically demultiplexed, with adapter trimming performed by Macrogen using the Nanopore sequencing system.

Data Analysis

Data were analyzed using the QIIME (Quantitative Insights into Microbial Ecology) method with high-throughput sequencing community data (Caporaso in Utami et al., 2024). The Nanopore MySeq 16S rRNA sequences obtained were grouped based on 97% sequence similarity and analyzed using the QIIME2 software package (version 3.5.3) (Swei & Kwan, 2017). The microbial profile was classified based on its environmental bioremediation potential and divided into groups of remediation-potential and non-remediation-potential microbes, as well as pathogenic and non-pathogenic properties.

Result and Discussion

This metagenomic exploration study of the Black Soldier Fly (Hermetia illucens) as an organic waste degrader for environmental bioremediation is an effort by the authors to understand the role of microbiota in BSF, from the larval stage to the adult fly, in facilitating organic waste processing into biomass-building substances. The study was conducted by rearing BSF insects from eggs to adult flies and performing metagenomic analysis at the GSI Laboratory, Jakarta.

Results 1: Microbiota Composition Structure and Profile in Larval and Adult Stages

a. Relative Abundance at the Genus Level

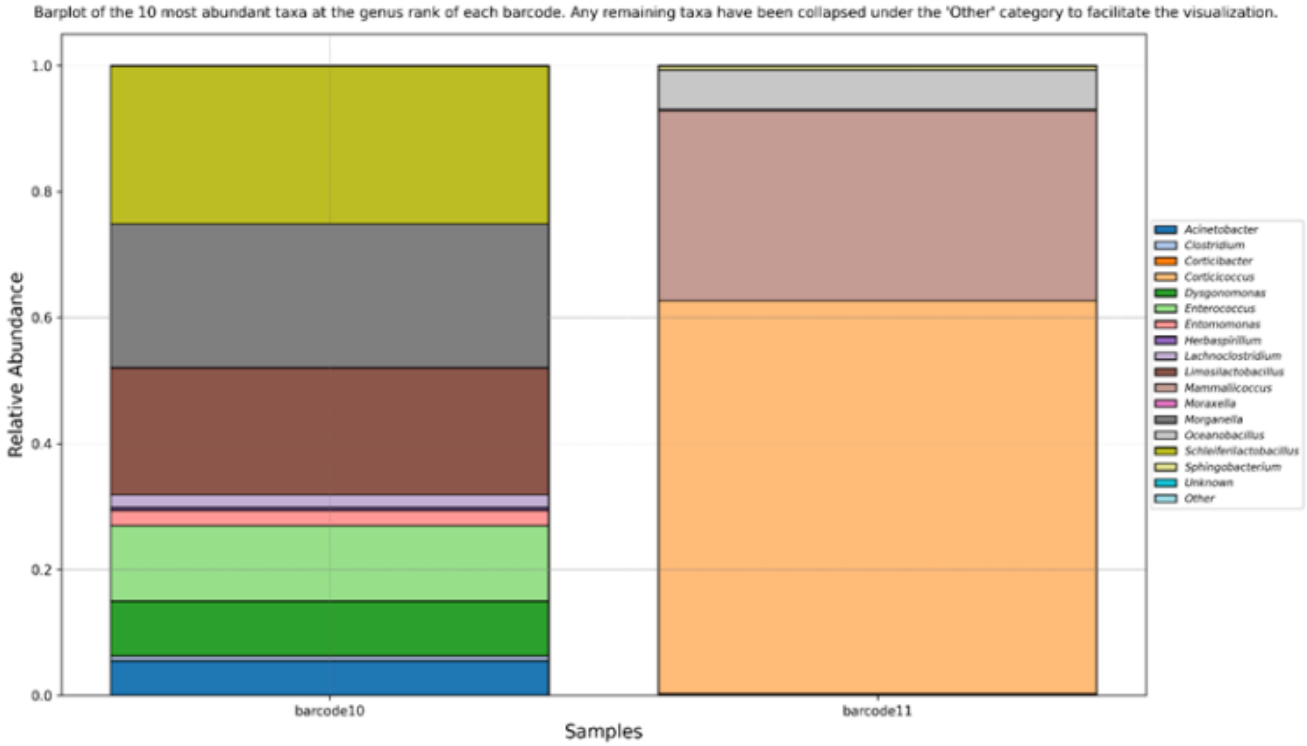


Figure 1. Relative Abundance of the Black Soldier Fly (Hermetia illucens) Metagenome at the Genus Level

The analysis of relative abundance at the genus level revealed significant differences between the larval samples (barcode 10) and the adult fly samples (barcode 11). In the larval samples, several microbiota genera were identified, including Schleiferilactobacillus, Morganella, Limosilactobacillus, Enterococcus, Dysgonomonas, Acinetobacter, Entomomonas, Lachnoclostridium, Clostridium, and Herbaspirillum, with Schleiferilactobacillus being the most abundant genus. In contrast, in the adult fly samples, the identified genera included Corticicoccus, Mammaliicoccus, Oceanobacillus, Sphingobacterium, Corticibacter, Moraxella, and Clostridium, with Corticicoccus being the most dominant genus.

b. Relative Abundance of Species Level

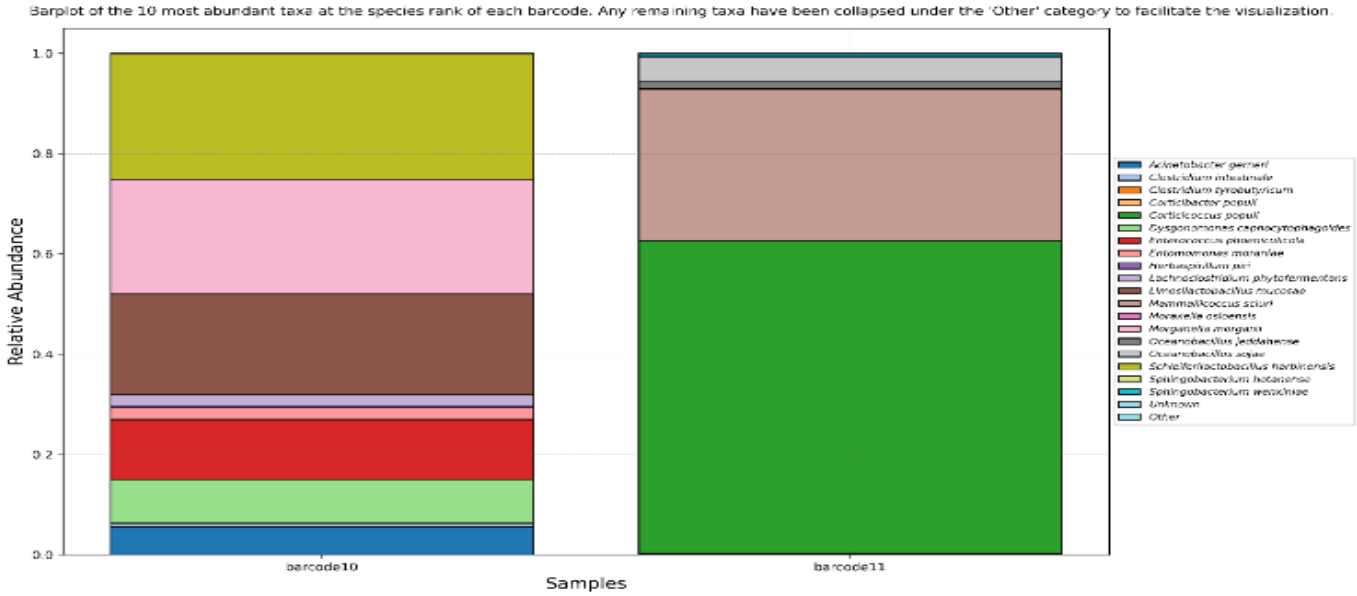


Figure 2. Relative Abundance of Black Soldier Fly (Hermetia illucens) Metagenome at Species Level

The results of the metagenome analysis of the Black Soldier Fly insect showed that the relative abundance at the species level contained many microbiota found. For the larval sample (barcode 10) at least 10 microbiota were detected, including Schleiferilactobacillus harbinensis, Morganella morganii, Limosilactobacillus mocosae, Enterococcus phoeniculicola, Dysgonomonas capnocytophagoides, Acinotebacter gerneri, Entomomonas moraniae, Lachnoclostridium phytofermentans, Clostridium intestinale and Herbaspirillum piri. The most dominant microbiota in this sample was the Schleiferilactobacillus harbinensis species and the least found was Herbaspirillum piri. In the fly sample (barcode 11), at least nine microbiota were found, including Corticicoccus populi, Mammaliicoccus sciuri, Oceanobacillus sojae, Oceanobacillus jeddahense, Sphingobacterium wenxiniae, Corticibacter populi, Moraxella osloensis, Sphingobacterium hotanense and Clostridium tyrobutyricum. The dominant species found in the sample was Corticicoccus populi and the least was Clostridium tyrobutyricum.

c. Heatmap Profile of Relative Abundance at Phylum Level

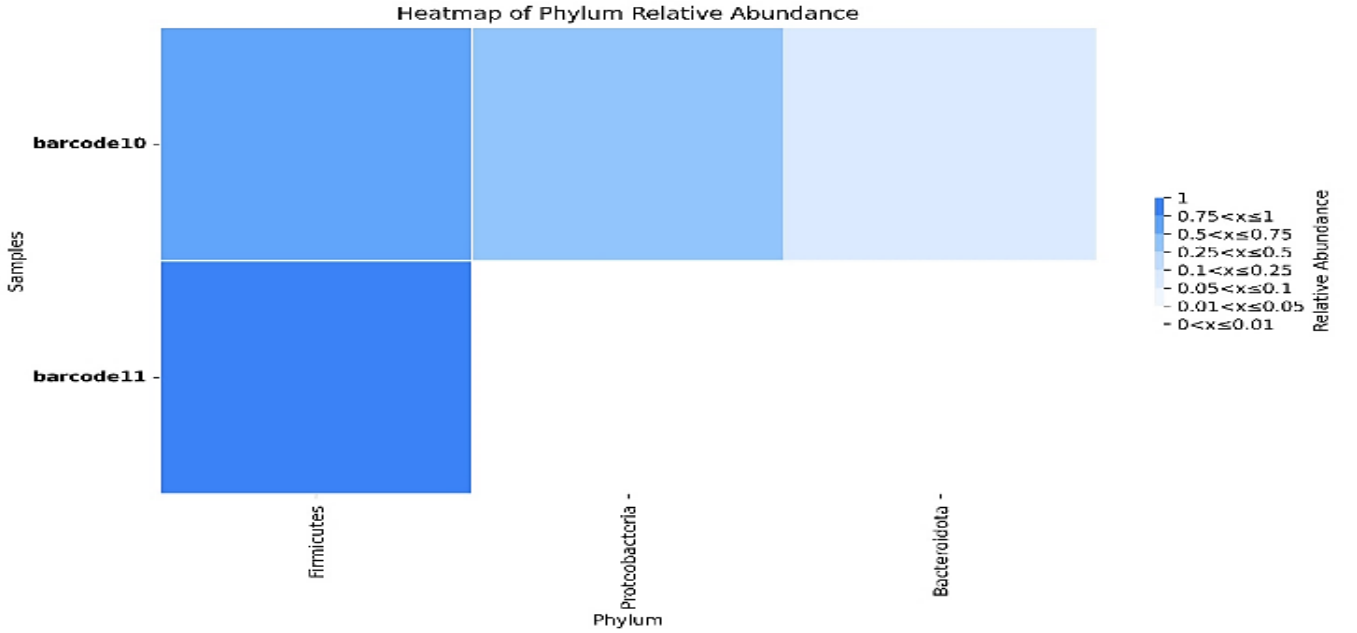


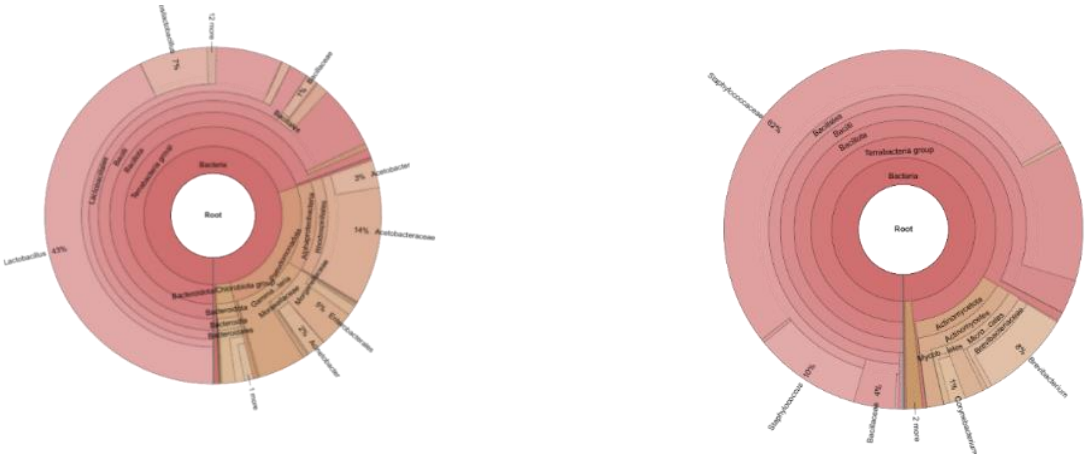
Figure 3. Heatmap Profile of the Black Soldier Fly (Hermetia illucens) Metagenome

Based on the heatmap analysis results, the metagenome of the Black Soldier Fly (Hermetia illucens) is categorized into three main abundance levels: low abundance: $0 < x \leq 0.01$ to $0.05 < x \leq 0.1$, next medium abundance: $0.25 < x \leq 0.5$ to $0.5 < x \leq 0.7$ and the last high abundance: $0.75 < x \leq 1$ to 1. The heatmap profile in Figure 3 shows that the larval sample is divided into three phylum groups: Bacteroidota in the low-abundance group, Proteobacteria in the medium-abundance group, and Firmicutes, which is classified under the high-abundance group. However, in the fly sample, there is a notable difference: Bacteroidota and Proteobacteria are categorized in the low-abundance group, and Firmicutes remains in the high-abundance group. This indicates that the phylum with the highest relative abundance in both larval and fly samples is Firmicutes.

The microbiota composition between the larval and fly samples shows significant differences (Figure 1). The larval sample was dominated by Lactobacillaceae, accounting for 45.3%. The fly sample was dominated by Staphylococcaceae, accounting for 92.7%.

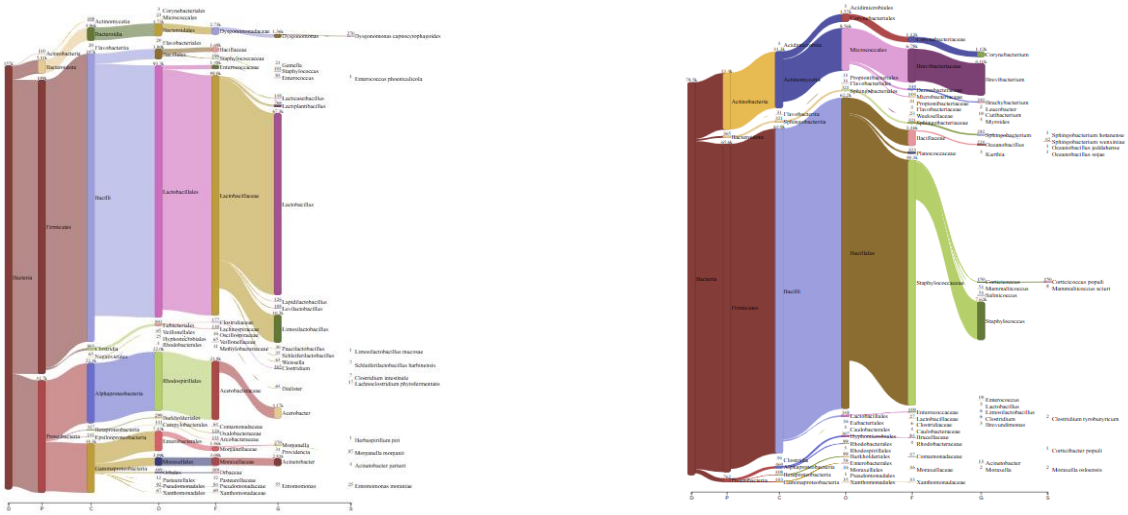
Microbiota abundance (family level) found in the larval sample: Lactobacillaceae: 27,688 (45.3%), Morganellaceae: 13,979 (22.9%), Enterococcaceae: 7,360 (12%), Dysgonomonadaceae: 5,305 (8.7%), Moraxellaceae: 3,375 (5.5%), Pseudomonadaceae: 1,452 (2.4%), Lachnospiraceae: 1,261 (2.1%), Clostridiaceae: 486 (0.8%), and Oxalobacteraceae: 270 (0.4%). Microbiota abundance (family level) found in the fly sample: Staphylococcaceae: 53,959 (92.7%), Bacillaceae: 3,661 (6.3%), Sphingobacteriaceae: 356 (0.6%, Comamonadaceae: 116 (0.2%), Moraxellaceae: 82 (0.1%), and Clostridiaceae: 27 (0.05%)

d. Prevalence Comparison



(a) (b)

Figure 4. Comparison of prevalence in the metagenome of the Black Soldier Fly (Hermetia illucens) in samples of (a) larvae and (b) flies.



(a) (b)

Figure 5. Metagenome Abundance Analysis Using Sankey Diagram

The most dominant abundance, as observed in the Sankey diagram (Figure 5), originates from the phylum Firmicutes (109k), primarily represented by the genus Schleiferilactobacillus (67.3k) and Limocilactobacillus (10.3k) in the larval samples. In contrast, the adult fly samples also showed that Firmicutes was the dominant phylum (65.6k), with the genus Corticicoccus (7.62k) and Mammaliicoccus (6.41k) being the most abundant.

Results-2: Microbiota Community in BSF Based on Pathogenicity and Bioremediation Potential

The abundance of microbiota in BSF larvae and their respective characteristics, based on their pathogenic nature and potential for organic waste bioremediation, are detailed as follows:

(1) Dysgonomonas capnocytophagoides (Hironaga et al., 2008) – Pathogenic, Potential for Bioremediation
(2) Clostridium intestinale (Wu et al., 2023) – Pathogenic, Not Potential for Bioremediation
(3) Enterococcus phoeniculicola (Byappanahalli et al., 2012) – Non-Pathogenic, Not Potential for Bioremediation
(4) Lachnoclostridium phytofermentans (Zaplana et al., 2023) – Non-Pathogenic, Potential for Bioremediation
(5) Schleiferilactobacillus harbinensis (Mashraqi et al., 2023) – Non-Pathogenic, Not Potential for Bioremediation
(6) Limosilactobacillus mucosae (Lee et al., 2024) – Non-Pathogenic, Not Potential for Bioremediation
(7) Morganella morganii (Laupland et al., 2022) – Pathogenic, Potential for Bioremediation
(8) Herbaspirillum piri (Xu et al., 2018) – Non-Pathogenic, Potential for Bioremediation
(9) Acinetobacter gerneri (Dortet et al., 2006) – Pathogenic, Potential for Bioremediation
(10) Entomomonas moraniae (Wang et al., 2020) – Non-Pathogenic, Not Potential for Bioremediation

The abundance of microbiota in adult BSF flies and their respective characteristics, based on pathogenic properties and potential for organic waste bioremediation, are described as follows:

(1) Corticicoccus populi (Li et al., 2017) – Non-Pathogenic, Potential for Bioremediation
(2) Mammaliicoccus sciuri (De Luca et al., 2022) – Pathogenic, Potential for Bioremediation
(3) Oceanobacillus sojae (Liu & Yang, 2014) – Non-Pathogenic, Not Potential for Bioremediation
(4) Oceanobacillus jeddahense (Khelaifia et al., 2016) – Non-Pathogenic, Not Potential for Bioremediation
(5) Clostridium tyrobutyricum (Mosconi et al., 2023) – Non-Pathogenic, Not Potential for Bioremediation
(6) Corticibacter populi (Fangt et al., 2015) – Non-Pathogenic, Not Potential for Bioremediation
(7) Moraxella osloensis (Tan & Grewal, 2001) – Pathogenic, Not Potential for Bioremediation
(8) Sphingobacterium wenxiniae (Chen et al., 2022) – Non-Pathogenic, Potential for Bioremediation
(9) Sphingobacterium hotanense (Xiao et al., 2013) – Non-Pathogenic, Not Potential for Bioremediation

Results-3: Comparison of Microbiota in Temperate and Tropical Climates

Table 1. Comparison of Microbiota in Temperate and Tropical Climates

Parameters	Temperate Region				Tropical Region	
	Belgia (Ijdema et al., 2022)	%	Austria (Klammsteiner et al., 2020)	%	Indonesia	%
Microbiota abundance at genus level	***Enterococcus***	87.7	*Actinomycess*	-	*Schleiferilactobacillus*	25.1
	Morganella	84.6	***Dysgonomonas***	-	*Morganella*	22.8
	Providencia;	80.0	***Enterococcus***	-	*Limosilactobacillus*	20.2
	Klebsiella	64.6			***Enterococcus***	12.0
	Scrofimicrobium	56.9			***Dysgonomonas***	8.7
					Acinetobacter	5.5
					Entomomonas	2.4
					Lachnoclostridium	2.1
					Clostridium	0.8
					Herbaspirillum	0.4

The table above presents the abundance of microbiota composition in Belgium based on core gut microbiota found in larval samples aged 4-14 days (young larvae), identified in each amplicon sequence, with a total of 7,666,062 sequences. The genus Enterococcus was found in amplicon sequence number 2 (87.7%). The genus Morganella was identified in amplicon sequence number 1 (84.6%). The genus Providencia appeared in amplicon sequence number 9 (80.0%). The genus Klebsiella was detected in amplicon sequence number 13 (64.6%), and the genus Scrofimicrobium was found in amplicon sequence number 21 (56.9%) (IJdema et al., 2022). The microbiota abundance reported in Austria did not include detailed prevalence data (Klammsteiner et al., 2020). The microbiota abundance from the present study was based on the composition of microbiota from the entire larval body at 16 days old (young larvae), without specifying

the microbiota composition found in each amplicon sequence.

Discussion

Discussion-1: Structure, Composition, and Microbiota Profile in Larval and Adult Fly Phases

The microbial composition structure in the larval phase is dominated by the Lactobacillaceae family followed by Morganellaceae, Enterococcaceae, Dysgonomonadaceae, Moraxellaceae, Pseudomonadaceae, Lachnospiraceae, Clostridiaceae and Oxalobacteraceae. At the genus level, the bacteria found include: Schleiferilactobacillus, Morganella, Limosilactobacillus, Enterococcus, Dysgonomonas, Acinetobacter, Entomomonas, Lachnoclostridium, Clostridium and Herbaspirillum. Previous research, the microbial composition structure in the larval phase is dominated by the Enterococcaceae, Morganellaceae, Enterobacteriaceae, Actinomycetaceae and Dysgonomonadaceae families. At the genus level, among others, Enterococcus, Morganella, Providencia, Klebsiella and Scrofimicrobium. Of the nine families found in larvae, there are four families that have pathogenic properties, namely Dysgonomonadaceae, Clostridiaceae, Morganellaceae and Moraxellaceae and five families that are non-pathogenic, namely Lactobacillaceae, Enterococcaceae, Lachnospiraceae, Oxalobacteraceae, Pseudomonadaceae.

The structure of the microbial composition in the fly phase is dominated by the Staphylococcaceae family followed by Bacillaceae, Sphingobacteriaceae, Comamonadaceae, Moraxellaceae and Clostridiaceae. Previous studies did not mention the composition at the genus level in detail. Of all the families found, there are two families that are pathogenic, namely Staphylococcaceae and Moraxellaceae and four families that are non-pathogenic, namely Bacillaceae, Sphingobacteriaceae, Comamonadaceae and Clostridiaceae.

The difference in microbial composition in the larval and fly phases is caused by environmental changes and physiological maturation. BSF larvae are kept in a different environment compared to adult flies. During the research period, BSF larvae were placed in transparent plastic containers with lids that were perforated for air circulation. Adult BSF flies were placed in transparent plastic containers separate from the BSF larvae containers with lids that were also perforated for air circulation. The research plastic containers were placed in the living room of the house to facilitate observation. During the research, there was an interaction between various microbes from the surrounding environment that affected the composition of microbes in the larval and adult fly phases. The next difference is the influence of physiological maturation of adult flies. When larvae develop into adult flies, changes in the physiology and biochemistry of the fly's body can affect the population of microbes living in it.

Discussion-2 (Microbial community in BSF insects based on pathogenic properties and potential for environmental bioremediation)

Based on the results of the analysis of the potential for pathogenic microbiota, it shows that the larvae and fly samples are dominated by non-pathogenic bacteria. In the larval samples, 6 out of 10 bacterial species are non-pathogenic bacteria, namely: Enterococcus phoeniculicola (Byappanahalli et al., 2012); Lachnoclostridium phytofermentans (Zaplana et al., 2023); Schleiferilactobacillus harbinensis (Mashraqi et al., 2023); Limosilactobacillus mucosae (Lee et al., 2024); Herbaspirillum piri (Xu et al., 2018); and Entomomonas moraniae (Wang et al., 2020).

In fly samples, 7 of the 9 bacterial species were non-pathogenic, namely: Sphingobacterium wenxiniae (Chen et al., 2022); Sphingobacterium hotanense (Xiao et al., 2013); Corticicoccus populi (Li et al., 2017); Oceanobacillus sojae (Liu & Yang, 2014); Oceanobacillus jeddahense (Khelaifia et al., 2016); Clostridium tyrobutyricum (Mosconi et al., 2023); and Corticibacter populi (Li et al., 2017).

The microbial community in an ecosystem consists of various species and genera that may have potential applications. Through metagenomic-based analysis, the microbial composition structure can be identified in both larval and adult fly samples. Based on the relative abundance analysis at the genus and species levels, significant differences were observed between the two samples (Figure 1). In the larval sample, the dominant genus was Schleiferilactobacillus. In the adult fly sample, the dominant genus was Corticicoccus. Additionally, confirmation of microbial composition structure at the species level (Figure 2) showed that: the larval sample was dominated by Schleiferilactobacillus harbinensis and the adult fly sample was dominated by Corticicoccus populi.

It is known that both genera exhibit distinct characteristics and are commonly found in the gut of Black Soldier Fly larvae and adult flies (Auger et al., 2023). The presence of these genera suggests that the sampling environment contained organic waste, supporting their dominance in the microbiota composition (Hegazi, 2023). This finding is further confirmed through the heatmap profile visualization (Figure 3), which illustrates the relative abundance distribution at the phylum level. The results emphasize that the most abundant phylum was Firmicutes, with Schleiferilactobacillus harbinensis and Corticicoccus populi belonging to this phylum.

A Sankey diagram analysis was conducted to accurately determine the microbiota composition from the domain level to the species level, allowing for the identification of the most dominant microorganisms in the tested samples. Additionally, through the Sankey diagram analysis, it was possible to distinguish between pathogenic and non-pathogenic microorganisms (Breitwieser & Salzberg, 2020; Platzer et al., 2018). The microbiota community observed (Figure 3) consisted of bacteria from the phyla: Firmicutes, Proteobacteria and Bacteroidota. These findings indicate that both larval and adult fly samples shared the same dominant phyla, suggesting that the microbiota composition in both samples was similar.

At the genus level, there was a difference in dominant microbiota between the larval and adult fly samples. This variation may be influenced by several factors, including growth environment, diet, and the digestive system. Diet is one of the most significant factors affecting microbial composition, as it is directly related to dysbiosis (negative changes in microbial communities), which impacts microbiota diversity. The microbiota that thrives adjust to the substrates consumed (IJdema et al., 2022). In a study conducted by Bruno et al. (2019), it was explained that pH levels also influence microbial composition. When the pH of the gut or body environment is acidic, only acid-tolerant microbiota can survive. Similarly, in neutral or alkaline conditions, microbial growth is adapted to those environments (Auger et al., 2023).

The presence of the Firmicutes or Bacillota phylum in the sample has functions and benefits for black soldier flies. In this study with kitchen waste substrate, the presence of the Firmicutes phylum in the larval sample was found as much as 60.1%, Proteobacteria 31.2% and Bacteroidota 8.7%, and other unknown phyla as much as 0.1%. Meanwhile, in the fly sample, the presence of the Firmicutes phylum was found as much as 99%, Bacteroidota 0.5%, Proteobacteria 0.3% and other unknown phyla as much as 0.1%.

Previous research stated that the dominance of Firmicutes in larval samples could reach more than 75% of its composition due to the composition of the animal-based substrate (Vandeweyer et al., 2023). With this dominance, the microbiota included in this phylum play a role in the process of controlling pathogens such as E. coli and Salmonella. In addition, this phylum can produce short fatty acid chains which are useful as a source of protein for larvae (IJdema et al., 2022). The ability of microbiota members of the Firmicutes phylum is also very important in the process of remediation of organic waste, where the microbiota produces enzymes to break down complex materials in waste such as cellulose and lignin into simpler ones so that they are easier to decompose. That way, the volume of organic waste can be decomposed and processed 50% faster and has great potential as a decomposing agent in environmental remediation (Rusli et al., 2022).

Based on the results of the abundance analysis at both the genus and species levels and reconfirmed through the Sankey diagram analysis, a microbiota community was found that could act as natural bioremediation in larvae, namely:

a) Morganella morganii

A study by Kumar et al. (2020) explains that M. morganii has the ability to absorb lead (Pb (II)), making it beneficial for designing heavy metal wastewater treatment systems. The absorption process occurs when M. morganii dies and produces biomass, which then binds and removes dissolved heavy metals from water. Another study (Princy et al., 2020) reported that M. morganii also has the ability to reduce chromium compounds in chromium-contaminated environments.

b) Herbaspirillum piri

This bacterium has the potential to remediate PAHs (polycyclic aromatic hydrocarbons) and pesticides. It can degrade and detoxify contaminants, such as 4-chlorophenol, from water and soil, making it a valuable agent for environmental remediation and detoxification. Additionally, H. piri plays a role in phytoremediation, aiding in the reduction of heavy metal concentrations in soil (Venkatachalam et al., 2023).

c) Dysgonomonas capnocytophagoides

A study by Sundberg et al. (2011) found that D. capnocytophagoides participates in the remediation of fresh household waste, often working in association with other bacteria to facilitate waste decomposition.

d) Acinetobacter gerneri

This bacterial species has been studied for its potential in degrading phenol degradation, nitrogen assimilation, chromium reduction, heavy metal bioremediation, hydrocarbon degradation, color removal process and breakdown of the chemical structure of dyes in textile industry waste, diesel degradation, polyurethane degradation, petroleum degradation, insecticide degradation, degradation of furan aldehyde compounds, degradation of the chemical insecticide fipronil (Dahal et al., 2023).

e) Lachnoclostridium phytofermentans

This bacterium has the ability to break down lignocellulose into simple sugars by secreting specialized enzymes for cellulose remediation (Zaplana et al., 2023).

In addition, microbiota that have the potential as candidates for bioremediation of organic waste in flies are:

a) Sphingobacterium wenxiniae, Bacteria that have the ability to break down cypermethrin (synthetic insecticide/pesticide) as the main source of carbon, PAHs (Polycyclic Aromatic Hydrocarbons), and plastic, oil and pesticide waste (Chen et al., 2022);
b) Mammaliicoccus sciuri, has the potential to remediate antibiotics in nature because it has antibiotic resistance genes, such as the mecA gene. In addition, M. sciuri is also able to remediate lignocellulose into simpler sugars (De Luca et al., 2022);
c) Corticicoccus populi, based on research conducted by (Li et al., 2017) shows that C. Populi has the potential to remediate lignocellulose and process organic waste.

Discussion-3 (Comparison of temperate and tropical climate microbiota)

Previous studies conducted in 2020 in Austria and 2022 in Belgium are countries located in subtropical (temperate) climate regions. This study is the first study in Indonesia, which is located in a tropical climate region. Based on the differences in climate in each region, microbial communities were found that were found in both temperate and tropical climate regions. Likewise, microbial communities were found in one climate region but not in another climate region. The differences in bacterial communities from these two types of climate regions are influenced by natural conditions so that environmental adaptation occurs.

Based on the climate comparison, it can be seen that the genera Enterococcus, Morganella and Dysgonomonas are genera found in both temperate and tropical climate regions. Meanwhile, the genera Providencia, Klebsiella, Scrofimicrobium and Actinomycess found in the intestines of BSF larvae in the temperate region were not found in the intestines of BSF larvae in the tropical region of Indonesia. Likewise, the genera Limosilactobacillus, Entomomonas, Lachnoclostridium and Clostridium were not found in the intestines of BSF larvae in temperate climate areas.

Conclusion

This study conducted a metagenomic survey of Black Soldier Fly (BSF) insects and bioinformatics in a tropical climate region, using data obtained from larvae and adult flies. This approach revealed species diversity within each sample. Through this meta-analysis, two dominant microbiota families were identified in the larval samples consist of Lactobacillaceae and Morganellaceae. In contrast, the adult fly samples were dominated by Staphylococcaceae and Bacillaceae.

Potential microbial candidates for organic waste bioremediation in larvae include by Morganella morganii, Herbaspirillum piri, Dysgonomonas capnocytophagoides, Acinetobacter gerneri and Lachnoclostridium phytofermentans Meanwhile, potential microbial candidates for organic waste bioremediation in adult flies include Sphingobacterium wenxiniae, Mammaliicoccus sciuri and Corticicoccus populi.

The genera Enterococcus, Morganella, and Dysgonomonas were found in both temperate and tropical climate regions. However, the genera Providencia, Klebsiella, Scrofimicrobium, and Actinomyces, which were identified in BSF larvae gut microbiota from temperate regions, were absent in BSF larvae from tropical Indonesia. Conversely, the genera Limosilactobacillus, Entomomonas, Lachnoclostridium, and Clostridium, which were found in BSF larvae from tropical regions, were not detected in BSF larvae from temperate regions.

The non-pathogenic bacterial composition identified as potential candidates for organic waste bioremediation in BSF larvae can be utilized for the development of organic waste management facilities based on bacterial consortiums.

Acknowledgments

The authors would like to express their gratitude to the Genomic Laboratory of Solidaritas Indonesia, Jakarta, and the Biotechnology Laboratory of the Faculty of Mathematics and Natural Sciences, Pattimura University, Ambon, for their support in data analysis and creative discussions.

Author Contributions

Conceptualization: Rony Marsyal Kunda and Maman Rumanta; Methodology: Irwanto and Rony Marsyal Kunda; Software: Rony Marsyal Kunda; Validation: Irwanto, Maman Rumanta, and Rony Marsyal Kunda; Formal Analysis: Irwanto, Maman Rumanta, and Rony Marsyal Kunda; Investigation: Irwanto, Maman Rumanta, and Rony Marsyal Kunda; Resources: Irwanto and Rony Marsyal Kunda; Data Curation: Irwanto, Maman Rumanta, and Rony Marsyal Kunda; Writing—Original Draft Preparation: Irwanto; Writing—Review & Editing: Irwanto, Maman Rumanta, and Rony Marsyal Kunda; Visualization: Rony Marsyal Kunda; Supervision: Maman Rumanta and Rony Marsyal Kunda; Project Administration: Irwanto; Funding Acquisition: Irwanto. All authors have read and approved the final published manuscript

Funding

This research was self-funded by Irwanto.

Conflicts of Interest

The authors declare no conflicts of interest." All claims expressed in this article are solely those of the authors and do

not necessarily represent the views of their affiliated organizations, publishers, editors, or reviewers.

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