UNIVERSITI TEKNOLOGI MARA

EVALUATION OF THE POTENTIAL LOCALLY ISOLATED FEATHER DEGRADING BACTERIA FOR CHICKEN FEATHER DEGRADATION IN VERMICOMPOSTING

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Thesis submitted in fulfilment
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CONFIRMATION BY PANEL OF EXAMINERS

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ABSTRACT

The increase of chicken production as one of affordable protein sources have led to increase in waste including chicken feather. Chicken feather is rich in keratinous protein which is a fibrous and insoluble structural protein. Conventional waste management is costly and not environmentally friendly. Recent studies found keratin degrading bacteria aids in degrading chicken feathers. Hence this study focusses on the locally isolated degrading bacteria (*Serratia* spp.) as composting agent along with *Eudrilus eugeniae* and the efficiency in the biodegradation of chicken feathers in vermicompost. Also, the physicochemical properties of vermicompost affect by composting agents whether applied singly or in combination. *Serratia* spp. was isolated from soil sample in coop area and identified through 16s rDNA sequencing followed by inoculating in pre-composted vermicompost. Physicochemical properties, microbial enzyme activity, feather degradation and earthworm population were observed and analysed statistically. Significant differences were observed in keratinase enzyme activity of isolate 5 at 3.8 U/mL which is 80.9% higher than isolate 15 ($p < 0.05$). Therefore isolate 5 was selected for further analysis. T3 (Mushroom Media Residue:Banana Trunk:Chicken Feather:Chicken Dung = 6:1:1.5:1.5) shows higher degradation coefficient as well as earthworm population significantly. However, there is no significant difference between treatments in pH value and EC of compost. N,P,K, Ca and Mg of T1-T4 is significantly higher than T5-T8 whereas organic matter content of T1-T4 is significantly lower than T5-T8 due to higher ratio of chicken dung. Further optimization of the environmental condition like pH, incubation time and temperature, carbon and nitrogen sources of isolate 5 (*Serratia* spp.) was recommended to enhance the keratinase production. The overall findings suggest that isolate 5 has a strong potential for biotechnological applications in chicken feather waste management. Further studies are recommended to optimize production conditions and evaluate the potential of isolate 5 for industrial-scale applications.

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LIST OF SYMBOLS

Symbols

CFU/mL	Colony Forming Units Per Millilitre
℃	Degrees Celcius (Temperature)
EC	Electrical Conductivity
U/mL	Enzyme Activity Unit Per Millilitre
g	Gram
mL	Mililitre
nm	Nanometer (Used For Absorbance Readings)
%	Percentage
pH	Potential of Hydrogen
× g	Relative Centrifugal Force
Kb	Vermicompost Biodegradability Coefficient

LIST OF ABBREVIATIONS

Abbreviations

BT	Banana Trunk
CF	Chicken Feather
CM	Chicken Manure
DNA	Deoxyribonucleic Acid
FAO	Food and Agriculture Organization
KCl	Potassium Chloride
MMR	Mushroom Media Residue
Na Salicylate	Sodium of Salicylic Acid
OD	Optical Density
rDNA	Ribosomal Deoxyribonucleic Acid
rpm	Revolutions Per Minute
SE	Standard Error
TCA	Trichloroacetic Acid
THAM	Tris (Hydroxymethyl) Aminomethane Buffer
Tris-HCl	Tris (Hydroxymethyl) Aminomethane Hydrochloride
UiTM	Universiti Teknologi MARA
UPM	Universiti Putra Malaysia

LIST OF NOMENCLATURE

Nomenclatures

Catalase	Enzyme That Decomposes Hydrogen Peroxide into Water and Oxygen
Crude enzyme	Initial Enzyme Extract Before Purification
Eudrilus eugeniae	African Nightcrawler Earthworm Used in Vermicomposting
Invertase	Enzyme That Breaks Sucrose Into Glucose
Keratin	Fibrous Protein, Main Component of Feathers
Keratinase	Enzyme That Breaks Down Keratin
Keratin azure	Substrate Used to Assay Keratinase Activity
Serratia spp.	Bacteria Genus Used in the Study
Polyphenol oxidase	Enzyme That Oxidizes Polyphenols, Involved in Browning Reactions
Urease	Enzyme That Hydrolyzes Urea Into Ammonia and Carbon Dioxide

CHAPTER 1
INTRODUCTION

1.1 Research Background

The term poultry refers to domestic birds raised for their eggs and meats and is important in providing a balance diet for human as a source of protein. Chicken is one of poultry and is consumed globally which is the reason for increase in production in the past years. According to Food and Agriculture Organization of the United Nations (2020) the global chicken population was over thirty-three billion birds in 2020, with 46% of these were in Asia. This increased in poultry production leads to increase in poultry waste like chicken feather and chicken dung. Up to these days, chicken feather commonly goes to waste although it is known to be a rich source of protein due to inadequate methods of degrading or recycling.

As the population grows, the demand for animal protein, particularly chicken, increases due to its affordability and nutritional value (Kleyn & Ciacciariello, 2021). This surge in demand results in significant waste production, including chicken manure and feathers, which have both benefits and drawbacks (Anandyawati et al., 2023). The costs associated with disposing of these wastes can drive up the price of poultry products (Zinina et al., 2022).

In recent years, a keratinous protein found in chicken feathers has drawn a lot of attention. It is the most prominent and abundant form of fibrous protein found especially in chicken feathers. The main component of chicken feather is keratin which is up to 90% of protein with β-keratin as the main component, a fibrous and insoluble structural protein that is strongly cross-linked by disulfide bonds (Aktayeva et al., 2022). The disulfide bonds, hydrogen bonds, and hydrophobic interactions between the amino acid residues in feather keratin, on the other hand, are insoluble in water and poorly digestible by pepsin-family enzymes. Keratinases that can break disulfide bonds are used for the enzymatic hydrolysis of keratin. Recent studies have been reported that a huge number of microorganisms that produce keratinases with bacteria being the most promising (Aktayeva et al., 2022; Almahasheer et al., 2022).

Numerous feather-degrading bacteria have been identified and reported from various environmental sources. These bacteria vary in their inherent ability to break down feather and produce keratinase enzyme (Ahmed et al., 2020). Previous studies shown that several microbes produce keratinase when keratin substrate is presence (Subugade et al., 2019). Microbial conversion has been a safer and reliable choice to degrade chicken feather compared to conventional disposal method which cause in greenhouse gases, however, still depends on efficient microbes which are hardly present (Opiyo et al., 2021). Hence, this study focusses on the potential locally isolated chicken feather degrading bacteria with the aid of *Eudrilus eugenia*e to enhance the degradation process of chicken feather as the substrate for composting.

Vermicomposting and traditional composting are process of conversion of organic waste but have different biological agents, composting time, and final product's quality. In this study, the organic waste chosen are chicken feather and chicken dung and a locally isolated bacteria as the composting agent. African nightcrawler (*Eudrilus eugeniae*) was used in vermicompost treatment.

1.2 Motivation for This Work

The motivation for this study comes from the significant gap in the current literature regarding chicken feather vermicompost and personal interest. The significance of this study lies in the potential of locally isolated feather-degrading bacteria. By studying the efficiency of the feather-degrading bacteria and earthworms in the biodegradation of chicken feathers, this study aims to contribute a deeper understanding of chicken feather vermicompost. Existing studies have primarily focused on feather-degrading bacteria, but none have addressed in using chicken feather as a substrate in vermicompost, which this study aims to fulfil.

This study provides new awareness into chicken feather degradation, chicken feather vermicompost, and feather-degrading bacteria. Practically, this study has the potential to produce vermicompost, benefiting both organic fertilizer and poultry industry.

1.3 Problem Statement

In the poultry industry, chicken feather is one of the waste products, which cause serious dumping problem (Prasanthi et al., 2016). The production of feathers is increasing as the poultry production increase causing pollutants that affect the environment and public health if left untreated (Gržinić et al., 2023). The former methods like burning and chemical treatment are not environmentally friendly and highly cost. Chicken feathers contain high keratin content which is difficult to degrade because of the cross-linking disulphide bonds of the keratin, an insoluble protein, which is why their disposal is a challenge (Opiyo et al., 2021).

According to Livestock Statistic Malaysia (Department of Veterinary Services, 2024), an increase of chicken production from 2021 (285,352,437 birds) to 2024 (326,057,949 birds) have been reported along with increase in the waste of chicken feathers (**Error! Reference source not found.**). Globally, increased chicken production generates approximately 68 billion tonnes of waste annually, including wastewater, feathers, eggshells, carcasses, blood, and manure, most of which have limited applications and often end up in landfills (McGauran et al., 2021).

JADUAL 1.5: BILANGAN TERNAKAN AYAM MENGIKUT NEGERI, MALAYSIA, 2021-2024[e]

Table 1.5: Number of Chicken by States, Malaysia, 2021-2024[e]

NEGERI *State*	*Ekor/Units*			
	2021	2022	2023	2024[e]
Perlis	844,573	867,301	523,860	530,609
Kedah	21,838,000	23,883,212	24,047,983	24,473,430
Pulau Pinang	13,197,995	15,574,537	16,213,609	16,633,268
Perak	42,031,500	41,894,500	41,097,500	42,866,242
Selangor	24,355,658	22,687,114	28,249,142	29,414,462
N. Sembilan	24,011,281	26,357,334	29,727,918	30,783,282
Melaka	30,861,607	30,356,503	33,286,808	34,943,095
Johor	51,862,940	54,170,061	53,192,051	55,103,317
Pahang	14,016,721	14,101,294	16,327,463	17,114,789
Terengganu	3,412,398	3,556,825	4,345,248	4,336,629
Kelantan	5,122,850	5,042,000	4,663,650	4,735,680
W. Persekutuan	0	0	-	-
Jumlah S. M'sia *Total For P. M'sia*	**231,555,523**	**238,490,681**	**251,675,232**	**260,934,803**
Sabah	6,640,295	9,643,735	9,095,891	9,294,445
Sarawak	47,156,619	51,288,777	55,414,307	55,828,701
JUMLAH BESAR *Grand Total*	**285,352,437**	**299,423,193**	**316,185,430**	**326,057,949**

Figure 1.1 Number of Chicken by States, Malaysia, 2021-2024 (DVS, 2024)

In Malaysia, all chicken feathers have mostly been disposed of through landfill and combustion all this while (Jayathilakan et al., 2012; Mozhiarasi & Natarajan, 2025).

These disposal methods lead to environmental problems such as foul odour release, as well as other water and air pollution issues (Ma et al., 2022). As a result of inappropriate handling of poultry waste, diseases spread in the poultry areas and dumping sites (Begum et al., 2023). Since feather wastes are difficult to decompose, there is a lack of knowledge on how to use them. These drawbacks compel us to seek a sustainable approach for managing chicken feather wastes through the vermicomposting process.

The vermicomposting technology has been a low cost and environmentally friendly practice to manage enormous amounts of bulky organic materials like chicken dung wastes. Prior to decomposition of the feather wastes, the fundamentals of promoting the efficiencies of the waste conversion must be studied. This research investigated this matter and provided scientific fundamental data that could lead to the future utilization of chicken feathers, as part of the aims of the Sustainable Development Goals number 12, which is to ensure sustainable consumption and production patterns.

1.4 Research Objectives

The objectives of this study were as follows:

i) To identify the most potential locally isolated feather-degrading bacteria.

ii) To determine the efficiency of the feather-degrading bacteria and earthworms in the biodegradation of chicken feathers.

iii) To characterize the physicochemical properties of by-products (vermicompost) produced by the feather-degrading bacteria and earthworms applied singly or in combination.

1.5 Research Question

i) What is the most potential locally bacterial isolates for degradation of chicken feathers?

ii) How do the most potential locally isolated feather degrading bacteria interact with earthworms in vermicomposting for biodegradation of chicken feathers?

iii) How do the most potential feather degrading bacteria and earthworms affect the physicochemical properties of by-product (vermicompost) when applied singly or in combination?

1.6 Significance of Study

The national production of chicken increases each year due to high consumption of chicken meat as a source of protein, which result in increase of chicken feather waste. Unlike chicken dung, chicken feathers are left unused despite having high protein contents due to keratin content. The significance of this study is to locally obtain bacterium that can be used to degrade chicken feathers. The earthworm interaction with the bacteria that break down chicken feathers improved the vermicomposting process, and higher quality compost produced. On the other hand, this study provides an alternative method for degrading chicken feathers using appropriate treatments, with the goal of converting the resulting decomposition materials into agriculturally beneficial products. Additionally, the research investigates whether the combined action of *Eudrilus eugeniae* and locally isolated bacteria can shorten the composting duration of chicken feathers. *Eudrilus eugeniae* is a well-known composting agent capable of breaking down organic matter, while the combination of feather degrading bacteria is efficient in degrading keratin and thus shortening the vermicomposting process of chicken feather. The focus of this study is on the synergistic interaction between these two organisms, particularly how variations in their population ratios influence the composting process. Moreover, the research assesses the chemical characteristics of the final compost produced, including pH and electrical conductivity

(EC), and examines the enzymatic activity created during the bioconversion of chicken waste by these decomposers.

1.7 Limitation

Despite the significant contributions of this study, several limitations must be acknowledged. The isolation and screening of feather-degrading bacteria was carried out under a standardized condition without any optimization which possibly might overlook the less dominant yet effective feather-degrading bacteria.

This study did not consider the suggested vermicomposting procedure's practicality or scalability. Future research should include cost-benefit assessments to determine the practicality and financial viability of using this approach on a wide scale.

By acknowledging these limitations, future research may focus on overcoming these issues in order to increase the reliability and application of the findings.

1.8 Thesis Scope

The main goal of this study is to identify the efficacy of locally isolated feather degrading bacteria and earthworm (*Eudrilus eugeniae*) in chicken feather vermicompost. This study was conducted at UiTM, Perlis and UPM, Serdang. Also, soil and feather samples collected from a local poultry farm. This study starts on 1st October 2022 to 31st August 2024.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

The increasing global demand for poultry products has led to rapid growth in the poultry industry, generating a substantial volume of by-products, particularly chicken feathers. These feathers often considered agricultural waste, which is a significant environmental challenge due to their complex keratin structure that resists natural degradation. As the poultry sector continues to expand, sustainable management of feather waste becomes an urgent priority, not only to prevent environmental pollution but also to explore its potential as a valuable organic resource.

Chicken feathers, which make up approximately 5–7% of chicken's total weight, are typically discarded through methods such as incineration or landfilling (Rahayu & Bata, 2015). These disposal techniques are not environmentally friendly and also represent a missed opportunity for waste valorisation. As more people become aware of sustainable agriculture and waste recycling, eco-friendly and efficient biological degradation methods are gaining attention. Vermicomposting, which uses earthworms and microorganisms to turn organic waste into nutrient-rich compost, is a great example of this approach.

To fully understand the value of utilizing chicken feathers in vermicomposting, it is essential to first understand the biological composition of feathers, especially the keratin they contain. Keratin is a highly stable, fibrous protein that imparts feathers with their structural rigidity and makes them resistant to degradation (Banasaz & Ferraro, 2015).

This chapter explores the essential concepts and scientific discoveries related to breaking down chicken feather waste and its use in vermicomposting. It begins with an overview of chicken feathers and their types, followed by keratin structure, function, and challenges in biodegradation. The discussion then shifts to vermicomposting, describing its biological basis, nutrient outcomes, and the role of earthworms in the decomposition process. Finally, the chapter explores recent developments in using

microbial and vermicomposting techniques for feather degradation, highlighting both their potential benefits and limitations.

2.2 Chicken Feather

Chicken feather is a great deal of waste product where the birds are processed mainly for their meats and eggs which leads to a major solid waste problem globally, including Malaysia. Feathers are frequently buried in landfills or piled in dumps. Feathers, on the other hand, are naturally resistant to deterioration and can last for decades in the environment. As a result, they take up a lot of space in landfills, and the bad odour from residual manure, blood, and other extraneous materials pollutes the environment. Waste feathers are burned in incineration plants in some countries. However, burning waste feather is costly, and the process emits greenhouse gases and causes ash disposal issues.

Chicken feather is difficult to degrade naturally, although a few methods have been used either not environmentally friendly or costly. According to Mousa et al. (2021), the latest treatment alternative to chicken feather waste that is environmentally friendly is microbial hydrolysis, where a study was conducted to isolate keratinolytic bacteria which enhance some physical parameters affecting the growth of bacteria and degrading ability. Shots of studies have been made to recycle and treat chicken feather wastes chemically and naturally; nevertheless, these management method rest on the poultry industries and the consumers.

Nowadays chicken feathers have been utilised as a by-product like protein supplement for animal feed. For instance, Musikoyo et al. (2021) conducted a study to degrade chicken feathers by bacteria isolated gathered from flamingo feathers which is then used to produce protein supplement for animal feed. Two bacteria isolates were used in the study however the result proves that *Bacillus agaradhaerens* have a higher ability to degrade feathers compared to LNN03. Many conducted studies were made upon converting these feathers into meal due to the high protein content. Furthermore, Kumari & Kumar (2020) conducted a study of chicken feather degradation effects on plant growth and found that the amino acid and protein release have improved soil nutritional value. The feathers were degraded with ease of *Alternaria tenuissima* within

25 days, which increase the pH value of the treated soil as well as the height of plant by 1.4 times.

Nevertheless, the dumping of this waste is still abundant, and research have been reported to utilise the chicken feather due to its high protein content. Chicken feathers vary in shape and function. They are classified as contour, down, semi plume, filoplume, or bristle. Still, regardless of type, chicken feathers are roughly half feather fibre (barbs) and half quill (rachis) by weight. The quill is a stiff central core with a hollow tube structure, and the barbs are fine fibrous materials that branch out from the quill (Figure 2.1). Both the feather fibre and the quill are made of approximately 90% of keratin that are highly durable and insoluble (Maurya & Singh, 2024).

Figure 2.1 Morphological structure of chicken feather (Belarmino et al., 2012)

Chicken feather literally serves as a protective layer to protect the birds from sun rays also as an insulator. Pigments and keratin in feathers contribute to the colours like brown, black, red, yellow, and white. Besides being processed and use as animal feed, feather keratin can also be used to make plastics, papers, pillow stuffing and padding for furniture insulation.

2.2.1 Types of Chicken Feathers

Chicken feathers vary in structure, function, and degradability. Each feather type contains different proportions of keratin and structural rigidity, which directly influence how easily it can be degraded during composting or microbial processes. The main types include down, contour (including flight feathers), semiplume, and filoplume (Bock, 2004).

i. Down Feathers

Down feathers are soft, fluffy feathers located beneath the outer contour feathers, especially around the breast and abdomen. They consist of short barbs that radiate from a central point without a defined shaft. Their loose structure traps air, providing insulation. Due to their low keratin density and lack of tightly interlocking barbules, down feathers are generally more biodegradable than other feather types.

ii. Contour Feathers

Contour feathers cover the surface of a chicken's body, wings, and tail, giving the bird its shape and coloration. They include the flight feathers, which are highly structured and adapted for aerodynamics. Each contour feather has a rigid central rachis (shaft) with barbs extending laterally, which are interlocked by microscopic barbules and hooklets to create a smooth surface.

iii. Semiplume Feathers

Semiplume feathers are located beneath contour feathers, providing additional insulation. They have a central rachis like contour feathers but lack barbule hooklets, making them softer and less rigid. Their structure is intermediate between contour and down feathers, and they are moderately degradable.

iv. Filoplume Feathers

Filoplume feathers are hair-like and found near the base of contour feathers. They have a thin, flexible shaft with few barbs and are connected to nerve endings that help position contour feathers. Their small size and simple structure make them less

significant in total feather mass, but they are relatively easier to degrade due to their low keratin content.

2.3 Keratin Overview

Keratin is a fibrous, structural protein that is highly insoluble, stable, and resistant to degradation. It is the primary constituent of many epidermal appendages, including feathers, wool, horns, nails, hooves, and hair. In chicken feathers, keratin comprises up to 90% of the total feather mass, making it the key factor influencing the durability and slow decomposition of poultry feather waste (Zhang & Fan, 2021).

2.3.1 Types of Keratins

According to Zhang & Fan (2021), keratin is broadly classified into two types based on its structural configuration:

- α-Keratin

Found predominantly in mammals (e.g., hair, nails, horns), α-keratin is organized in coiled coils of α-helices, giving it flexibility and elasticity. It is comparatively less rigid than β-keratin.

- β-Keratin

Found in birds and reptiles, β-keratin is the primary form in chicken feathers. It is composed of β-pleated sheet structures, which provide greater rigidity, crystallinity, and resistance to enzymatic breakdown. This high mechanical strength makes β-keratin more durable and significantly more challenging to degrade through natural processes.

2.3.2 Structure and Composition of Keratin

Keratin is made up of over 90 amino acids, the majority of which are cystine, lysine, proline, and serine (Figure 2.2). These amino acids tend to cross-link with one another via disulfide or hydrogen bonds, resulting in tough, strong, lightweight fibres with good thermal and insulating properties. In its native state, its tight secondary structure and the fact that the peptide chains are held together by disulfide linkages,

feather keratin is insoluble and not degradable by commonly known proteolytic enzymes such as trypsin, pepsin, and papain but feathers do not accumulate in nature and can be efficiently degraded by a wide range of microorganisms thanks to the action of keratinolytic proteases – keratinases (Subugade et al., 2019). The unique properties of keratin sparked interest in researching alternative uses for waste chicken feathers for a variety of potential applications.

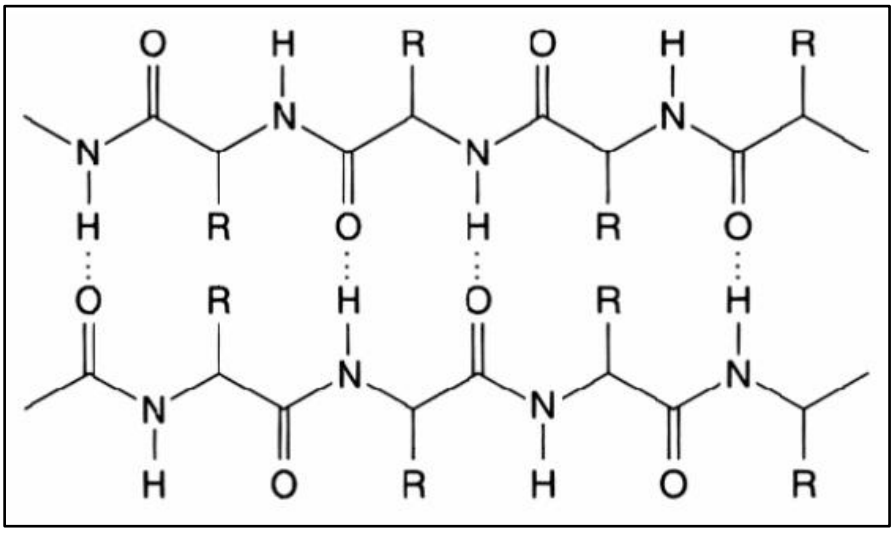

Figure 2.2 Chemical structure of keratin in chicken feather (Menandro, 2010)

There are many different types of keratinolytic organisms in nature. These organisms were isolated from a variety of sources on all continents. Many of them were isolated from keratin waste dumps, particularly from the slaughterhouse and feather dumps. The majority of studies on keratinase production were conducted on bacteria, primarily *Bacilli* (Yahaya, 2022; Cavello et al., 2021; Yadav & Khosla, 2021). *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus subtilis*, *Bacillus cereus*, and *Bacillus pumilus* are different *Bacillus* species that produce keratinase (Aktayeva & Khassenov, 2024; Derhab et al., 2024; Avdiyuk & Roy, 2021; Sun et al., 2021). Keratinases are subtilisin, serine protease (S8 family) enzymes (Azrin et al., 2022). Bacteria from other genera besides *Bacilli* have also been reported including *Stenotrophomonas* (Peng et al., 2024), *Serratia* (Gerlicz et al., 2024), and *Pseudomonas* (Ramalingum et al., 2024).

Apart from bacteria, fungi and actinomycetes are also involved in the degradation of keratin wastes. Most actinomycetes are isolated from various soil sites and could hydrolyse a wide range of keratinous wastes, and their keratinase has been used in a variety of industries. In general, all keratin-degrading organisms are found in

natural environments such as soil and water, where they aid in the natural recycling of carbon, nitrogen, and sulphur compounds in keratins. However, the degree of degradation and ability to utilise various keratin waste varies between organisms. Some individuals are more aggressive than others. While some rely entirely on keratin as their sole source of carbon and nitrogen, others benefit from the addition of non-keratin sources to the medium.

Some keratinolytic organisms are highly substrate specific because they only grow on specific keratin waste like feathers. However, some like *B. pumilus* A1, *B. licheniformis* ER-15, *Chryseobacterium* sp RBT, *Purpuriocillium lilacinum*, *Scopulariopsis brevicaulis* and *Streptomyces thermoviolaceus* SD8 can utilise different keratin waste (Yusuf, 2016).

2.3.3 Challenges in Degrading Chicken Feather Waste

Chicken feathers, though seemingly soft and lightweight, present one of the most resilient and difficult-to-degrade organic wastes generated by the poultry industry (Gupta et al., 2023). The primary reason for this recalcitrance lies in their biochemical composition, dominated by β-keratin — a fibrous, structural protein rich in disulfide bonds. While the keratin content gives feathers their strength, elasticity, and water resistance, it also significantly limits the effectiveness of natural decomposition, conventional composting, and even some industrial treatment methods (Maurya & Singh, 2023).

Keratin is known for its high stability due to its tightly packed polypeptide chains, extensive hydrogen bonding, and abundant disulfide bridges formed by cysteine residues. These molecular features render keratin insoluble in water and resistant to degradation by common proteolytic enzymes like trypsin and pepsin. In comparison to other proteins, keratin exhibits exceptional mechanical strength and thermal stability, making it an unfavourable substrate for microbial or enzymatic attack without specialized conditions or pretreatment (Giteru et al., 2023).

From a waste management perspective, this biochemical resilience poses several challenges. Traditional disposal techniques such as landfilling and incineration are energy-intensive, polluting, or both. Landfilling keratinous waste contributes to long-term environmental persistence, as decomposition may take years or even decades (Sypka et al., 2021). Incineration, while faster, results in the loss of organic nutrients

and generates harmful emissions. Additionally, the centralized infrastructure required for these methods may not be available or feasible for small-scale poultry operations, especially in rural or resource-limited regions.

Chemical and physical methods for feather degradation including hydrolysis using strong acids or alkalis, or thermal pressure treatments (autoclaving) have been explored (Isembart et al., 2025). These approaches can partially or fully denature keratin, making it more amenable to further processing. However, these methods often suffer from several limitations:

- High energy requirements, which make them economically unsustainable.
- Use of hazardous chemicals, which introduce new environmental risks and require neutralization before disposal.
- Loss of nutrient value, where the nitrogen-rich composition of the feathers is degraded or volatilized.
- Generation of secondary waste, such as chemical residues and process by-products.

Biological degradation offers a more sustainable and environmentally friendly alternative. However, even within this domain, significant challenges remain. Most microorganisms are incapable of breaking down the robust keratin matrix without specialized enzymes, such as keratinases (Sypka et al., 2023). These keratinolytic enzymes are secreted by certain bacteria and fungi capable of cleaving disulfide bonds and peptide linkages in keratin. However, the efficiency of keratinase production depends on multiple factors, including species or strain specificity, growth conditions, substrate concentration, pH, temperature, and nutrient availability (Yahaya et al., 2021).

Moreover, large-scale application of keratin-degrading microbes or enzymes has not yet been widely adopted due to cost, scalability issues, and regulatory limitations. Many microbial strains with keratinolytic potential have only been studied under laboratory conditions, and their field performance may vary significantly. There is also limited standardization regarding optimal fermentation conditions or process parameters for maximizing keratin degradation across different feather types and waste volumes (Sypka et al., 2021).

Another critical challenge is the time required for biological feather degradation, which can be relatively long compared to the speed of mechanical or chemical

treatments. Without the aid of pretreatment steps or process optimization, microbial degradation of keratinous waste can take weeks to months. Additionally, the physical structure of the feathers such as size, density, and compactness can hinder microbial penetration and enzymatic access to internal keratin fibers, slowing down the overall rate of decomposition (Grima & Cornish, 2024).

Furthermore, integrating feather waste into existing organic waste recycling systems, such as composting or vermicomposting, requires careful balancing of the carbon-to-nitrogen (C:N) ratio, moisture content, and aeration. Feathers are rich in nitrogen but poor in readily degradable carbon sources (Gupta et al., 2023). Without co-composting with high-carbon materials (e.g., straw, leaves, sawdust), the degradation process may become inefficient or produce unpleasant odors due to ammonia volatilization or anaerobic conditions.

Despite these challenges, research has shown promising results when combining feather-degrading microbes with other organic treatment systems, including vermicomposting (Tesfaye et al., 2017; Nad et al., 2024). By leveraging the synergistic action of microbes, enzymes, and earthworm gut flora, feather degradation can be accelerated and converted into nutrient-rich compost (Meilander & Caporaso, 2024). Still, further studies are needed to refine the protocols, improve efficiency, and evaluate long-term effects on soil health and crop productivity.

In summary, the degradation of chicken feathers is hindered by the intrinsic properties of keratin, limitations of conventional treatment methods, and biological complexity. Overcoming these challenges requires innovative, multidisciplinary approaches that blend microbiology, biotechnology, and sustainable waste management practices. As the poultry industry continues to grow, so does the need for scalable, cost-effective, and environmentally sound solutions to manage feather waste positioning biological degradation and integrated composting methods as promising areas for future development.

2.3.4 Microbial Degradation of Keratin

Microbial degradation of keratin has emerged as one of the most promising and sustainable approaches to manage keratin-rich waste materials, such as chicken feathers. Unlike conventional chemical or thermal methods, microbial degradation utilizes naturally occurring bacteria and fungi that possess the enzymatic machinery

necessary to break down the complex and recalcitrant keratin structure into simpler, environmentally friendly by-products (Vikash et al., 2025). This process offers a low-energy, cost-effective, and eco-conscious alternative to traditional feather waste treatment techniques.

The primary biological agents responsible for keratin degradation are keratinolytic microorganisms. These include several species of bacteria (e.g., *Bacillus*, *Pseudomonas*, *Streptomyces*) and fungi (e.g., *Aspergillus*, *Trichophyton*, *Chrysosporium*) that produce specialized enzymes known as keratinases (Anbesaw, 2022). Keratinases are extracellular proteolytic enzymes capable of cleaving the peptide bonds and disulfide bridges in keratin, thereby enabling the complete hydrolysis of the protein into peptides and free amino acids. This enzymatic process renders the feather material bioavailable for microbial metabolism and subsequent conversion into valuable end-products such as compost, amino acid-rich liquid fertilizers, or animal feed supplements.

Bacteria from the genus *Bacillus* are among the most extensively studied keratin-degrading organisms due to their ability to grow under a wide range of environmental conditions, fast enzymatic activity, and ease of cultivation (Gerlicz et al., 2024). *Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus cereus* are commonly isolated from poultry farm soils, compost piles, and slaughterhouse waste sites (Yadav & Khosla, 2021). These bacteria are particularly efficient in feather hydrolysis and keratinase secretion, especially under optimized conditions such as pH 7.5–9.0 and temperatures ranging from 37°C to 55°C (Tamreihao et al., 2019).

According to Peng et al., (2019), the process of microbial keratin degradation generally occurs in three stages:

i. Adsorption and colonization: Microbial cells attach to the surface of keratinous material, forming biofilms and initiating the secretion of keratinases.

ii. Enzymatic breakdown: Keratinases cleave the protein into soluble peptides and amino acids, often with the help of disulfide reductases that break disulfide bonds.

iii. Metabolite assimilation: Microbes utilize the hydrolyzed products as nitrogen and carbon sources, promoting microbial biomass production and further enzymatic activity.

Numerous studies have demonstrated that the efficiency of keratin degradation depends not only on microbial strain selection but also on environmental factors such as temperature, pH, substrate concentration, and nutrient availability (Yahaya et al., 2022; Mozejko & Bohacz, 2022). For instance, supplementation with glucose (carbon source) and tryptone (nitrogen source) can enhance keratinase production and microbial growth (Al-Amery & Alyousif, 2025; Akhter et al., 2020). Additionally, feather pre-treatment methods such as grinding, steam explosion, or mild alkali soaking have been shown to increase surface area and porosity, improving microbial access to keratin fibers (Wang et al., 2025).

The potential applications of microbial keratin degradation are massive. The hydrolysates obtained from feather biodegradation contain essential amino acids like cysteine, serine, and glycine, which are useful in agriculture as biofertilizers (Bhari et al., 2021). Moreover, microbial keratinase enzymes themselves have attracted commercial interest for use in detergent formulations, leather processing, pharmaceutical production, and biodegradable plastics.

Despite these advantages, the large-scale industrial application of microbial keratin degradation is still in its initial stages. Challenges remain regarding strain stability, scalability of production systems, downstream processing, and regulatory approvals for using microbial hydrolysates in food or feed industries. However, there is now more interest in developing and bringing to market microbial keratin degradation technologies due to the growing need for sustainable waste management and the movement toward circular economy models.

In the context of vermicomposting, the integration of keratinolytic microbes can enhance the breakdown of chicken feathers by pre-degrading the keratin structure before or during the vermicomposting process. The combination of earthworm digestion and microbial activity may speed up the rate of degradation, enhance the quality of compost, and increase the variety of organic materials that can be degraded (Zhang et al, 2000). Current research efforts are exploring such integrated

biotechnological approaches to develop efficient and eco-friendly solutions for managing poultry waste at both local and industrial scales.

In conclusion, microbial degradation of keratin represents a biologically sustainable and versatile approach to managing chicken feather waste. Through careful selection of keratinolytic microbes and optimization of growth conditions, it is possible to harness nature's own biological systems to convert a problematic waste material into a valuable resource paving the way for more sustainable agriculture and waste management practices.

2.4 Vermicompost

Vermicompost is the process of conversion of organic wastes with the aid of earthworms and microbes into finely degraded substances that can be used as planting media or commonly applied as fertilizer. Earthworms consume various organic wastes, reducing their volume by 40-60%. Each earthworm weighs about 0.5 to 0.6 g, consumes waste proportional to its body weight, and produces cast roughly equivalent to about 50% of the waste it consumes in a day. Worm casts contain a higher percentage, almost double the content of macro and micronutrients than garden compost. Previous research has shown that vermicompost provides all nutrients in readily available form and improves nutrient uptake by plants (Rehman et al., 2023).

Vermicomposting is an environmentally friendly technique that employs earthworms to degrade crop waste and other solid wastes into organic fertilizer, which supply essential nutrients, which fertilize the soil (Saha et al., 2022). Over 4000 species of earthworms found worldwide can be used for composting including *Eisenia foetida* and *Eisenia Andrei* (the red wiggler) which are commonly used can be found on the soil surface, while other species like *Lumbricus rubellus* sps found in deeper layer. Besides earthworms, biodegradable wastes like animal wastes are commonly used in the preparation of vermicompost. The application of vermicompost increases soil fertility as well as enhance soil health, also develop roots of plants. Furthermore, vermicompost helps maintaining and assisting with plant development, seed germination, and crop yield, also serves as a biofertilizer and is utilized in organic farming since it produces superior results. The pH of the soil is balanced with the aid of vermicomposting without infections, insects, or hazardous substances.

According to Mohite et al. (2024), vermicompost is a high-nutrient biofertilizer containing a variety of microbial communities that is thought to significantly increase the growth and yield of many field crops, vegetables, flowering plants, and fruit trees. Vermicomposting, an alternative waste management technique, is the process of turning organic wastes into finely degraded peat-like substances using earthworms. Vermicompost is created through this process and has a relatively higher nutrient content than compost and manures.

Vermicomposting of chicken feathers through the interaction between bacteria isolated from feathers and earthworms. The composting process of chicken feathers is difficult and time-consuming because of the keratins that are resistant to degradation. A conversion of chicken feathers through the composting process as one of the biological treatments for these wastes can be improved and accelerated by the addition of activators in the form of suitable microorganisms for facilitating such a decomposition process.

2.4.1 Nutrient Content in Vermicompost

Heavy doses of chemical fertilisers and pesticides have increased social and environmental risks around the world as well as affecting the fertility, soil microbes and agricultural yields, besides minimizing the resistance of plant against insect-pest diseases. Organic fertilizer which includes vermicompost is one of the attempts to supply nutrient to plant without affecting the environment or even the yield. In order to grow healthily, a plant needs 17 elements which are obtained from soil except C, H and O which are taken from water and air. The primary nutrients (N, P and K) are needed by the plants in a large amount and often supplemented as fertilizers. The secondary nutrients (Ca, Mg, and S) are needed in quite a large amount but are adequately supplied and are usually readily available. Whereas the micronutrients or trace elements (Fe, Zn, Mo, Mn, B, Cu, Co, and Cl) are needed in small amount. According to Amante (2024), the organic matter formed during vermicomposting process benefits in binding mineral particles such as potassium, calcium, magnesium, and in the form of humus and clay colloids, which allows solid clumps of soil particles for optimum porosity and better growth of plants. The humic substances present in vermicompost formed effects on plants comparable to those caused by auxin. The higher chlorophyll concentrations

identified in plants treated with vermicompost were caused by the humic substances in the vermicompost, which potentially enhanced plant photosynthesis and yield qualities.

A recent study shows that vermicompost has a higher nutrient content compared to compost (Syarifinnur et al., 2023). The pH range of 6.5 to 7.5 is ideal for vermicomposting. Vermicomposting performed best with 50% aeration and temperatures ranging from 52 to 55℃ (Katiyar et al., 2023).

Table 2.1
Nutrient composition of vermicompost

Nutrient	Content
Organic Carbon	21.26 to 34.66%
Total Nitrogen	3.04 to 4.26 %
Available Phosphorus	775.39 to 1277.62 mg/kg
Available Potassium	3740.40 to 7327.7 mg/kg
Calcium	26.8 to 35.2 m.e/100 g
Magnesium	10 to 13.2 m.e/100 g
Sulphur	128 to 548 ppm
Copper	100 ppm
Iron	1800 ppm
Zinc	50 ppm

Source (Geremu et al., 2020)

2.4.2 Types of Vermicomposting

In a recent study by Bhari et al. (2021), conversion of organic wastes is a mesophilic operation that involves symbiotic microbial activities, while earthworms are well-known earth dwellers which can work synergistically with the bacteria to turn organic wastes into nutrient-rich and valuable end products for farming in the procedure of vermicomposting. Vermicomposting can be carried out using different systems depending on the scale of production and the resources available (Kaur & Bhatt, 2024). Broadly, these methods are categorized into batch systems and continuous flow systems, with further variations as outlined below.

i. Batch System

In the batch system, bedding material and feed are mixed together, after which earthworms are introduced. The system is then left undisturbed until the process is complete, at which point the vermicompost is harvested in bulk.

ii. Continuous Flow System

Continuous flow systems involve placing worms in bedding material and subsequently providing small amounts of fresh feed and bedding at regular intervals. This ensures continuous composting activity and allows for ongoing production.

iii. Windrow Vermicomposting

Windrow vermicomposting is the most common commercial method in agriculture, typically using wide rows of cow manure. Farmers prepare windrows that are around 30 meters long, 0.9 meters high, and 0.6 meters wide. Worms are introduced into these rows, and moisture is maintained throughout the process. Additional manure is often added to the ends of the rows to encourage worm migration and accelerate composting. Several windrow variations exist:

a) Static Pile Windrows (Batch Type):

Piles of bedding and feed are inoculated with worms and left in place until the process is complete. These piles are typically long and rounded but may also be square or rectangular, with a maximum height of one meter.

b) Top-Fed Windrows (Continuous Flow):

Feed is applied in thin layers (less than 10 cm) on top of the bedding and worms. Worms consume the feed from the top and leave castings at the bottom, forming a stratified heap with finished compost at the base and fresh feed at the top.

c) Wedges (Continuous Flow):

Worms are introduced into a small enclosure filled with bedding and feed. Fresh feed is added progressively from one side, encouraging worms to migrate towards the new feed. Once the material at the back is fully processed, it is harvested.

iv. Beds or Bin Vermicomposting

This method involves using containers made of plastic or untreated wood. A mixture of soil and bedding materials such as shredded paper, composted manure, or dried leaves is placed in the bin, providing habitat for worms. Organic food wastes such as fruit and

vegetable scraps, tea bags, and coffee grounds serve as feed, while crushed eggshells help regulate pH and provide calcium.

v. Top-Fed Beds (Continuous Flow)

Similar to top-fed windrows, beds are enclosed on all four sides and usually have a base or floor. Fresh feed is applied in successive layers on the top, while processed material accumulates at the bottom. Insulation with straw or sacks may be used in colder conditions.

vi. Stacked Bins (Batch or Continuous Flow)

Stacked bins incorporate a vertical component, addressing space limitations. Pre-mixed bedding and feed are placed in bins with worms, which are then stacked. The process may be managed as batch or continuous flow, but routine monitoring is required.

vii. Flow-Through Reactors

In this system, worms process feed continuously, and mature compost is harvested from the bottom. A set of breaker bars powered hydraulically loosens the material, allowing the finished vermicompost to fall through.

viii. Trough Vermicomposting

This method uses cemented troughs filled initially with a thin layer of pre-matured manure. Worms are added and allowed to process the material before subsequent layers of manure are applied every 10 days. The process continues until the trough is filled with vermicompost.

ix. Tower Method

The tower method involves vertically installed PVC pipes perforated with multiple holes. Worms are introduced into the pipe, and kitchen waste is added regularly. The worms consume the feed, and the castings accumulate around the tower in the surrounding soil. Alternatively, farmers may use lined pits filled with organic residues and worms to achieve a similar effect.

x. Vermiwash

Apart from solid composting, vermiwash is another valuable by-product of vermicomposting. It is a liquid extract that accumulates when water passes through worm-inhabited compost. Vermiwash contains mucus secretions, excretory products of worms, and soluble nutrients derived from organic matter. This pale-yellow liquid is rich in enzymes, plant growth hormones, vitamins, and macro- and micronutrients. It is used as a foliar spray, promoting plant growth and enhancing resistance against diseases.

2.4.3 Morphology of Earthworm

Earthworms are vital biological agents in vermicomposting due to their capacity to fragment, ingest, and biologically transform organic matter (Devi & Khwairakpam, 2022). Their anatomical and physiological characteristics directly influence their efficiency in decomposition and nutrient cycling. *Eudrilus eugeniae*, commonly known as the African Nightcrawler is among the various species used in vermicomposting. It is a preferred choice due to its high biomass turnover, tolerance to a broad range of organic substrates, and prolific reproduction under suitable conditions (Singh & Devi, 2024).

Figure 2.3 African night crawler (*Eudrilus eugeniae*)

Eudrilus eugeniae (Figure 2.3) exhibits a reddish-brown coloration posteriorly, while the anterior region shows an iridescent blue or green sheen, resulting from cuticular diffraction (Blakemore, 2015). Mature individuals typically measure between 90–185 mm in length, although under optimal conditions, lengths of 250–400 mm have been recorded. The average adult body weight ranges between 5–6 grams.

The body of *E. eugeniae* is elongated, cylindrical, and segmented, consisting of approximately 250–300 segments. Each segment, except for the first (prostomium) and the terminal segment (pygidium), contains eight setae arranged in pairs. These bristle-like structures assist in locomotion and provide anchorage within the substrate. The prostomium is small and open epilobous in form, functioning as a tactile and sensory organ that aids in navigating soil environments.

Internally, earthworms possess a well-developed digestive system adapted for the breakdown of complex organic compounds. As they consume decomposing matter, their gut-associated microbiota contributes to the biochemical degradation of organic residues, thereby enhancing microbial diversity and accelerating compost maturation.

Earthworms also secrete mucus and castings that further stimulate microbial activity and improve the structure and fertility of the resulting vermicompost.

In vermicomposting systems, the morphological and behavioural traits of *E. eugeniae* make it particularly efficient in processing high loads of organic waste, including fibrous and proteinaceous materials such as chicken feathers. Their burrowing behavior aerates the substrate, while their feeding habits help homogenize and stabilize the composting material.

2.4.4 Earthworm Gut Microbiota and its Role in Decomposition

Earthworms play a vital role in organic waste degradation, not only through mechanical fragmentation and ingestion but also through the biological activity of their gut microbiota (Sun et al., 2020). The earthworm gut is a dynamic and complex micro-ecosystem that houses a diverse community of microorganisms, including bacteria, fungi, and actinomycetes, all of which contribute significantly to the biochemical transformation of organic materials (Chopra & Bajya, 2023). In the context of vermicomposting, this gut-associated microbial activity greatly enhances the breakdown of resistant compounds such as lignin, cellulose, and even recalcitrant proteins like keratin (Chowdhury, 2025).

The earthworm gut environment is characterized by high moisture content, neutral to slightly alkaline pH, and a relatively stable temperature — conditions that are highly conducive to microbial activity (Yang et al., 2023). As earthworms consume organic matter, it passes through their muscular gizzard, where it is ground into finer particles. This physical breakdown increases the surface area of the material, making it more accessible to enzymatic and microbial action. Once in the gut, the organic matter is further digested with the help of digestive enzymes secreted by both the earthworm and its symbiotic microorganisms (Walia & Kaur, 2024).

Studies have shown that earthworm gut microbiota are responsible for producing a wide array of hydrolytic enzymes, including cellulases, proteases, lipases, amylases, and phosphatases. These enzymes facilitate the decomposition of complex organic substrates into simpler, bioavailable forms (Biswas & Banerjee, 2025). Particularly relevant to feather degradation is the presence of keratinolytic bacteria in the earthworm gut. Certain microbial isolates from *Eisenia fetida* and *Eudrilus eugeniae*

have been identified as keratin degraders, capable of producing keratinases that cleave the tough keratin structure found in chicken feathers (Arthanari et al., 2021).

The symbiotic relationship between earthworms and their gut microbiota creates a mutual benefit: the microbes gain a nutrient-rich habitat and steady food supply, while the earthworms benefit from the enhanced digestive efficiency provided by microbial enzymatic activity (Edward & Arancon, 2022). This relationship not only accelerates the decomposition process but also improves the nutrient profile of the resulting vermicompost. For instance, microbial mineralization of nitrogen and phosphorus in the gut converts organic forms into plant-available nutrients such as ammonium, nitrate, and orthophosphate, which enrich the vermicast (Marumure et al., 2023).

Another important function of gut microbiota is the suppression of pathogens. Earthworm digestion, assisted by its gut flora, has been shown to reduce populations of harmful microorganisms in organic waste, including *Escherichia coli*, *Salmonella spp.*, and *Helminth* eggs (Edwards & Arancon, 2022). This natural sanitization process enhances the safety and microbiological quality of the final vermicompost product, making it suitable for agricultural use. *E. eugeniae* needed around three and a half hours, resulting in seven gut fillings per day. Analysis of gut and vermicompost bacteria revealed the presence of *Bacillus* spp. and *Klebsiella* sp. (Singh & Devi, 2024).

The interplay between the gut microbiota and the host earthworm also influences the microbial composition of the castings (Baskar et al., 2023). As the digested material exits the earthworm body, it carries with it a rich microbial load that continues to decompose remaining organic material in the vermicompost bed. This post-excretion microbial activity further enhances the maturity, stability, and nutrient richness of the compost (Vermiere et al., 2021).

In conclusion, the earthworm gut microbiota is a central component of the vermicomposting process, significantly contributing to organic matter breakdown, nutrient mineralization, and pathogen suppression. In the context of chicken feather degradation, these microorganisms offer an underutilized but promising biological tool. Understanding and leveraging this micro-ecosystem opens new pathways for improving the efficiency and scope of vermicomposting, particularly for managing challenging organic wastes such as keratinous poultry by-products.

2.4.5 Vermicomposting of Unconventional Materials

Traditional vermicomposting has typically focused on common biodegradable wastes such as kitchen scraps, livestock manure, garden waste, and agricultural residues. However, recent advances in waste management and circular bioeconomy have pushed the boundaries of vermicomposting toward the inclusion of unconventional materials — organic wastes that are often recalcitrant, protein-rich, or underutilized, such as hair, wool, feathers, slaughterhouse waste, sewage sludge, and textile fiber residues. These substrates pose significant disposal and environmental challenges but may be effectively managed through carefully designed vermicomposting systems.

Unconventional materials often differ from conventional feedstocks in several important aspects: they typically have a low carbon-to-nitrogen (C:N) ratio, high protein content, resistance to microbial degradation, and, in many cases, potential pathogenicity or chemical contamination (Das et al., 2022). This presents both opportunities and obstacles for their use in vermicomposting. While such materials are rich in nutrients, their composition can lead to the release of ammonia, offensive odours, and potentially toxic metabolites if not properly pre-treated or balanced with carbon-rich bulking agents such as straw, sawdust, or dried leaves.

Chicken feathers are a prime example of such unconventional waste. Composed of over 90% keratin, feathers are protein-dense yet resistant to natural degradation due to the presence of disulfide bonds and tightly packed polypeptide chains (Maurya & Singh, 2024). On their own, feathers are not easily decomposed by earthworms and may not be suitable as a sole feedstock. However, when combined with other compostable materials or pre-treated using microbial or enzymatic methods, they can be effectively integrated into vermicomposting systems.

Studies have demonstrated the feasibility of vermicomposting partially degraded feathers mixed with farmyard manure or vegetable waste (Dume et al., 2022). These mixtures reduce the overall nitrogen concentration and create a more favorable substrate for both earthworm activity and microbial succession (Gupta et al., 2023). The result is a balanced composting environment that promotes rapid decomposition while enhancing the final vermicast with added nitrogen and sulfur derived from feather proteins.

Similarly, other unconventional materials such as wool waste, human hair, slaughterhouse residues, and food industry by-products have been experimented for

vermicomposting (Limeneh et al., 2022). Wool and hair, like feathers, are composed of keratin and thus share similar degradation challenges. Pre-treatment using keratinolytic microbes, fungal fermentation, or mechanical size reduction has been employed to make these wastes more accessible to earthworms and their symbiotic microbes (Isembart et al., 2025). Some research also reports improved cast quality and elevated micronutrient levels, especially when such proteinaceous materials are used in small proportions within a diversified compost medium (Lirikum et al., 2022).

Sewage sludge, while controversial due to heavy metal and pathogen content, has also been studied for vermicomposting (Georgi et al., 2022). When adequately stabilized and mixed with organic bulking agents, sewage sludge can serve as a nutrient-rich substrate (Wu et al., 2015). The key lies in appropriate processing conditions, such as pH adjustment, aeration, temperature control, and pre-composting, to reduce toxic compounds and ensure earthworm survival. The final vermicompost is often high in organic matter, nitrogen, and microbial biomass, although its safety and applicability depend on rigorous contaminant testing.

The incorporation of agro-industrial wastes, such as brewery spent grains, fruit pulp, coffee husk, or sugarcane bagasse, is another innovative area of vermicomposting (Garg & Gupta, 2009). These materials are usually rich in nutrients and fiber but may be acidic or prone to microbial imbalance if used alone. Their co-composting with livestock manure or green waste helps maintain optimal moisture, pH, and nutrient ratios for earthworm development (Waqas et al., 2023).

Several studies emphasize the importance of earthworm species selection when dealing with unconventional feedstocks. *Eisenia fetida* and *Eudrilus eugeniae* are among the most adaptable species, capable of tolerating a wide range of feedstock compositions (Gebrehana et al., 2023). However, extreme substrates may still require acclimatization periods or gradual feedstock introduction to prevent worm mortality.

In conclusion, the scope of vermicomposting has evolved beyond traditional organic waste to include a broad spectrum of unconventional materials. When appropriately handled, these wastes offer not only a sustainable solution to complex waste streams but also produce high-quality organic amendments. The integration of chicken feathers into such systems, aided by microbial pre-treatment and balanced compost recipes, stands as a promising approach in both waste valorisation and soil fertility enhancement.

2.5 Policies and Regulations Related to Organic Waste Recycling

The management and recycling of organic waste, including poultry by-products such as chicken feathers, are governed by a combination of environmental, agricultural, and waste management policies. These policies vary across regions but generally aim to promote sustainable waste utilization, minimize environmental pollution, and encourage the adoption of organic alternatives in agriculture. This section outlines relevant national and international policies, regulations, and institutional frameworks that influence the recycling of organic waste through processes such as composting and vermicomposting, with specific reference to feather waste.

2.5.1 National Policies on Organic Waste Management

In many countries, including Malaysia, several organizations, including the Department of Environment (DOE), the Ministry of Agriculture and Food Security (MAFS), and local municipal councils, oversee regulating organic waste. Recycling food and agricultural waste is encouraged by several national frameworks and initiatives:

i) **Malaysia's National Solid Waste Management Policy (2016)** emphasizes the reduction, reuse, and recycling (3R) of waste, including organic fractions, with composting and bio-conversion methods encouraged as alternatives to landfilling (Malaysia National Solid Waste Management, 2016).

ii) **The 12th Malaysia Plan (2021)** prioritizes green growth and sustainable development, calling for integrated waste management approaches and the promotion of bio-based industries (Shafie, 2021).

Under these policies, the use of compost and vermicompost in agriculture is encouraged, especially in organic farming and integrated nutrient management systems. However, the regulation of animal-derived waste such as feathers remains less defined and often lacks specific guidelines for composting or reuse, creating challenges in implementation and standardization.

2.5.2 International Guidelines and Best Practices

International organizations such as the Food and Agriculture Organization (FAO), United Nations Environment Programme (UNEP), and the European Union (EU) have developed guidelines and directives that support organic waste recycling and composting:

i) **Sustainable Development Goals** emphasize the sustainable consumption and production patterns.

ii) **FAO's Guidelines for Sustainable Agricultural Waste Management** highlight composting and vermicomposting as cost-effective, decentralized solutions for nutrient recovery from organic waste, including animal by-products.

iii) **EU Animal By-Products Regulation (EC No. 1069/2009)** governs the safe handling and processing of animal waste, including feathers. Feathers must be treated (e.g., pressure sterilized or composted) to prevent disease transmission before being applied to land.

iv) **Circular Economy Action Plan (EU, 2020)** and **EU Fertilizing Products Regulation (EU 2019/1009)** support the transformation of biowaste into marketable organic fertilizers, opening avenues for feather-based compost products to enter regulated markets.

Countries with mature composting industries, such as Germany, the Netherlands, and India, have implemented certification and quality standards for compost and vermicompost, often requiring testing for pathogens, heavy metals, maturity indices, and nutrient content. These standards ensure the safety and efficacy of compost products, increase consumer trust, and enable the development of formal organic waste recycling markets.

2.5.3 Organic Certification Standards

For feather-based vermicompost to be accepted in organic agriculture, it must comply with organic farming regulations and certification standards. These include:

i) **Malaysia Organic Scheme (myOrganic)**, implemented by the Department of Agriculture Malaysia, which outlines the permissible inputs and composting practices for certified organic farms.

ii) **IFOAM (International Federation of Organic Agriculture Movements) Standards**, which allow composts made from animal waste provided they are well-composted, pathogen-free, and free of synthetic additives.

iii) **USDA Organic Regulations (7 CFR Part 205)**, which permit the use of composted animal materials if managed under approved composting processes that meet temperature and time thresholds.

Adhering to these standards is essential for feather-based vermicompost producers targeting the organic farming market, both locally and for export purposes.

CHAPTER 3
RESEARCH METHODOLOGY

3.1 Introduction

This chapter begins with the soil samples collection, substrates collection, followed by isolating keratinolytic bacteria from soil samples and identification through 16s rDNA sequencing. The isolated and identified bacteria were then inoculated along with African nightcrawler earthworm to degrade chicken feather and other substrates used, producing a nutrient-rich vermicompost.

3.2 Soil Sample Collection

Soil samples were collected from 3 different locations where potential feather degrading bacteria might be presence: compost house area, chicken coop area, and chicken feather dump site. The samples were collected using random sampling method. Soil sampler auger was used to dig a hole to 15 cm depth. Five soil cores were collected and composited to produce one sample, 10 g of soil sample from each location with 3 replications were sealed in zipper plastic bag, labelled and stored in polystyrene ice box while being transported to the laboratory (Makmal Agroteknologi, UiTM Perlis).

3.3 Substrates Collection

All substrates used for composting in this study were agricultural wastes which are chicken feather, chicken manure, banana trunk and mushroom media residue. The substrates were collected from local farms in Perlis and Kedah. All substrates were collected and prepared for pre-composting process and monitor daily until electrical conductivity (EC) is below 2.0 mS/cm, prior to inoculation of composting agent. All preparation and composting process took place in Unit Ladang, UiTM Perlis.

3.3.1 Chicken Feather Collection

Chicken feathers were collected from a local poultry processing centre in Alor Setar, Kedah. The feathers were rinsed on the same day with tap water three times until the water runs clear. The cleaned feathers were spread on a canvas and dried under the sun. The dried feathers were then cut into 2 cm long before pre-composting process (Zhang et al., 2000).

3.3.2 Chicken Manure Collection

Chicken manure was collected from a local poultry farm in Pokok Sena, Kedah. It was then placed in a cement culvert with drainage hole. The chicken manure was water daily for 2 weeks until the electrical conductivity (EC) is less than 2.0 (mS/cm).

3.3.3 Banana Trunk Collection

Banana trunk was collected from a local farm in Alor Sena, Perlis prior to the pre-composting process. It was then cut into 2 cm long before being used in pre-composting.

3.3.4 Mushroom Media Residue Collection

Mushroom media residue (MMR) was collected in sacks from Pertubuhan Peladang Kawasan (PPK) Paya, Perlis. The MMR was crushed by hand and put in a cement culvert pipe with drainage hole. It was watered daily until the electrical conductivity (EC) is less than 2.0 mS/cm.

3.4 Earthworm Collection

The composting agent used in this study is African nightcrawler earthworm (*Eudrilus eugeniae*) was obtained from Department of Agriculture, Perlis. African nightcrawlers can decompose organic matter quickly compared to red wigglers as they consume a significant amount of organic matter and turning it into vermicompost (Sarimong & Legaspi, 2019). African nightcrawlers also thrive best in warm climates, hence they are ideal for Perlis' climate.

3.5 Screening for Chicken Feather Degrading Bacteria

3.5.1 Crude Enzyme Production

The collected soil samples were used for growing bacteria culture by inoculating in nutrient broth followed by incubating for 48 hours at 30℃ and 150 rpm in an incubator shaker (Biosan). After 48 hours, the culture was sub-cultured three more times in a fresh nutrient broth to increase the bacterial population.

The crude enzyme production was obtained by Sultana & Saha (2018) method with slight modification where 1 mL of 24-hours old culture inoculated into basal salt medium supplemented with 0.5% chicken feather (autoclaved) and incubated at 30℃ for 4 days under shaking conditions (150 rpm). On the final day, the chicken feathers were filtered using filter paper (Whatman No. 1) to remove bacteria mass and protein. The feathers were then rinsed with distilled water followed by oven-dried at 60℃ until constant weight was obtained. The bacterial culture was filtered through 0.22 µm pore size membrane to remove microparticles and collect the crude enzyme (Aktayeva et al., 2022).

3.5.2 Keratinase Enzyme Assay

The keratinase enzyme activity was determined according to Navone & Speight (2018) with slight moderation by using 5 mg keratin azure (Sigma Aldrich), ground using mortar and pestle, added in 0.8 mL of 50 mM Tris-HCl (pH 8) as substrate, followed by constant agitation until keratin azure was fully suspended. Then, 0.2 mL of crude enzyme was added to the substrate solution and incubated for 1 hour at 37℃ with shaking (200 rpm). The reaction was then terminated by the addition of 0.2 mL of 10% trichloroacetic acid (TCA) and centrifuged (Hettich) at 4500 x g for 10 minutes. The absorbance of the supernatant was measured at 595 nm using spectrophotometer (Thermo scientific). The unit of keratinase activity was defined as 0.1 increase in the absorbance at 595 nm after 30 minutes incubation under the experimental conditions described. Control sample was prepared without the addition of enzyme solution.

3.6 Isolation and Secondary Screening of Feather Degrading Bacteria

The bacterial culture with highest keratinase enzyme activity based on keratinase assay and feather weight loss analysis was chosen for feather degrading bacteria screening and isolation. 0.1 mL of the bacterial culture was mixed in 0.9 mL distilled water and diluted to 10^{-3}, 10^{-4} and 10^{-5}. Then, 50 µL of each diluted aliquot was cultured on skimmed milk agar using streaking technique followed by incubation for 3 days at 30℃. Surviving bacterial colonies were sub-cultured a three times and the final surviving colonies were inoculated in nutrient broth individually and incubated for 24 hours at 30℃ under shaking condition (150 rpm). The culture was sub-cultured twice and incubated as the experimental condition described. Then, 1 mL of the suspension from 24 hours old culture was inoculated into 100 mL basal salt medium supplemented with 0.5% feather and incubated at 30℃ for 4 days under shaking condition (150 rpm). After that, 2 mL of incubation culture was taken daily for keratinase enzyme assay. Keratinase activity was assessed as the procedure described earlier. The bacterial culture with the highest keratinase enzyme activity was chosen for the next procedure.

3.7 Identification of Feather Degrading Bacteria

3.7.1 Gram Staining

The bacterial culture with the highest keratinase enzyme activity was identified based on morphological characteristics using Gram staining method according to Becerra et al. (2016). Gram staining method was developed by a Danish physician, Hans Christian Gram. This method requires four chemicals: iodine, crystal violet, alcohol, and safranin to stain bacteria. This way, most bacteria are separated into two groups based to composition of cell wall: Gram-positive bacteria with purple stain while Gram-negative bacteria with pink stain.

Bacterial smear was prepared using 1 µL of bacterial culture; spread on a microscope slide creating a thin film using inoculating loop. The smear was then heat fixed by passing the slide through a flame. The heat-fixed smear was then flooded with crystal violet dye for 1 minute, allowing the bacteria to stain. Then, it was rinsed with distilled water before iodine solution was applied for another 1 minute. Using acetone, the slide was rinsed for a few seconds. Finally, it was counterstained with safranin for

1 minute. The slide was then rinsed carefully using distilled water to remove excess stain and was blotted dry.

The stained slide was examined under a light microscope (Olympus Corporation), starting with 4× magnification to 100× using oil immersion lens. Gram-positive bacteria appeared purple, whereas Gram-negative bacteria appeared pink. The shape of the bacteria (Figure 3.1); cocci, bacilli, or spirilla was noted as well as the arrangements including clusters, chains, or single (Cowan & Talaro, 2006).

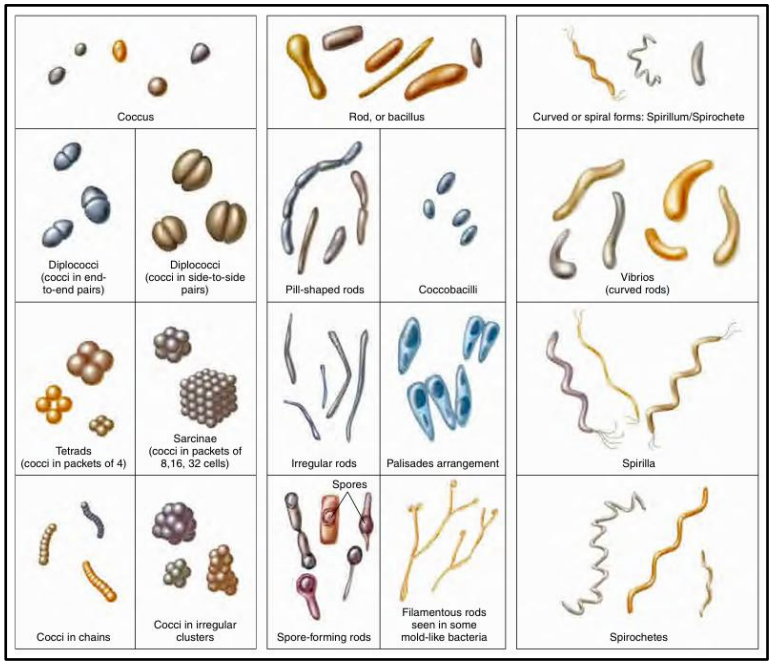

Figure 3.1 Shape and arrangement of bacteria (Cowan & Talaro, 2006)

3.7.2 Molecular Identification of Feather Degrading Bacteria

Molecular identification of the feather degrading bacteria was performed using the 16S rDNA sequence analysis using a 24-hour culture of the bacterial cell using Bacterial DNA Extraction kit (New England Biolabs) according to the manufacturer's procedure. For cell lysis, 2 mL of 24-hours-old bacterial culture were transferred to microcentrifuge tube and pellet by centrifugation at 1000 x g for 1 minute and resuspend in 100 µL cold phosphate buffer saline (PBS) by pipetting up and down. The pellet was resuspended completely. Then, 1 µL of Proteinase L and 3 µL RNase A was added to the resuspended pellet and mixed by vortexing briefly to ensure the enzymes are efficiently dispersed. Next, 100 µL of Cell Lysis Buffer was added and vortex immediately and thoroughly. The solution became viscous rapidly. The solution was

then incubated for 5 minutes in water bath at 56℃, and vortex for 1 minute. For genomic DNA binding and elution, 400 µL of gDNA Binding Buffer were added to the sample and mix thoroughly by pulse-vortexing for 10 seconds. Then, 600 µL of the binding buffer mix was transferred to a gDNA Purification Column which was pre-inserted into a collection tube, without touching the upper column area. Next, the mixture was centrifuged for 3 minutes at 1000 x g to bind the gDNA, followed by another minute at maximum speed to clear membrane. The flow-through and the collection tube was discarded. The column what transferred to a new collection tube and added with 500 µL gDNA Wash Buffer. The cap was closed and inverted (without vortex) a few times so that the wash buffer reached the cap. Then, the sample was centrifuged for 1 minute at maximum speed before the flow through was discarded. The collection tube was tapped on a paper towel to remove residual buffer before the column being replaced into the collection tube. Next, 500 µL gDNA wash buffer was added, and centrifuged immediately for 1 minute at maximum speed. The collection tube and the flow through was discarded. Then, the gDNA purification column was placed in a DNase-free (autoclaved) 1.5 mL microcentrifuge tube. 100 µL of preheated (60℃) gDNA elution buffer was added and incubated at room temperature for 1 minute. After 1 minute, the sample was centrifuged for 1 minute at maximum speed to elute the gDNA.

Amplification of the partial 16S RNA gene was carried out using universal primers. The PCR mixtures consist of 1 µL of 5mM 27F (50-AGA GTT TGATCC TGG CTC AG-30) and 1429R (50-TAC GGT TACCTT GTT ACG ACTT-3-) of forward and reverse primer, 1 µL of DNA sample, 12.5 µL of Master mix 2 × Taq and 1 µL of deionized water to a final volume of 25 µL. The polymerase chain rection was completed using a gradient thermocycler under the following conditions: 3 minutes initial denaturation at 94℃, 29 cycles denaturation for 1 minute at 95℃, 1 minute of annealing at 58℃, 2 minutes of extension for 10 minutes, and final extension at 72℃ for 10 minutes with incubation at 4℃. Successfully amplified DNA fragments was analysed on 1% (w/v) agarose gel. The amplified DNA fragments was purified and submitted to Nextgene Sdn. Bhd. to sequence DNA using the same primers.

3.7.3 Sequence Analysis and Phylogenetic Analysis

The selected sequence was analysed using BLASTn. The sequence alignment with more than 95% similarity was described as identical and used for further

phylogenetic analysis as Operational Taxonomic Unit (UTO). The evolutionary tree was based on distances complied using the nearest neighbour algorithm.

3.8 Vermicomposting

This part of the experiment was conducted in Vermicompost House, Plantation Unit, UiTM Perlis. There were eight vermicomposting treatments with different ratio in this study. The earthworm and feather degrading bacteria were applied in combination or singly and contrasted with no earthworm or no bacteria control, to determine the vermicomposting of the difficult to degrade chicken feathers. The vermicomposting lasted for 60 days.

3.8.1 Experimental Design

This research was conducted using randomised complete block design (RCBD) with two factors which were the composting agent; potential feather degrading bacteria, and earthworm (*Eudrilus eugeniae*) and substrates; mushroom medium residue, banana trunk, chicken manure, and chicken feather (Table 2). There were eight treatments with three replications each treatment.

3.8.2 Experimental Layout

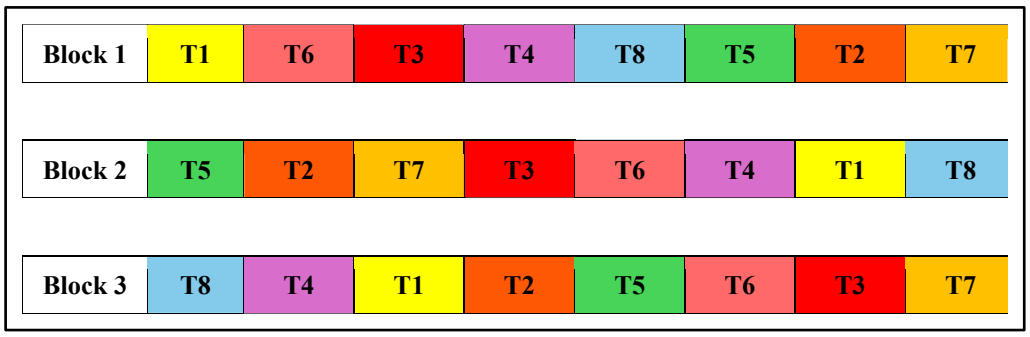

Figure 3.2 Experimental Layout

Table 3.1

List of treatments in compost

Treatment		Substrate and Ratio			
		Mushroom Media Residue	Banana Trunk	Chicken Manure	Chicken Feather
Bacteria	T1	6	1	1.5	1.5
Earthworm	T2	6	1	1.5	1.5
Bacteria and Earthworm	T3	6	1	1.5	1.5
No Composting Agent	T4	6	1	1.5	1.5
Bacteria	T5	6	1	-	3
Earthworm	T6	6	1	-	3
Bacteria and Earthworm	T7	6	1	-	3
No Composting Agent	T8	6	1	-	3

3.8.3 Substrate Preparation

There were 4 different substrates used in this experiment including poultry wastes and plant wastes: chicken feather, chicken manure, banana trunk and mushroom media residue. The substrates were collected earlier were prepared accordingly before mixing in boxes for the composting process to take place. Chicken feathers were cut into smaller pieces (2 cm) using scissors. Banana trunk was cut into smaller pieces (10-15 cm), while mushroom medium residue was crushed using hand. All substrates were mixed thoroughly and accordingly.

3.8.4 Pre-composting

All substrates were gathered according to ratio by weight and subjected to precomposting process prior to vermicomposting, where each mixture was watered daily with tap water for 14 days. The water was changed daily until the electrical conductivity (EC) of the wastes was below 4.0 (mS/cm) by the end of the 14 days of

pre-composting. After 14 days, the substrates were air-dried for 2 days before adding the selected composting agent accordingly.

3.8.5 Composting Agent

There were 2 composting agents used in this experiment which were earthworms (*Eudrilus eugeniae*) and bacteria.

3.8.5.1 Inoculation of Chicken Feather Degradation Bacteria

The selected bacterial isolate, isolate 5, was chosen due to its highest keratinase activity and superior feather degradation percentage compared to other isolates. This feather-degrading bacterium was then inoculated into the pre-composted substrates at the rate of 1×10^{-9} CFU mL^{-1}. The bacteria strain isolated was first inoculated in nutrient broth and incubated at 30℃ for 24 hours under shaking condition (150 rpm). It was then sub-cultured to multiply the bacteria population, and a series of serial dilution was done to count the colony forming unit (CFU). Next, 0.1 mL of each dilution was cultured on nutrient agar plate using spread plate technique and incubated at 37℃ for 24 hours. The colony formed was counted using the formula in (3.1).

$$CFU\ mL = \frac{Number\ of\ colonies\ \times Dilution\ factor}{0.1\ ml} \tag{3.1}$$

3.8.5.2 Inoculation of Eudrilus eugeniae

For the treatments involving earthworms, 30 African night crawler earthworm (*Eudrilus eugeniae*) with the same size were introduced to the pre-composted substrate on the same day as the bacteria inoculation. The earthworms were weighed prior to being introduced to the pre-composted substrate.

3.9 Data Collection

The vermicompost lasted for 60 days after adding composting agent. The interaction between the vermicomposting agent and substrate combination was evaluated according to the following parameters:

3.9.1 Computation of Vermicompost Biodegradability Coefficient (K_b)

The organic matter volumes of all treatments were measured at the beginning of vermicomposting and every 15 days (day 15, 30, 45, and 60) until end of vermicomposting using a measuring cylinder to determine the organic matter conversion during vermicomposting.

3.9.2 Microbial Enzyme Activities

Destructive sampling (100 g) were collected from the compost box at 15-, 30-, 45- and 60-days bioconversion (aerobic degradation) for enzyme activity assays. The enzymes analysed include catalase which is a significant antioxidant enzyme widely exist in animals, plants, and microorganisms and uses hydrogen peroxide as specific substrate and decompose into water and oxygen (Man et al., 2022). Urease plays an important role in nitrogen cycle (Koçak, 2020) which involved in the peptide bond hydrolysis of organic matter and decomposition of urea into ammonia. Invertase is one of the significant enzymes in sucrose metabolism (Topcu et al., 2022). Polyphenol oxidases is a significant deteriorative enzyme that speed up oxidation and degradation of polyphenols (Toro-Uribe et al., 2020).

3.9.2.1 Catalase

Catalase enzyme activity was determined by measuring the amount of oxygen released from hydrogen peroxide decomposition, according to Hadwan et al. (2024) and Kadhum & Hadwan (2020) method with slight modification. 1 g of compost were added to an Erlenmeyer flask and thoroughly mixed with 10 mL of 50mM phosphate buffer (pH 7). The mixture was then centrifuged 10 000 × g for 10 minutes to obtain the supernatant which contain the catalase enzyme. In a clean flask, 2 mL of the supernatant

was added with 1 mL hydrogen peroxide to start the reaction. Catalase activity was measured by the difference in absorbance of the mixture using spectrophotometer (Thermo scientific) at 240 nm. The absorbance was observed every 30 seconds for 3 minutes. A control was prepared by using 2 mL of buffer instead of compost extract (supernatant). The catalase enzyme activity was calculated using the following formula in (3.2.

$$Catalase\ Activity\ \left({}^{U}/_{g}\right) = \frac{(\Delta A240) \times 4\ mL}{43.5M\ \times\ 1\ g} \tag{3.2}$$

3.9.2.2 Urease

Urease enzyme activity determines the ability to hydrolyse urea into ammonia and carbon dioxide. The method was obtained from Tabatabai & Bremner (1972) with slight modification. Compost samples were air-dried and sieved through 2 mm screen. Then, 5 g of compost sample was added into a 50 mL Erlenmeyer flask and added with 0.2 mL of toluene and 9 mL of tris(hydroxymethyl) aminomethane (THAM) buffer. The flask was swirled a few times to combine the mixture. It was then covered with stopper and incubated at 37℃ for 2 hours.

After 2 hours, 1 mL of 2 M potassium chloride (KCl) was added to the mixture to stop the reaction. The mixture was shake on an orbital shaker for 30 minutes. The suspension was then filtered. Next, the filtrate was diluted with 10 mL distilled water and added with 5 mL Na salicylate solution and 2 mL 0.1% Na dichlorisocynurate. The absorbance was observed at 690 nm using a spectrophotometer (Thermo scientific) after 30 minutes of being incubated at room temperature (Kandeler & Gerber, 1988).

3.9.2.3 Invertase

50 mL of citrate buffer was added to a 100 mL beaker containing 5 g of compost. The mixture was mixed thoroughly and incubated at room temperature for 30 minutes. Then, 5 mL of aliquot was transferred to a test tube and added with 0.2 M sucrose solution. The mixture was then incubated at 37℃ for 1 hour. After an hour, 3 mL of 3,5-dinitrosalicylic acid (DNS) solution was added to stop the reaction. The mixture

was incubated in boiling water bath for 5 minutes and let cool to room temperature after. The mixture was diluted with 20 mL distilled water. The absorbance was observed using a spectrophotometer (Thermo scientific) at 540 nm (Frankenberger & Johanson, 1983).

3.9.2.4 Polyphenol Oxidase

200 µL of compost extract was added to 50 µL L-3,4-dihydroxyphenylalanine. The absorbance was observed using spectrophotometer (Thermo scientific) at 500 nm for 5 minutes with 30 seconds interval (Mayende et al., 2006).

3.9.3 Population growth of potential feather-degrading bacteria and *Eudrilus eugeniae*

Population growth and reproduction of earthworms and the known vermicomposting efficiency indicator. The results of vermicomposting efficiency using chicken manure and chicken feathers were shown by the earthworm populations' growth and reproduction rate in vermicompost. The earthworm activity in vermicomposting was observed through earthworm growth rate, weight gain of earthworms and the total number of earthworms in the vermicompost (Lim et al., 2014). The number of potential feather-degrading bacteria colonies were determined at the commencement of vermicomposting and after completing the bioconversion process.

3.9.4 Chemical Properties of Vermicompost

Analysis of the properties of the composts obtained from vermicomposting with the most potential feather degrading bacteria and earthworms, singly or in combination according to the procedure above, was conducted in the laboratories UiTM Perlis. The analysis of the chemical properties of the different composts described below.

3.9.4.1 Electrical Conductivity (EC), pH, and Biodegradability coefficient (K_b)

Electrical conductivity (EC) and pH were recorded every 15 days on day 15, 30, 45 and 60 using EC meter and pH meter probe. Biodegradability coefficient (K_b) and earthworm population was gathered on the final day of composting.

3.9.4.2 Total nitrogen determination

The vermicompost and compost products collected after completing the vermicomposting procedure were air-dried according to Kumar et al. (2020). Total nitrogen content was determined by complete combustion method by using N-analyser following AOAC-Official Method 993.13.

3.9.4.3 Organic matter determination

The organic matter of vermicompost and compost products above was determined according to the Loss-On-lgnition Method (Nelson & Sommers, 1996). Empty crucibles were first heated at 400 °C for 2 h in a muffle furnace, cooled in a desiccator containing $CaCl_2$ as dessicant, and weighed to obtain the tare weight. Approximately 1–3 g of air-dried, ground (<0.4 mm) compost was added to each crucible. The samples were dried at 105 °C for 24 h, cooled in a desiccator, and weighed to determine the oven-dry weight. Subsequently, the samples were ignited at 400 °C for 16 h, cooled, and reweighed. The percentage of organic matter was calculated based on weight loss according to the formula (3.3).

$$LOI\ (\%) = \frac{W_{105} - W_{400}}{W_{105}} \times 100 \qquad (3.3)$$

Where W_{105} is the sample weight after drying at 105 °C, and W_{400} is the sample weight after ignition at 400 °C.

3.9.4.4 Available phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) determination

The available P, K, Ca, and Mg was determined by digestion method following MS 417:1994-The analysis of fertilizers using Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES).

3.9.5 Statistical Analysis

The data was analysed using R Studio 4.4.1 software. The data was analysed following Two-way ANOVA and LSD multiple comparison test was performed for mean differences at a 5% level of significance to reveal the importance of the feather degrading bacteria in converting the difficult-to-degrade chicken feathers into useful by-products (vermicompost).

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Screening for Chicken Feather Degrading Bacteria

A total of 17 pure colony bacteria obtained and screen for keratinase enzyme activity and feather degradation rate. *Bacillus subtilis* (Isolate 18) is use as positive control.

4.1.1 Keratinase Enzyme Activity

The keratinase enzyme activity of the bacterial isolates was quantified using keratin azure as substrate. After 96 hours of incubation (under shaking condition) at 37℃, isolate 5 shows significantly higher enzyme activity (3.8 U/mL) as compared to isolate 15 (2.1 U/mL) as shown in Figure 4.1. Isolate 5 was 80.9% superior in keratinase activity compared to isolate 15. In this study, isolate 5 was obtained from coop area. Comparing between isolate 5 with control (*Bacillus subtilis*, isolate 18), isolate 5 shows relatively high keratinase enzyme activity. Isolate 1, 2, 3 and 4 which are also from coop area shows relatively higher keratinase activity, 3.2, 3.6, 3.1 and 3.2 U/mL respectively compared to chicken feather dumpsite and vermicompost area. Whereas bacterial isolate 15 shows the lowest keratinase enzyme activity of 2.1 U/mL, isolated from vermicompost area. Isolate 15 shows 32.3% keratinase activity lower than control (*Bacillus subtilis*, isolate 18). Isolate 11, 12, 13, 14, 16, and 17 which are also from vermicompost area shows relatively low keratinase activity.

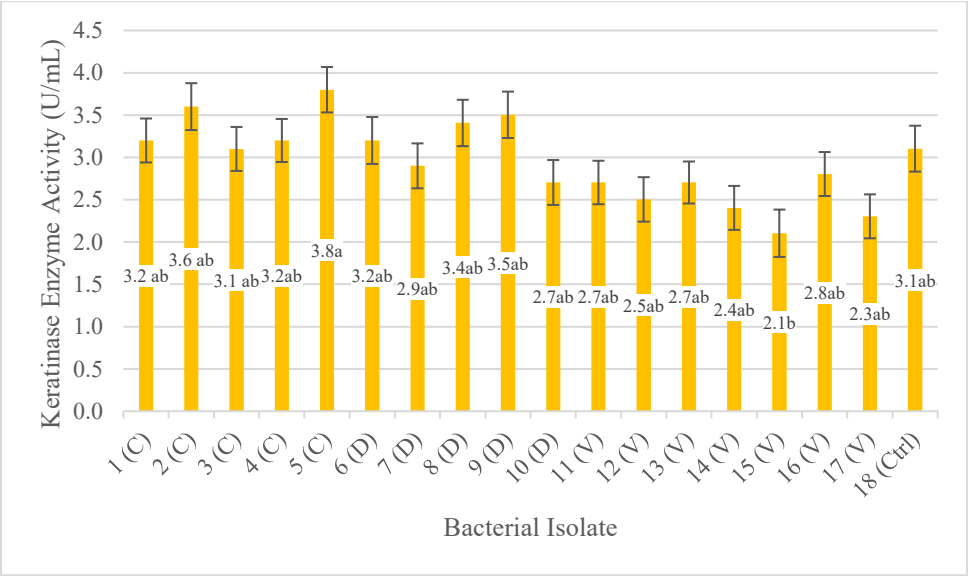

Figure 4.1 Keratinase Enzyme Activity

Note: Error bar represents standard error of different keratinase enzyme activity. Different letters indicate significantly different levels of keratinase enzyme activity between different bacterial isolates. C = Coop, D = Dumpsite, V = Vermicompost House, Ctrl = Control

Isolate 5, isolated from coop area able to produce high keratinase enzyme among other isolates which suggests that the bacterial strain was adapted to environment rich in keratin like chicken feathers and skin debris, hence producing keratinase enzymes efficiently (Jaouadi et al., 2013). Keratinases is a special hydrolytic enzyme produced by microorganisms, which can catalyse the degradation of keratin (Yan et al., 2024). Keratinase is one of the proteolytic enzymes that specifically breaks down keratin, a fibrous protein found in chicken feather. Keratinase accelerates chicken feather decomposition in compost, hence reducing composting time. Isolate 15 has the lowest potential to produce keratinase enzyme, as well as isolate 11, 12, 13, 14, 16, and 17 which are isolated from vermicompost area suggesting that the environment might be influenced by the presence of other decomposing enzymes. Differences in keratinase enzyme activity between bacteria can be influenced by various factors such as the type of bacterial species, genetic expression of the keratinase enzyme, environmental conditions, and enzyme regulatory mechanisms (Yuliatmo et al., 2024).

4.1.2 Chicken Feather Weight Loss

The high keratinase enzyme production of isolate 5 was further confirmed by the relatively high weight loss percentage of chicken feathers incubated in basal salt medium and the bacterial culture. Isolate 5 degraded 42.8% of 10 g/L chicken feathers as compared to isolate 6 which degraded only 26.2% of chicken feather in 96 hours. Isolate 5 managed to degrade 63.4% more chicken feather than isolate 6. Isolate 18 (*Bacillus subtilis*) shows relatively lower weight loss (34.8 %) than isolate 5, which is 17.2 % higher. Isolate 15 however, shows relatively higher chicken feather weight loss (35.5 %) compared to isolate 18 although the keratinase activity shows otherwise. However, there is no significant difference between bacterial isolates for chicken feather weight loss.

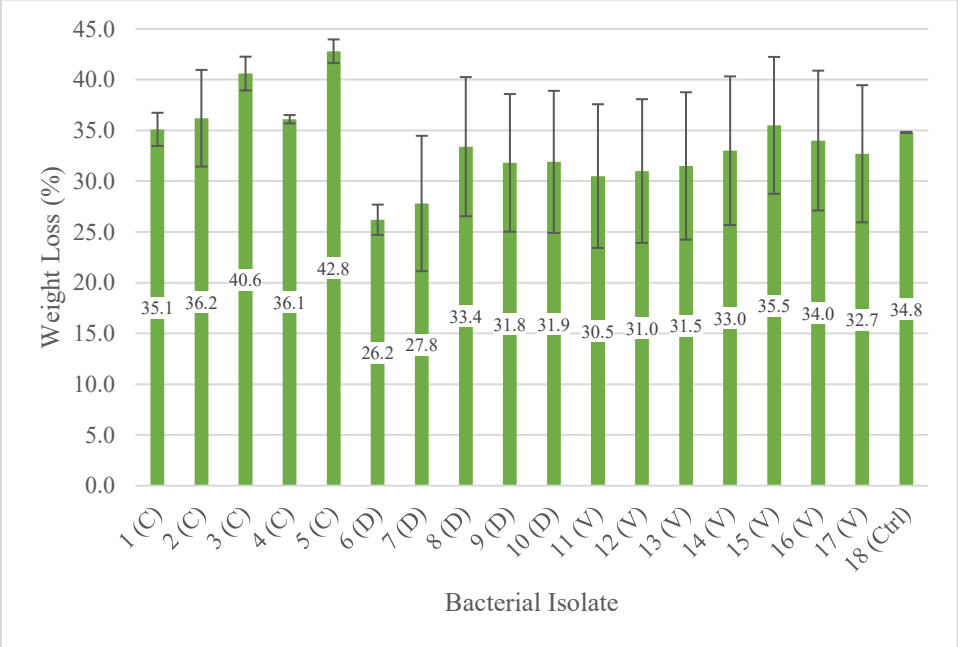

Figure 4.2 Chicken feather weight loss

Note: Error bar represents standard error of different chicken feather degradation rate of bacterial isolates. C = Coop, D = Dumpsite, V = Vermicompost House, Ctrl = Control

Isolate 5 shows relatively high chicken feather weight loss compared to other isolates suggesting that bacterial isolate from chicken coop might have undergone natural selection which enhanced the keratinase production, hence higher weight loss due to more keratin breakdown. However, isolate 15 able to degrade relatively high chicken feather although the keratinase activity is significantly the lowest, suggesting

other proteases produced by isolate 15 that help break down keratin or decompose other proteins and lipids in chicken feather.

4.1.3 Correlation

There is a moderate positive correlation ($r = 0.514$) between keratinase activity and chicken feather weight loss, meaning that higher values of keratinase activity tend to be associated with higher values of chicken feather degradation. The relationship is statistically significant ($p < 0.001$), suggesting that the Pearson correlation coefficient is unlikely to be due to random chance.

Table 4.1
Correlation coefficient between keratinase enzyme activity and percentage of chicken feather weight loss

Variables		**Keratinase Enzyme Activity (U/mL)**	**Feather Degradation (%)**
Keratinase Enzyme Activity (U/mL)	r	1	0.514
	p		0.000069
Feather Degradation (%)	r	0.514	1
	p	0.000069	

The positive correlation underscores the fundamental function of keratinase in feather biodegradation, as keratinase facilitates the hydrolysis of the insoluble keratin protein, which constitutes the primary structural element of chicken feathers (Sinkiewicz et al., 2018). Since keratin contains extensive disulfide bonds and a tightly packed structure, its degradation is challenging, and microorganisms capable of secreting keratinase enzymes are therefore essential for initiating and sustaining the breakdown process (Harland et al., 2022). The present result is consistent with previous studies, which reported that keratinolytic bacteria accelerate feather degradation by producing keratinase that cleaves peptide bonds in keratin, resulting in measurable feather weight loss (Brandelli et al., 2010; Gupta & Ramnani, 2006).

Despite the significant correlation, the moderate strength of association suggests that keratinase activity alone does not fully account for feather degradation. Other factors, such as the presence of auxiliary enzymes (e.g., proteases, disulfide reductases)

and microbial community interactions, also contribute to the process. Environmental conditions such as temperature, pH, and nutrient availability can further influence both enzyme secretion and degradation efficiency. These findings emphasise that feather biodegradation is a multifactorial process, where keratinase activity is a major but not the only determinant of degradation efficiency.

From an applied perspective, the significant positive correlation suggests that keratinase enzyme activity may serve as a biological indicator for predicting feather degradation performance. This is particularly relevant for poultry waste management strategies such as composting and vermicomposting, where the rate of feather decomposition determines the overall efficiency of the process. Enhancing keratinase production, either by selecting highly efficient microbial strains or through process optimisation, could therefore accelerate feather waste conversion into value-added products such as organic fertilizers and protein supplements. This study thus supports the potential application of keratinase-producing bacteria in sustainable waste management while recognising the need to consider complementary microbial and environmental factors to achieve complete feather biodegradation.

4.2 Morphological Characterisation and Molecular Identification of Chicken Feather Degrading Bacteria

Isolate 5 being the most promising chicken feather degrading bacteria selected from the preliminary screening was characterised morphologically and classified phylogenetically.

4.2.1 Colony Morphology

Bacteria of different species have different morphology; hence, the first step of characterisation in this study was the examination of bacterial morphology and characteristics on agar medium. Once bacteria have been isolated and cultured on milk agar, observation the visual appearance of the colony is crucial as the colony characteristics might provide useful information on identifying the bacteria (Jones et al., 2003). The colony morphology of isolate 5 grown on milk agar at 37°C after 24 hours are summarized in Table 4.2. The colony morphology of different isolate was described according to Shapiro (1995).

Table 4.2
Colony morphology of different isolates selected from different locations

Isolate	Location	Pigmentation	Margin	Form	Elevation	Opacity
1	C	Yellowish white	Entire	Circular	Flat	Opaque
2	C	White	Entire	Punctiform	Convex	Opaque
3	C	Red	Entire	Circular	Convex	Opaque
4	C	Yellowish white	Entire	Punctiform	Convex	Opaque
5	C	Yellowish white	Entire	Circular	Flat	Opaque
6	D	Red	Entire	Circular	Convex	Opaque
7	D	Red	Entire	Punctiform	Convex	Opaque
8	D	Red	Entire	Circular	Convex	Opaque
9	D	White	Entire	Circular	Convex	Opaque
10	D	Yellowish white	Entire	Punctiform	Convex	Opaque
11	V	White	Undulate	Irregular	Flat	Opaque
12	V	White	Entire	Circular	Convex	Opaque
13	V	White	Undulate	Irregular	Flat	Opaque
14	V	White	Entire	Punctiform	Convex	Opaque
15	V	White	Entire	Irregular	Flat	Opaque
16	V	White	Entire	Circular	Flat	Opaque
17	V	White	Entire	Punctiform	Convex	Opaque
18	-	White	Undulate	Irregular	Convex	Opaque

Note: V=Vermicompost House, C=Coop, D=Dumpsite

4.2.2 Cell Morphology

Gram staining of the bacterial isolates obtained from different sampling sites (vermicompost house, chicken coop, and dumpsite) revealed a diversity of Gram-positive and Gram-negative bacteria with varying morphological shapes (Table 4.3). Out of 18 isolates, both Gram-positive (+) and Gram-negative (–) types were present, and their cell morphologies were either rods or cocci. Isolate 5 was Gram-negative bacilli upon observation under a light microscope. The Gram staining results of isolate 5 is provided in APPENDIX 1.

Table 4.3
Cell Morphology of different isolates selected from different locations

Isolate	Location	Gram Stain	Shape
1	C	+	Rod
2	C	+	Cocci
3	C	+	Cocci
4	C	-	Cocci
5	C	-	Rod
6	D	-	Rod
7	D	-	Rod
8	D	-	Rod
9	D	+	Cocci
10	D	+	Cocci
11	V	-	Rod
12	V	+	Rod
13	V	+	Cocci
14	V	-	Rod
15	V	-	Rod
16	V	+	Rod
17	V	+	Cocci
18	Control	+	Rod

Note: V=Vermicompost House, C=Coop, D=Dumpsite

The presence of both Gram-positive and Gram-negative bacteria among the isolates suggests that keratin degradation is not confined to a specific bacterial group but rather involves a wide range of microorganisms. Gram staining is the most important differential staining technique in bacteriology which allows us to divide bacteria into two distinct groups: Gram-positive and Gram-negative. While *Bacillus subtilis* is a well-known Gram-positive keratin degrader, however many Gram-negative bacteria (*Pseudomonas, Serratia, Proteus*) have been reported to produce keratinase enzyme which breaks down keratin in chicken feather (Olusegun-Awosika et al., 2024, Fuke et al., 2017). The detection of both types in this study reinforces previous findings that keratinolytic ability is not restricted to one taxonomic group but is distributed across diverse microbial populations.

The observation that isolate 5 was a Gram-negative bacilli highlights the potential of Gram-negative bacteria in keratin degradation. While Gram-positive bacteria like *Bacillus* are traditionally associated with feather degradation, increasing evidence shows that Gram-negative species also play significant roles, often producing

keratinases with different biochemical properties and substrate specificities. This microbial diversity may enhance the overall efficiency of feather degradation in natural environments, as different bacterial taxa may act synergistically to break down the highly recalcitrant keratin matrix.

Despite being Gram-negative, isolate 5 demonstrated the highest keratinase activity among all isolates tested. This finding underscores that keratinolytic potential is not strictly determined by Gram reaction, but rather by the enzymatic capacity of individual strains. The higher keratinase activity of isolate 5 makes it a promising candidate for further study, as high enzyme production directly correlates with feather degradation efficiency.

4.2.3 Phylogenetic Analysis

A phylogenetic tree was constructed to determine the evolutionary relationship among bacterial isolate 5 based on 16s rRNA gene sequencing. The Neighbour Joining method was used for tree construction, with bootstrap analysis (1000 replicates) to ensure reliability. The phylogenetic tree (Figure 4.3) included both the isolates obtained in this study and reference sequences retrieved from NCBI GenBank using 16s ribosomal RNA sequences (Bacteria and Arcaea) database. Isolate 5, obtained from chicken coop area, clustered closely with *Serratia rubidaea* (bootstrap value 99%), suggesting it is a closely related species within *Serratia* genus. This finding is consistent with previous studies that identify *Serratia* sp. as an effective keratin degrader (Olusegun-Awosika et al., 2024).

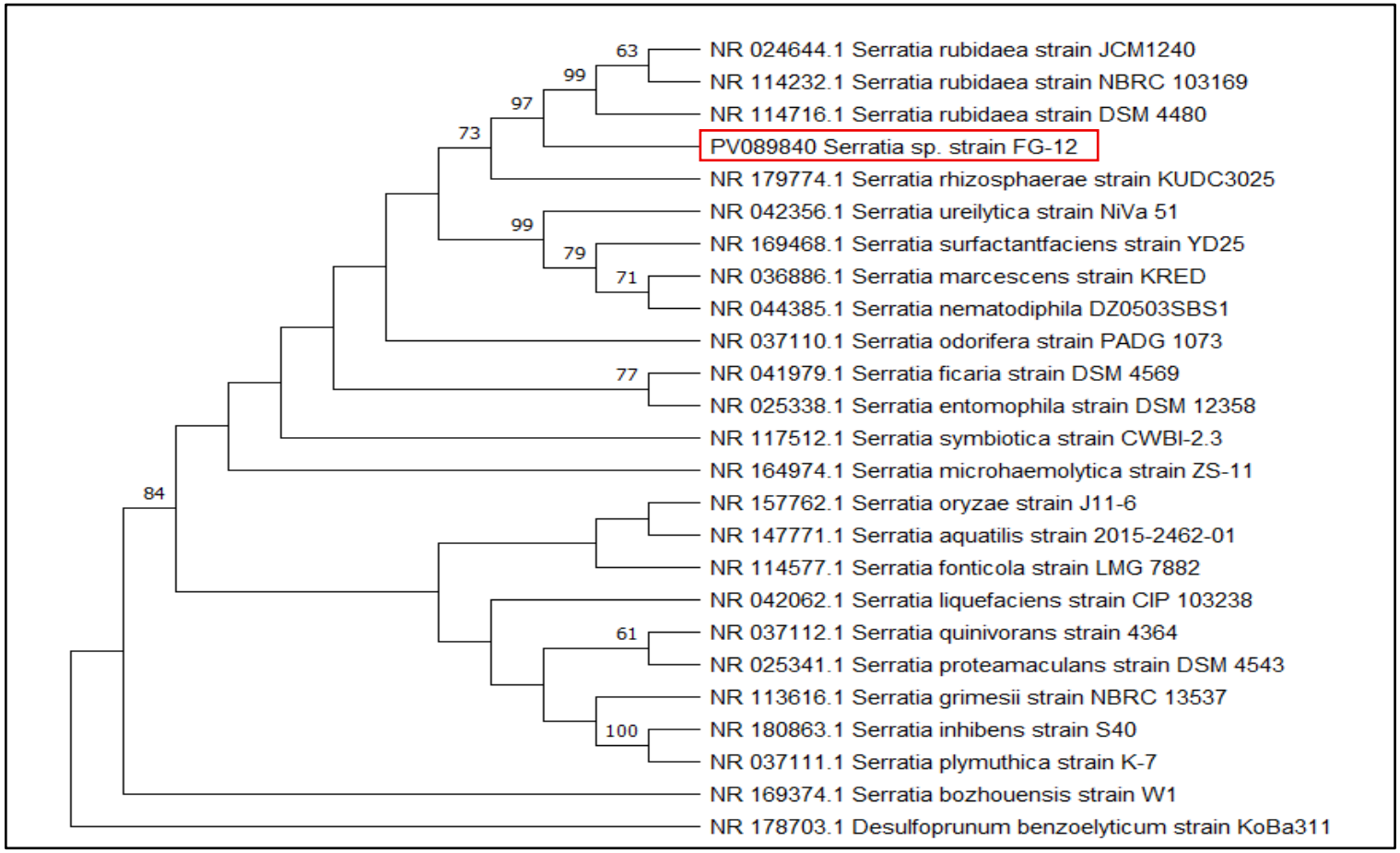

Figure 4.3 Phylogenetic tree based on 16s rRNA nucleotide sequences

Note: The tree was constructed using neighbour joining method algorithms with 1000 bootstrap replicates. Only bootstrap values > 50 are shown. A red square indicates isolate 5 isolated in this study

From an ecological and functional perspective, the association with *S. rubidaea* is notable because members of the *Serratia* genus have been reported to possess extracellular keratinases, enzymes capable of degrading keratin in feathers (Olusegun-Awosika et al., 2024). In poultry environments like chicken coops, keratin-rich waste (chicken feathers) accumulates in large quantities, making keratin-degrading bacteria ecologically beneficial. The presence of a *S. rubidaea* strain in this environment suggests a potential role in natural keratin turnover, which could be utilised in biotechnological processes such as waste bioconversion, animal feed enrichment, or biofertilizer production.

This result also strengthens the link between environmental source and functional potential where previous studies have documented *Serratia* isolates from similar settings with demonstrated feather degradation ability and keratinase activity. Future work, such as whole-genome sequencing and enzyme activity assays, could clarify whether isolate 5 produces keratinases with comparable or superior efficiency to known *Serratia* strains. If confirmed, it could represent a viable candidate for industrial keratin waste valorisation, reducing environmental impact while generating value-added products.

4.3 Vermicompost

4.3.1 Vermicompost Biodegradability Coefficient (K_b) and Population growth of *Eudrilus eugeniae*

Result showed that earthworm populations have significant differences on different substrate ratio, where the earthworm population is higher on final day of composting in treatment T3 compared to treatment T6 and T7. The biodegradability coefficient increases in T3 after completing the vermicomposting process due to the presence of earthworms and bacteria. The biodegradability coefficient increases when complete vermicomposting because of the presence of microorganisms, which decompose organic matter and make it more accessible to other decomposers like bacteria and fungi.

Table 4.4
Earthworm populations and biodegradability coefficient of vermicompost

Treatment	Earthworm population	Biodegradability coefficient (%)
T1	No earthworm	28.4 ± 7.2
T2	35 ± 1.7 a	32.6 ± 4.6
T3	40 ± 2.5 a	41.1 ± 8.4
T4	No earthworm	33.2 ± 1.3
T5	No earthworm	39.2 ± 8.6
T6	34 ± 0.9 b	40.5 ± 9.7
T7	33 ± 0.9 b	38.4 ± 10.8
T8	No earthworm	33.7 ± 6.8
p-value	*	ns

Note: different letter indicates different levels between vermicompost treatment, *shows significant difference between vermicompost treatment, ns=no significant difference between vermicompost treatment ($p<0.05$). The data are mean ± SD.

4.3.2 Microbial Enzyme Activity

4.3.2.1 Keratinase

Figure 4.4 shows the keratinase enzyme activity of different vermicompost treatment every 15 days from Day 15 to Day 60 (complete composting). T6 shows the highest activity among all treatments, starting from 6.82 U/mL on Day 15, increasing to 7.54 U/mL by Day 30 and 9.69 U/mL on Day 45, and drops to 9.19 U/mL on the final day of composting. T2 has the lowest keratinase activity on Day 15 however keeps increasing as the compost mature from day 15 to day 60 with the activity 4.14, 4.84, 5.48, 6.14 (U/mL) respectively.

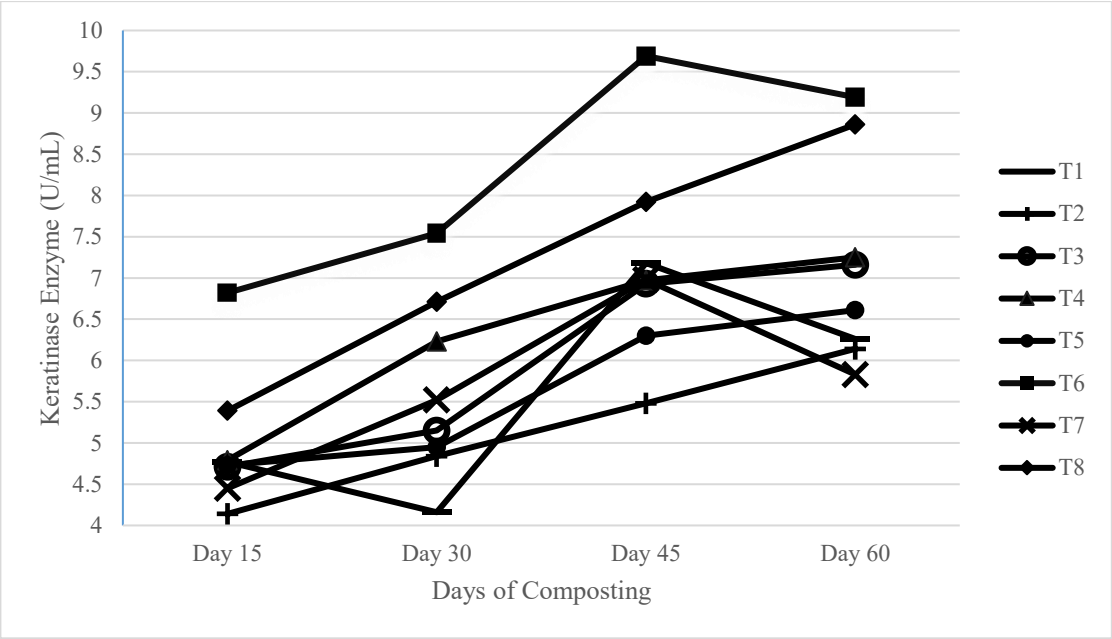

Figure 4.4 Keratinase enzyme activity trendline

During the early stages of composting, microbial communities are still establishing themselves. As the compost matures, keratinolytic bacteria increase, leading to enhanced keratinase production (Nagarajan et al., 2018). As compost matures, the accumulation of organic acids and microbial metabolites further facilitates keratin hydrolysis (Reuter et al., 2015). The degradation of keratin releases amino acids, nitrogen, and sulfur, enriching the compost and making it more beneficial for plant growth (Tamreihao et al., 2019). Some treatments have keratinase activity drops slightly towards Day 60 as the compost matures, because most keratin has been degraded, hence reducing enzyme synthesis. Also, as compost matures, microbes begin utilizing alternative nitrogen sources, reducing reliance on keratin degradation (Duan et al., 2020).

4.3.2.2 Urease

Figure 4.5 shows the urease enzyme activity in vermicompost every 15 days (Day 15, 30, 45, 60). On day 15, T2 shows the highest activity (0.35 U/mL), while T5 shows the lowest activity (0.01 U/mL). As the vermicompost starts maturing, all treatments have decreasing urease activity on day 30 towards day 45 and final day of composting. On day 60, T3 shows the highest urease enzyme activity (0.14 U/mL) while T5 shows the lowest urease activity (0.07 U/mL). T3 (MMR:BT:CF:CD=6:1:1.5:1.5)

has both worm and bacteria with both chicken feather and chicken dung as substrates, whereas T5 only has chicken feather as substrate and bacteria as a composting agent. Vermicompost has been reported to have a higher urease activity compared to conventional compost (without earthworm) (Usmani et al., 2019).

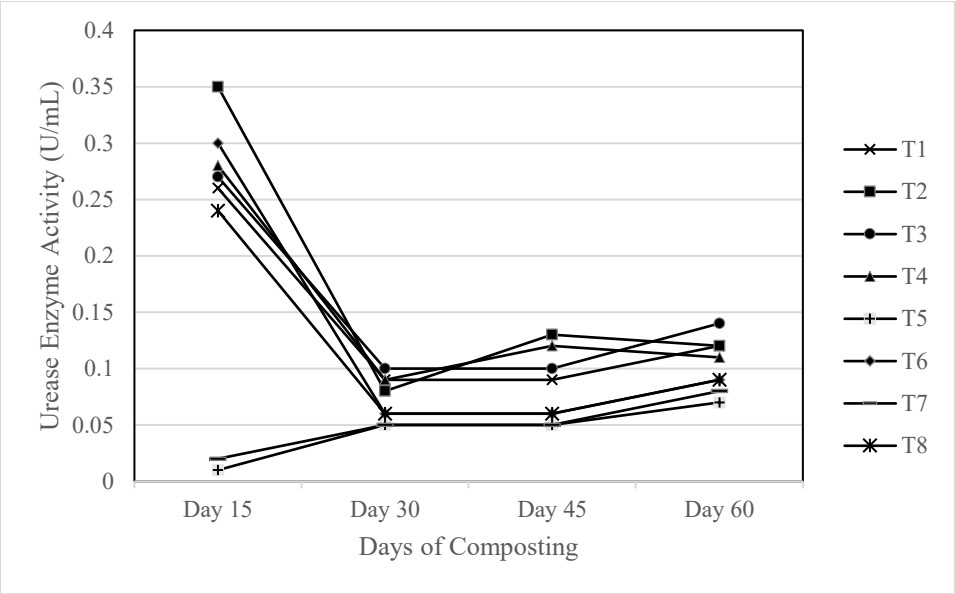

Figure 4.5 Urease Enzyme Activity (U/mL)

At day 15, both earthworms and *Serratia* spp. play complementary roles in enhancing urease activity during vermicomposting. The combined presence of earthworms and *Serratia* spp. creates a synergistic effect that amplifies urease activity during early stage of vermicomposting process. Earthworms promote the distribution and activity of *Serratia* spp. through their digestive processes and cast deposition, while *Serratia* spp. accelerate the breakdown of nitrogenous substrates, boosting the overall efficiency of nitrogen mineralization. Earthworms enhance microbial growth and substrate conditioning, while *Serratia* spp. directly contribute to urease production. This synergy results in improved nitrogen transformation, leading to higher-quality compost with better agronomic value. Meanwhile, the urease enzyme activity is observed to decrease at day 30, as urea has been converted into ammonia and carbon dioxide, hence reducing urease activity. Urease is a critical enzyme in the nitrogen cycle, responsible for hydrolysing urea into ammonia and carbon dioxide. This transformation is vital during vermicomposting, as it contributes to the mineralization of organic nitrogen and improves the nitrogen availability in the final compost product (Amante, 2024; Matczuk & Siczek, 2021).

4.3.2.3 Invertase

Figure 4.6 shows that the invertase activity steadily from Day 15 to Day 45, peaking around Day 45. All treatments start with relatively low enzyme activity (around 3.0–3.6 U/mL). T6 has the highest activity of invertase which is 3.6 U/mL, while T8 and T1 have the lowest (2.9–3.0 U/mL).

T6 shows significantly high invertase enzyme activity from day 15 to day 60 among other treatments as the earthworm activity boosts microbial biomass and aerates the substrates due to higher ratio of chicken feather as substrate. On day 15 and 30, T1 shows significantly lowest invertase activity among other treatments. T6 consistently outperforms other treatments, suggesting it may be the most effective for promoting invertase activity. A slight decline by Day 60 indicates that composting may be nearing maturation, where enzymatic activity stabilizes or reduces.

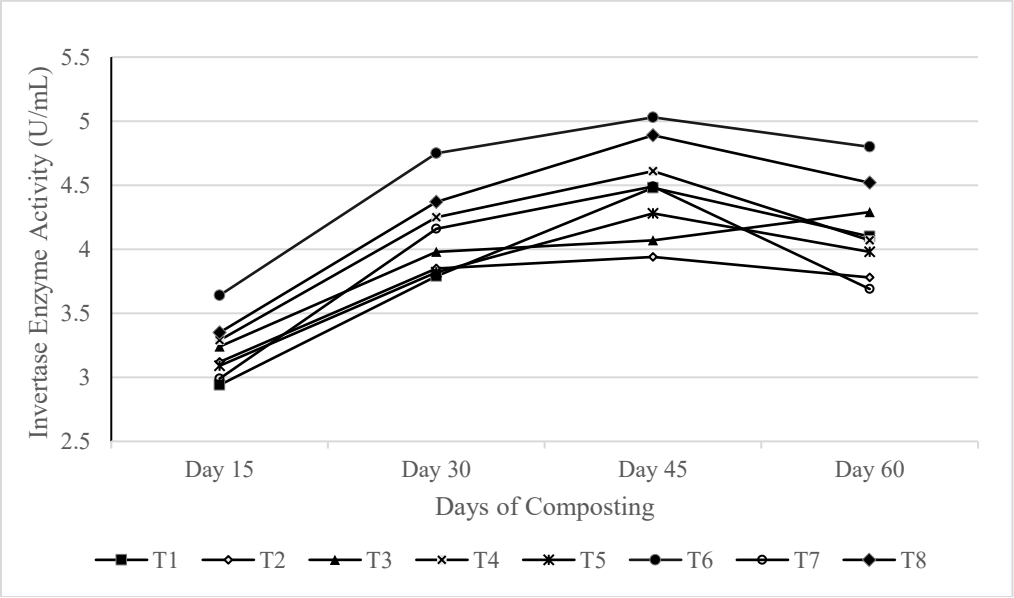

Figure 4.6 Invertase Enzyme Activity (U/mL)

T6 exhibited the highest invertase activity at 5.0 U/mL on Day 45. This proposes that T6 is the most effective in microbial enzyme production. T4 and T5 also showed high activity, 4.5–4.6 U/mL, indicating strong microbial response, meanwhile T3 and T7 followed with moderate activity. In this study, the mutual interaction of *E. eugeniae* and *Serratia* spp. for treatment 6 during showed a synergistic effect, enhanced invertase activity throughout vermicomposting process from day-15 to day-60. In this study, earthworms promote the physical breakdown of organic matter and stimulate microbial

activity, while *Serratia* spp. contribute enzymatic capacity to degrade sugars and other carbohydrates. This interaction improves the breakdown of complex organic matter, accelerates composting, and contributes to the formation of stable humus. Earthworms, such as *Eudrilus eugeniae* and *Eisenia fetida*, significantly influence invertase activity during vermicomposting (Aira et al., 2007). According to Lazcano & Domínguez (2011), the mucus and other secretions of earthworms provide a rich microenvironment for microbial growth and enzymatic synthesis through their ingestion and digestion of organic matter, earthworms stimulate microbial proliferation and enhance enzymatic activity in the substrate.

4.3.2.4 Polyphenol Oxidase

Figure 4.7 shows polyphenol oxidase activity across day 15, 30, 45, and 60. for eight treatments. Polyphenol oxidase is an important oxidative enzyme comprised with lignin degradation and phenolic compound oxidation, indicating microbial activity and breakdown of organic matter. The polyphenol oxidase activity begins low, peak in mid composting and drop towards the end of composting. There is no significant different among all treatments from day 15 to day 60. On day 15, T2 shows relatively high polyphenol oxidase activity (0.59 U/mL) as compared to T8 (0.51 U/mL). Towards the end of composting, T2 still shows relatively highest activity (0.65 U/mL), whereas T4 shows relatively lowest activity (0.55 U/mL).

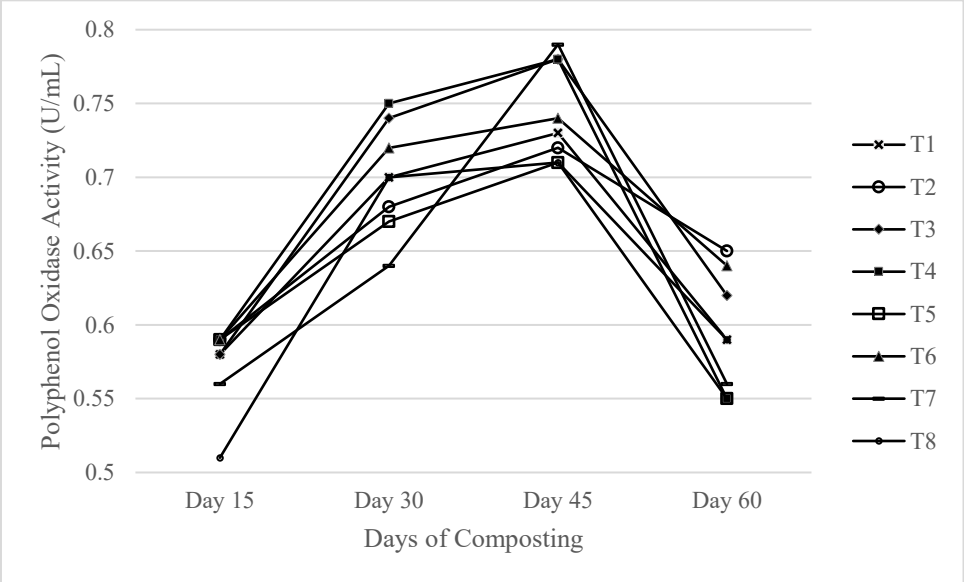

Figure 4.7 Polyphenol Oxidase Activity (U/mL)

The ratio of phenolic-rich materials, mushroom media residue used in this study is similar in all treatments, hence the polyphenol oxidase activity does not differ significantly between treatments. The pH of compost treatments is stable across treatments which influence the polyphenol oxidase activity as well. T1, T3, and T6 have the highest values, indicating a strong phenolic degradation. According to Wang et al. (2024), polyphenol oxidase enzyme reflects the thermophilic phase, which activity is due to high microbial growth and optimal temperature. Generally, the enzyme such as polyphenol oxidase is increases during the thermophilic phase due to the intensive microbial degradation of complex organics like lignin and phenolics, then decrease during the maturation phase as substrate availability reduced (Wang et al., 2024). The finding of Day 45 in this study agrees with this previous study by Wang et al. (2024), reflecting peak microbial oxidation. The reduction of polyphenol oxidase at Day 60 is consistent with compost maturation and reduced in substrate availability.

4.3.2.5 Dehydrogenase

Figure 4.8 shows dehydrogenase enzyme activity every 15 days from day 15 to day 60 of composting. On day 15, T3 shows significantly highest dehydrogenase activity among other treatments, whereas T4, T5, T6 show significantly low activity. Treatments with both composting agent (T3 and T7) shows significantly high activity regardless of the substrate ratio. At the early composting stage, the earthworm aids in fragmentation of organic matter and releasing nitrogenous waste that feed microbes. Also, the burrowing of earthworms creates aeration, which is an optimal environment for aerobic bacteria, in this case *Serratia* spp.

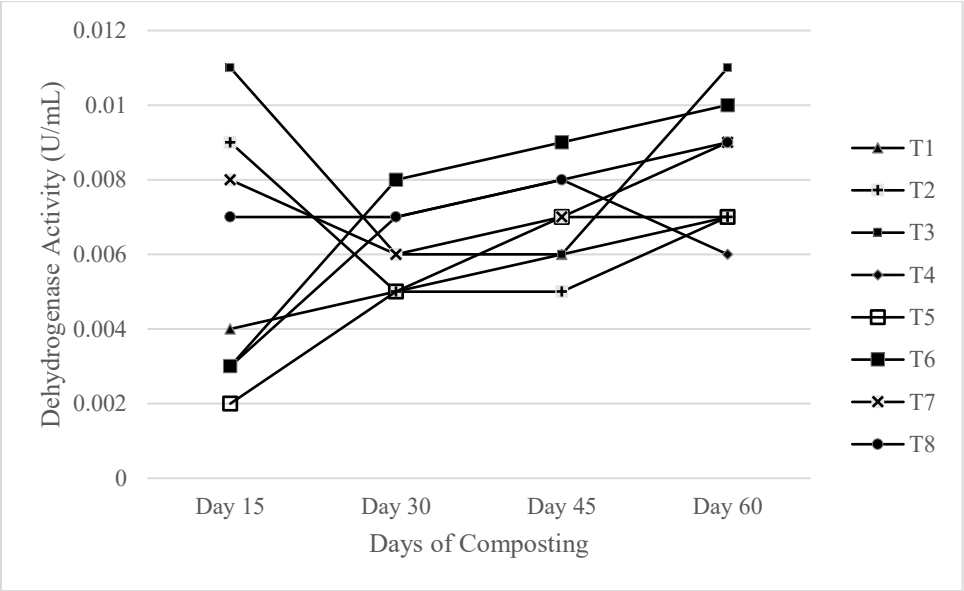

Figure 4.8 Dehydrogenase enzyme activity (U/mL)

4.3.3 Chemical Properties of Vermicompost

4.3.3.1 Electrical Conductivity (EC) and pH

Figure 4.9 that both substrate ratio and composting agent significantly affect the electrical conductivity of compost on day 15 of composting, indicating that treatments without chicken manure (T5-T8) has lower electrical conductivity level ranging from 2.80 to 2.95 mS/cm, and treatment with only earthworm has lowest electrical conductivity level. There is also significant interaction between treatments indicating presence of composting agents vary based on substrate ratio, as T7 has higher EC level (3.44 mS/cm) compared to other treatments without chicken manure. As the compost reached day 30 up to day 45 of composting, only substrate ratio significantly affects the electrical conductivity of compost, where treatments without chicken manure has lower levels of electrical conductivity ranging from 2.34 to 2.76 mS/cm. At the end of the composting process, both substrate ratio and composting agent significantly affects the level of electrical conductivity. The EC value of mushroom medium residue, chicken dung and chicken feather were decreasing from the first week until the final week of observation. The downfall of EC value was due to the flushing of mushroom medium residue by using water multiple times a day.

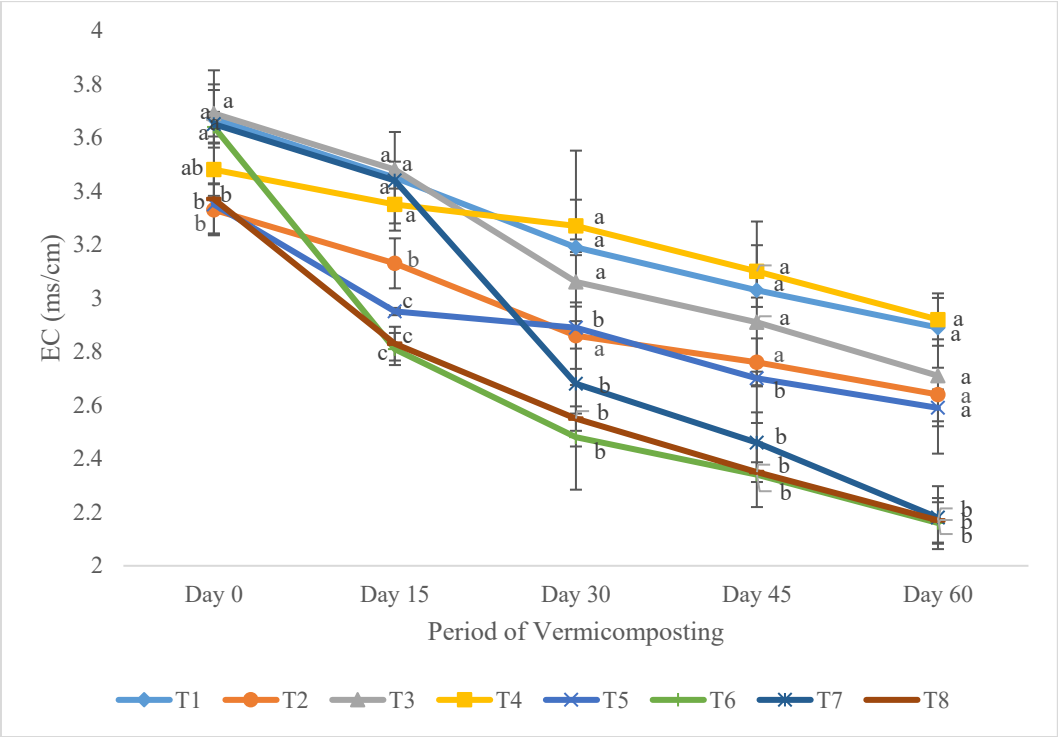

Figure 4.9 Trendline of EC during vermicomposting process

Note: different letter indicates significantly different levels between vermicompost treatment

Result showed that different substrate ratio and composting agent did not significantly influence pH value of compost (Figure 4.10). There is also no significant interaction between different substrate ratio and composting agent on pH value of the compost. The pH values were not significantly different between treatment and was very close to neutral on the final day of composting indicating degradation of substrates mixture which is in conformity with the findings of Biruntha et al. (2020). According to Omar et al. (2024), the difference in pH value ($p<0.05$) of chicken dung between the initial and final observation during vermicomposting process. In first week of pre-composting process, the initial pH for mushroom medium residue, chicken dung and chicken feather were in alkali condition.

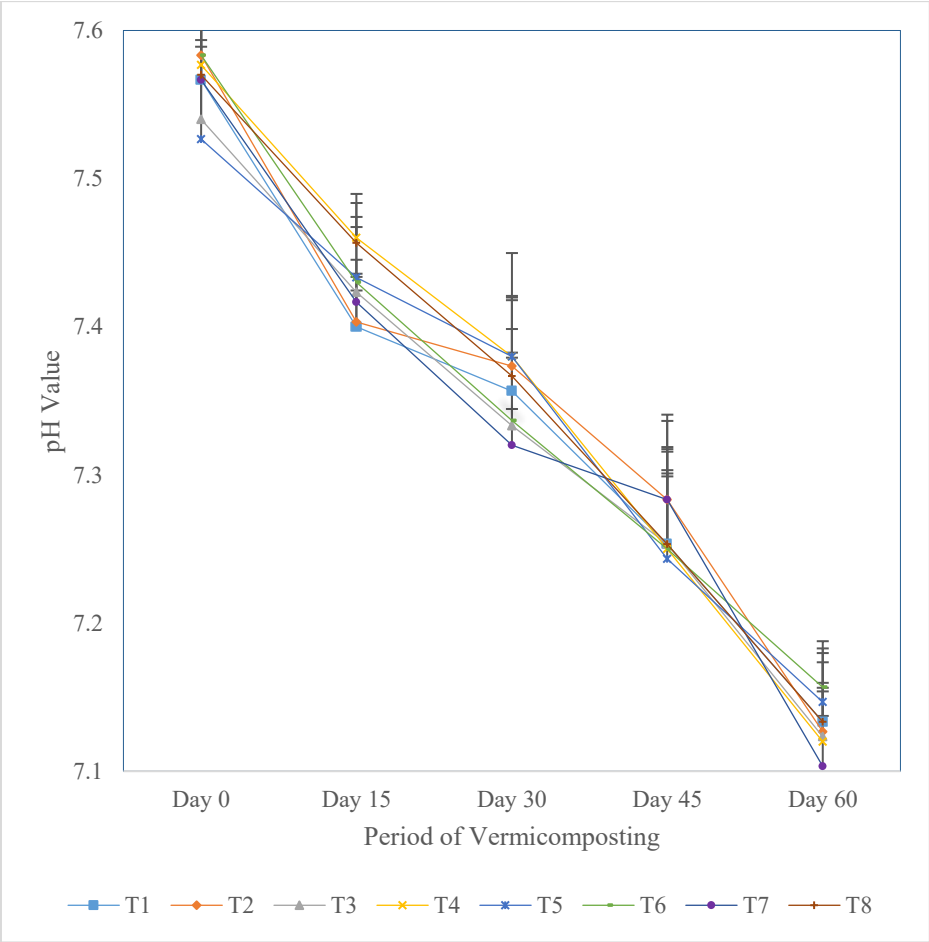

Figure 4.10 Trendline of pH level during vermicomposting process

4.3.3.2 Total Nitrogen, Organic Carbon, Available Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg)

Table 4.5 shows the chemical analysis of vermicompost produced from different substrate formulations and composting agents revealed clear differences in nutrient composition. Treatments T1–T4, which incorporated mushroom media residue, banana trunk, chicken manure, and chicken feathers in a 6:1:1.5:1.5 ratio, consistently exhibited higher nutrient concentrations compared to treatments T5–T8, which excluded chicken manure and replaced it with a higher proportion of feathers (6:1:0:3). Among all treatments, T3 (bacteria + earthworm) produced the most enriched nutrient profile, with phosphorus (2.9%), calcium (4.4%), and magnesium (1.6%) exceeding other treatments, while nitrogen (2.2%) remained within the recommended range for vermicompost. Although T1 (bacteria only) recorded the highest nitrogen (2.8%), the overall nutrient spectrum in T3 was superior, offering a more balanced supply of macro

and secondary nutrients. T4 (no agents) recorded the lowest nutrient content within the manure-based group.

In contrast, treatments without manure (T5–T8) showed significantly lower nutrient concentrations, especially in nitrogen and phosphorus, although they contained very high organic matter (92.7–96.2%), indicating slower decomposition and incomplete mineralization. The no-agent treatments (T4, T8) consistently produced the lowest nutrient enrichment, confirming the importance of biological agents in accelerating decomposition.

Table 4.5
Total N, P, K, Ca, Mg in vermicompost

Treatment	Substrate Ratio (MMR:BT:CM:CF)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	OM (%)
T1 (Bacteria)	6:1:1.5:1.5	2.8 ± 1.03 a	2.7 ± 0.46 a	0.4 ± 0.05 a	4.0 ± 0.56 a	1.4 ± 0.16 a	81.3 ± 3.70 b
T2 (Earthworm)	6:1:1.5:1.5	2.6 ± 1.15 a	2.3 ± 1.02 a	0.4 ± 0.14 a	3.5 ± 1.22 a	1.3 ± 0.53 a	84.4 ± 6.06 b
T3 (Bacteria and earthworm)	6:1:1.5:1.5	2.2 ± 0.73 a	2.9 ± 1.17 a	0.5 ± 0.26 a	4.4 ± 1.67 a	1.6 ± 0.53 a	78.2 ± 11.1 b
T4 (No composting agent)	6:1:1.5:1.5	1.7 ± 0.62 a	1.9 ± 0.30 a	0.3 ± 0.03 a	3.1 ± 0.15 a	1.2 ± 0.07 a	86.9 ± 1.08 b
T5 (Bacteria)	6:1:0:3	1.0 ± 0.06 b	0.4 ± 0.03 b	0.3 ± 0.02 b	1.8 ± 0.10 b	0.7 ± 0.05 b	94.3 ± 0.69 a
T6 (Earthworm)	6:1:0:3	1.4 ± 0.32 b	0.4 ± 0.07 b	0.3 ± 0.07 b	2.1 ± 0.36 b	0.9 ± 0.15 b	93.6 ± 0.93 a
T7 (Bacteria and earthworm)	6:1:0:3	1.0 ± 029 b	0.3 ± 0.01 b	0.2 ± 0.01 b	1.7 ± 0.14 b	0.7 ± 0.06 b	92.7 ± 3.39 a
T8 (No composting agent)	6:1:0:3	0.7 ± 0.18 b	0.4 ± 0.16 b	0.3 ± 0.12 b	2.1 ± 0.66 b	0.8 ± 0.30 b	96.2 ± 1.98 a
p-value		*	*	*	*	*	*

Note: different letter indicates different levels between substrate ratios, *show significant difference ($p<0.05$) between substrate ratios. The data are mean ± SD. N, nitrogen; P, phosphorus; K, potassium; Ca, calcium; Mg, magnesium; OM, organic matter; MMR, Mushroom Media Residue; BT, Banana Trunk; CM, Chicken Manure; CF, Chicken Feather.

The results highlight the advantage of using both bacteria and earthworms in vermicomposting. T3 demonstrated the most comprehensive nutrient enrichment, with higher phosphorus, calcium, and magnesium compared to single-agent treatments. This aligns with previous studies reporting that synergistic interactions between earthworms and microbes enhance organic matter degradation and nutrient solubilization (Saharan,

2024). Earthworms fragment substrates and stimulate microbial activity through gut-associated enzymes, while inoculated bacteria accelerate the mineralization of resistant compounds such as keratin from chicken feathers (Kooch & Kuzyakov, 2024; Gupta et al., 2023). The combined activity therefore leads to greater nutrient release than either agent alone.

Although nitrogen was slightly lower in T3 compared to T1, this may be attributed to more complete mineralization, where nitrogen was converted into stable organic and inorganic forms within vermicast. According to Baskar et al., (2023) such stabilization reduces the risk of nitrogen loss through leaching or volatilization and ensures gradual nutrient availability to plants. The higher phosphorus and calcium values in T3 are particularly beneficial, as these nutrients are often limiting in soils and are critical for root development, structural integrity, and reproductive growth (Khan et al., 2023).

The relatively weaker performance of bacteria-only (T1) and earthworm-only (T2) treatments suggests that single agents, while effective, cannot match the nutrient enrichment achieved through their combined action. Treatments without composting agents (T4, T8) produced the least favourable nutrient composition, highlighting that passive decomposition is insufficient for efficient mineralization of complex substrates such as chicken feathers. High organic matter values, particularly in T5–T8, improve soil structure and microbial activity but dilute the nutrient concentrations, making these treatments more suitable as soil conditioners rather than nutrient-rich fertilizers (Thakur et al., 2023).

Overall, T3 can be considered the best treatment, as it provided the most nutritionally rich vermicompost with a broad spectrum of macronutrients and secondary nutrients. Its balanced nutrient profile makes it more suitable for enhancing soil fertility compared to other treatments. However, the consistently low potassium levels across all treatments suggest the need for supplementary K sources during application.

CHAPTER 5
CONCLUSION

In conclusion, the objectives of this study – identifying the most potential locally isolated feather-degrading bacteria, to determine the efficiency of the feather-degrading bacteria and earthworms in the biodegradation of chicken feathers, and to characterize the physicochemical properties of by-products (vermicompost) produced by the feather-degrading bacteria and earthworms applied singly of in combinations were accomplished.

Among 17 isolates obtained from soil samples of 3 different locations (coop area, dumpsite, vermicompost house), isolate 5 was the most potential isolated chicken feather degrading bacteria with 3.8 U/mL keratinase enzyme activity as well as relatively high weight loss percentage of chicken feathers at 42.8% of 10g/L chicken feathers. Isolate 5, a Gram-negative bacterium was isolated from chicken coop area in Kedah, Malaysia. This bacterium was identified as *Serratia* sp. using 16s rRNA sequencing method, based on 99% gene sequence homology it showed with *Serratia rubideae* species.

The efficiency of the feather-degrading bacteria and earthworm revealed that T3 (MMR:BT:CF:CD = 6:1:1.5:1.5) with both composting agents has significantly high earthworm populations and biodegradable coefficient as compared to T1 and T2 (same substrate ratio) with single composting agent. T2 shows a higher biodegradable coefficient than T1 suggesting the presence of earthworm enhanced the degradation of chicken feathers in vermicompost.

The ratio of substrates in vermicompost affects the total nitrogen, organic carbon, available phosphorus, potassium, calcium and magnesium with treatments (T1-T4) containing equal ratio of chicken feather:chicken dung at 1.5:1.5 exhibiting higher nutrient content compared to treatments (T5-T8) containing only chicken feather. Treatments without chicken dung (T5-T8) shows a higher organic matter content suggesting slower degradation and higher carbon content of chicken feathers (higher ratio). Hence, the treatments with equal ratio of chicken feather and chicken dung results in more complete degradation and lower organic matter residual.

The use of keratin degrading bacteria and earthworm in degrading chicken feather in vermicompost presents a sustainable waste management strategy, an

environmentally friendly alternative to conventional disposal methods such as incineration or landfilling, which contribute to pollution and resource loss. The enhanced nutrient content of the resulting vermicompost suggests its suitability as an organic fertilizer, offering benefits for soil health and crop productivity while reducing reliance on chemical fertilizers in agriculture.

However, the results obtained in this study was limited to laboratory-scale composting conditions, which may not wholly represent the large scale. The duration of the vermicomposting was fixed to 60 days, and long-term assessments of compost maturity and soil impact were not conducted. Therefore, future studies are recommended to focus on long-term experiments to assess the effects of the resulting vermicompost on soil fertility and crop performance. Also, it is recommended for a further optimization of environmental condition of the bacterium. Overall, this study highlights the potential of chicken feather degrading bacteria as a sustainable solution for managing poultry waste.

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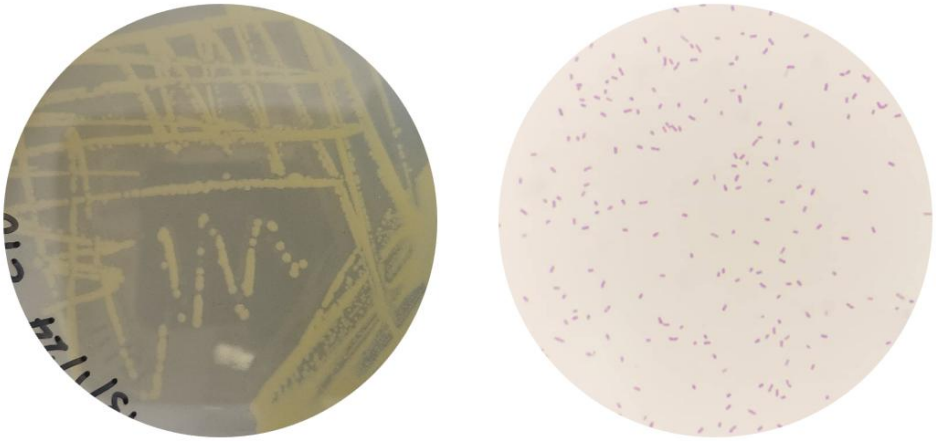
APPENDICES

APPENDIX 1

Photographs of Milk Agar (a) and Gram stain (b; 1000 × magnification) of isolate 5 (*Serratia* spp.)



(a) (b)

APPENDIX 2

SPSS Output for Keratinase Activity, Chicken Feather Degradation, and Correlations

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Ker	Between Groups	8.214	17	.483	2.286	.018
	Within Groups	7.609	36	.211		
	Total	15.823	53			
Deg	Between Groups	262.057	17	15.415	.164	1.000
	Within Groups	3380.869	36	93.913		
	Total	3642.926	53			

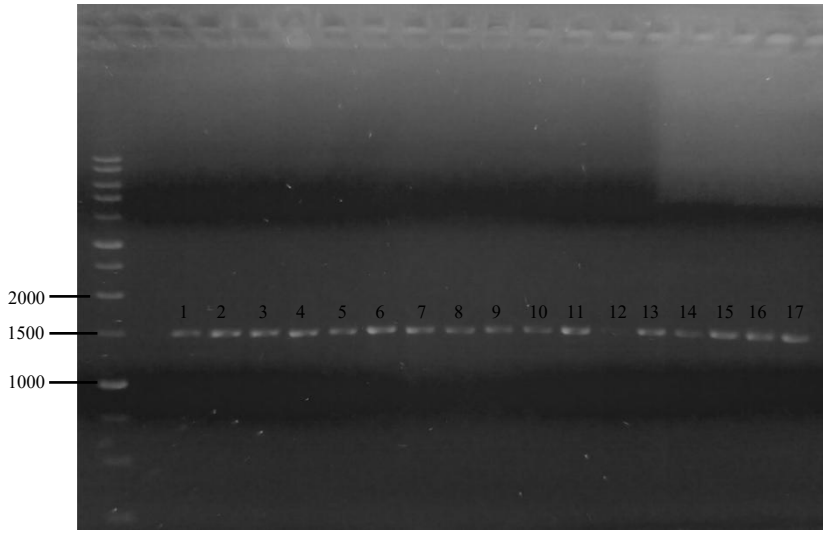
Correlations

		Ker	Deg
Ker	Pearson Correlation	1	.514**
	Sig. (2-tailed)		.000
	Sum of Squares and Cross-products	15.823	123.486
	Covariance	.299	2.330
	N	54	54
Deg	Pearson Correlation	.514**	1
	Sig. (2-tailed)	.000	
	Sum of Squares and Cross-products	123.486	3642.926
	Covariance	2.330	68.734
	N	54	54

**. Correlation is significant at the 0.01 level (2-tailed).

APPENDIX 3

Gel image of agarose electrophoresis of touchdown PCR using marker genes.



AUTHOR'S PROFILE

Nour Shazana Shahiful Hizam obtained Bachelor of Science in Agrotechnology (Hons.) Horticulture Technology in 2023 from Universiti Teknologi MARA, Perlis and currently completing Masters of Science at Universiti Teknologi MARA. Her research focuses on the biodegradation of chicken feather using keratinase-producing bacteria and earthworms as composting agents. She is particularly interested in sustainable agriculture and organic waste. Upon graduation, she hopes to use research and development in both academic and industrial contexts to support agricultural innovation.

LIST OF PUBLICATION:

Shahiful Hizam, N. S., Omar, N. F., Sabri, N. F., Samsudin, N. Z., Ahmad, S. A., Ismail, I. S., & Zainol Abidin, M. A. (2025). Sustainability of Poultry Waste: Bacterial Isolates for Efficient Biodegradation of Chicken Feathers. *Advances in Agricultural and Food Research Journal*, *6*(2). https://doi.org/10.36877/aafrj.a0000593

Shahiful Hizam, N. S., Omar, N. F., Sabri, N. F., Samsuddin, N. Z., & Zainol Abidin, M. A. (2024). Evaluation of Physicochemical Properties and Nutrient Composition of Chicken Feather Vermicompost for Sustainable Waste Management System. *IOP Conference Series: Earth and Environmental Science*, *1426*(1). https://doi.org/10.1088/1755-1315/1426/1/012014