World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Characterizing thermophilic bacteria isolated from surface layers of compost in the pre-decomposition stage

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World Journal of Advanced Research and Reviews, 2025, 27(01), 1087-1097

Publication history: Received on 03 June 2025; revised on 08 July 2025; accepted on 11 July 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.27.1.2630

Abstract

Thermophilic bacteria are potential bacteria for degrading organic waste. This study aims to isolate and characterize thermophilic bacteria in surface compost in the pre-decomposition phase of PT. Great Giant Pineapple Compost Plant. This study used an exploratory descriptive method including isolation, purification, macroscopic characterization, microscopic characterization, an enzymatic activity test, and determination of the Enzymatic Index (EI) value. The results of this study obtained 7 isolates of thermophilic bacteria that have irregular and spreading, and round shapes with flat, raised, and umbonate elevations. Colony edges are undulate, entire, lobate, and irregular with cream color. Isolates ZS1, ZS2, ZS4, and ZS5 have a monobacillary cell shape, while isolates ZS3, ZS6, and ZS7 have a streptobacillary cell shape; all Gram positive except ZS1 and only ZS1 does not form spores. All isolates of thermophilic bacteria were able to produce catalase enzyme, protease enzyme except isolate ZS7; amylase except isolate ZS5; and cellulase in isolates ZS1, ZS5, ZS6, and ZS7. The highest Enzymatic Index (IE) of protease, amylase, and cellulase in order were ZS4 with a value of 6.1, ZS2 with a value of 4.37, and ZS1 with a value of 6.5.

Keywords: Composting; Thermophilic Bacteria; Compost; Isolation; Characterization

1. Introduction

Organic waste management is a major challenge in the agricultural industry. One common approach to deal with organic waste is composting, which is the process of bioconversion of organic matter into compost with the help of microbial activity. Composting proceeds through mesophilic, thermophilic, cooling, and maturation phases [1]. Among these stages, the thermophilic phase has a crucial role. This phase occurs at 45-70°C, accelerates the decomposition of organic matter, and reduces the number of pathogenic microbes [2]. High temperatures in this phase induce the emergence of thermophilic microbes, including extracellular enzyme-producing bacteria that actively decompose organic matter [3].

PT. Great Giant Pineapple (PT. GGP) is an agricultural industry that implements composting. At the PT. GGP Compost Plant, there is a pile of pre-decomposition phase compost that is dominated by protein, amylum, and cellulose substrates, which require enzymatic degradation by protease, amylase, and cellulase for fast composting. One effort to accelerate this process is the addition of microbial inoculum [4]. Masi et al. [5] reported that thermophilic bacterial isolates from household waste can produce protease, amylase, and cellulase enzymes that support the decomposition of organic matter during composting. Therefore, enzyme-producing thermophilic bacteria are indispensable to optimizing composting.

Temperature in the pre-decomposed compost pile is in the thermophilic phase, around 50°C with an average pH of 5.0. The surface of the compost pile is a strategic location to obtain thermophilic bacteria because it has high aeration.

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Aeration is related to oxygen availability and helps maintain thermophilic temperatures [6]. The combination of oxygen and temperature creates an ideal environment for thermophilic bacteria to thrive on the compost surface [7].

This study aims to isolate, characterize macroscopically and microscopically, and test the enzyme activity of catalase, protease, amylase, and cellulase against thermophilic bacteria from the surface of pre-decomposed compost at the Compost Plant PT. GGP. This research is expected to obtain potential thermophilic bacterial isolates as inoculum to accelerate the composting process.

2. Material and Methods

2.1. Sampling

Compost sampling was conducted on the surface compost of the pre-decomposition phase at the compost plant of PT. Great Giant Pineapple, Central Lampung, Lampung Province. Compost samples were taken with 3 repetitions at a depth of 5-10 cm from the surface. Furthermore, the samples were isolated at the Research and Development Laboratory of PT. Great Giant Pineapple.

2.2. Isolation and Purification of Thermophilic Bacteria

A total of 25 g of compost sample was put into a sterile Erlenmeyer containing 225 mL of 0.9% NaCl, then homogenized to obtain a dilution of 10^{-1}. The suspension of the 10^{-1} dilution was taken as much as 1 mL and then put into a test tube containing 9 mL of 0.9% NaCl, then homogenized using a vortex so that a 10^{-2} dilution was obtained. The working procedure was carried out in the same way up to the 10^{-10} dilution level. A total of 0.1 mL from dilutions 10^{-6} to 10^{-10} was taken using a micropipette and then inoculated into Petri dishes containing Nutrient Agar (NA) media modified using Nutrient Broth (NB) + Agar 6% and leveled using Drigalski, then incubated at 50°C for 24 hours [8].

Single colonies formed on Petri dishes were then selected and then taken as much as 1 ose to be scratched by the streak plate method on the surface of NA media modified using NB media with the addition of 6% agar. Furthermore, it was incubated at 50°C for 24 hours [9]. The purpose of purification is to get a single colony. The single colony obtained was then inoculated as much as 1 ose on modified NA slant media as a stock to be characterized macroscopically and microscopically, as well as enzymatic activity tests.

2.3. Macroscopic Characterization of Thermophilic Bacteria

Macroscopic characters were observed directly on 24-hour-old isolates, including the shape, elevation, edges, and color of colonies [10].

2.4. Microscopic Characterization of Thermophilic Bacteria

2.4.1. Gram Staining

Bacterial isolates aged 24 hours were made on a glass slide. It was then stained with crystal violet (Gram A) for 1 minute, lugol iodine (Gram B) for 1 minute, washed with alcohol (Gram C) for 30 seconds, and stained with safranin (Gram D) for 1 minute. Then rinsed with distilled water and observed under a microscope [11].

2.4.2. 3% KOH Test

The 3% KOH test was performed as a confirmation test for Gram staining. The 3% KOH test was carried out by taking 1 ose of a 24-hour-old bacterial isolate, then placed on a glass object. Furthermore, it is added with 1 drop of 3% KOH and flattened slowly. If the mixture produces mucus, the bacteria tested are classified as Gram-negative bacteria, while if it does not produce mucus, the bacteria tested are classified as Gram-positive bacteria [12].

2.4.3. Spore Staining

Bacterial isolates aged 72 hours are made on a glass object. Then it was stained with malachite green for 10 minutes. After rinsing, safranin was added for 30 seconds. Then rinsed with running water and observed under a microscope [13].

2.5. Enzymatic Activity Test of Thermophilic Bacteria

2.5.1. Catalase Test

The catalase test was carried out by dropping 3% H_2O_2 as much as 1 drop on a glass object, then adding 1 isolate of thermophilic bacteria and homogenized. If gas bubbles form, the catalase test results are positive, while if no gas bubbles form, the catalase test results are negative [14].

2.5.2. Protease Test

Bacterial isolates aged 24 hours were inoculated by the point method on Skim Milk Agar media, then incubated at 50°C for 24 hours. A clear zone around the colony was observed as a positive result, and then the Enzymatic Index (EI) was determined [8].

2.5.3. Amylase Test

Bacterial isolates aged 24 hours were inoculated by the point method onto modified Starch Agar media using 6% NB + Agar with the addition of 1% starch, then incubated at 50°C for 24 hours. Then watered using 1% iodine solution. Observed the clear zone around the colony as a positive result and then determined the Enzymatic Index (EI) [8].

2.5.4. Cellulase Test

Bacterial isolates aged 24 hours were inoculated by the point method onto NA media modified using NB + 6% agar with the addition of 1% Carboxymethyl Cellulose (CMC). Then incubated at 50°C for 24 hours. Then watered with 0.1% Congo red solution, and the remaining 0.1% Congo red solution was discarded and then rinsed with 1 M NaCl for 15 minutes. The clear zone around the colony was observed as a positive result, and then the Enzymatic Index (EI) was determined [8].

2.6. Enzymatic Index (EI)

The enzymatic index (protease, amylase, cellulase) was determined by comparing the colony area and the clear zone area formed. The colony area and clear zone area were calculated using the gravimetric method, as described by Sumardi et al. [15].

- Colony patterns and clear zones formed were made replicas by drawing on clear plastic mica.
- Replicas of colonies and clear zones were then weighed using an analytical balance.
- Paper pieces measuring 1 cm × 1 cm were made, then weighed using an analytical balance.
- The area of colonies and clear zones was then calculated using the formula:

$$\text{Colony area} = \frac{\text{Weight of colony replica}}{\text{Weight of 1 cm} \times \text{1 cm paper}} \times 1\ \text{cm}^2 \qquad \text{Clear zone} = \frac{\text{Weight of clear zone replica}}{\text{Weight of 1 cm} \times \text{1 cm paper}} \times 1\ \text{cm}^2$$

$$\text{Average Total Clear Zone Area (ATz)} = \frac{Za1+Za2+Za3}{3} \qquad \text{Average Total Colony Area (ATc)} = \frac{Ca1+Ca2+Ca3}{3}$$

The enzymatic index (EI) was determined based on the ratio of the average clear zone area to the average colony area, as described by Rosa et al. [16].

$$\text{Enzymatic Index (EI)} = \frac{ATz-ATc}{ATc}$$

The IE value was then categorized as very high (EI value > 5.0), high (EI value > 2.0–5.0), and low (EI value < 2.0) [17].

3. Results and Discussion

3.1. Isolation and Macroscopic Characterization of Thermophilic Bacteria

Seven isolates of thermophilic bacteria were obtained, each of which was coded ZS1, ZS2, ZS3, ZS4, ZS5, ZS6, and ZS7. The seven isolates of thermophilic bacteria have different morphological characteristics. The colony shape of the seven

isolates of thermophilic bacteria is dominated by irregular and spreading, with flat elevation. Colony edge characteristics show diverse results, namely undulate, entire, lobate, and irregular while the color of the colonies shows cream results (Table 1).

Table 1 Macroscopic observation results of thermophilic bacterial isolates from surface compost in the pre-decomposition phase

Isolate Code	Macroscopic Characteristics			
	Shape	**Elevation**	**Edge**	**Color**
ZS1	Irregular and spreading	Raised	Undulate	Cream
ZS2	Round	Raised	Entire	Cream
ZS3	Irregular and spreading	Flat	Irregular	Cream
ZS4	Round	Flat	Entire	Cream
ZS5	Irregular and spreading	Flat	Lobate	Cream
ZS6	Irregular and spreading	Flat	Undulate	Cream
ZS7	Irregular and spreading	Umbonate	Irregular	Cream

3.2. Microscopic Characterization of Thermophilic Bacteria

3.2.1. 3% KOH Test and Gram Staining

The 3% KOH test was conducted as an initial identification test to determine the nature of the bacterial Gram stain. It is known that isolated ZS1 forms mucus threads after being dripped with 3% KOH. Meanwhile, the other six isolates, namely ZS2, ZS3, ZS4, ZS5, ZS6, and ZS7, did not form mucus threads after 3% KOH. Thus, isolate ZS1 is classified as Gram-negative, while isolates S2, ZS3, ZS4, ZS5, ZS6, and ZS7 are classified as Gram-negative. The results of Gram staining in Figure 1 with 1000× magnification show that isolate ZS1 (A) is Gram-negative, characterized by red cells. Meanwhile, isolates ZS2 (B), ZS3 (C), ZS4 (D), ZS5 (E), ZS6 (F), and ZS7 (G) are Gram-positive, indicated by purple cells. Isolates ZS1, ZS2, ZS4, and ZS5 have a single bacillus cell form (monobacillus), while isolates ZS3, ZS6, and ZS7 have a streptobacillus cell form (Figure 1). The majority of isolates found in this study (isolates ZS2-ZS7) are classified as Gram-positive bacteria. This is in line with previous findings that many thermophilic bacteria are known to be Gram-positive. In line with the research of Ortega-Villar et al. [18], 18 isolates of thermophilic bacteria from hot springs are known to be classified as Gram-positive bacteria, and research by Masi et al. [5], that thermophilic bacterial isolates from landfills are Gram-positive and bacillus-shaped. Meanwhile, one isolate, ZS1, was identified as Gram-negative. This shows that Gram-negative bacteria can have adaptations to high temperatures [19].

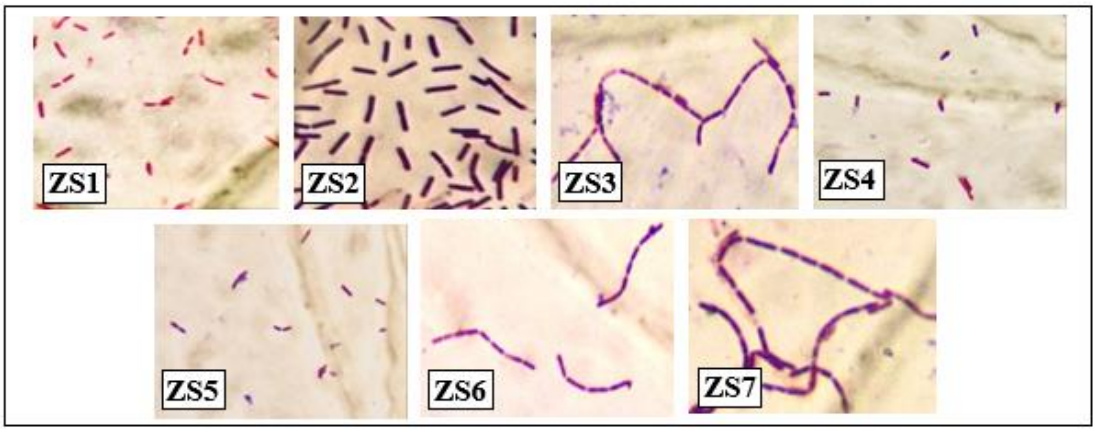


Figure 1 Results of observations of cell shape and Gram properties of seven thermophilic bacterial isolates with a magnification of 1000×

3.2.2. Spore Staining

The results of spore staining on the seven isolates of thermophilic bacteria show that isolate ZS1 has no spores, while isolates ZS2, ZS3, ZS4, ZS5, ZS6, and ZS7 have spores. The presence of spores is a strong characteristic in thermophilic bacteria. Spores are structures that function to protect bacterial cells from unfavorable conditions. Spores are referred to as resistant structures because they are resistant to extreme conditions such as high temperatures, extreme pH, nutrient deficiencies, and others. Spores will be stained with malachite green paint so that they appear green, while vegetative cells will be stained with safranin so that they appear red [20] (Figure 2).

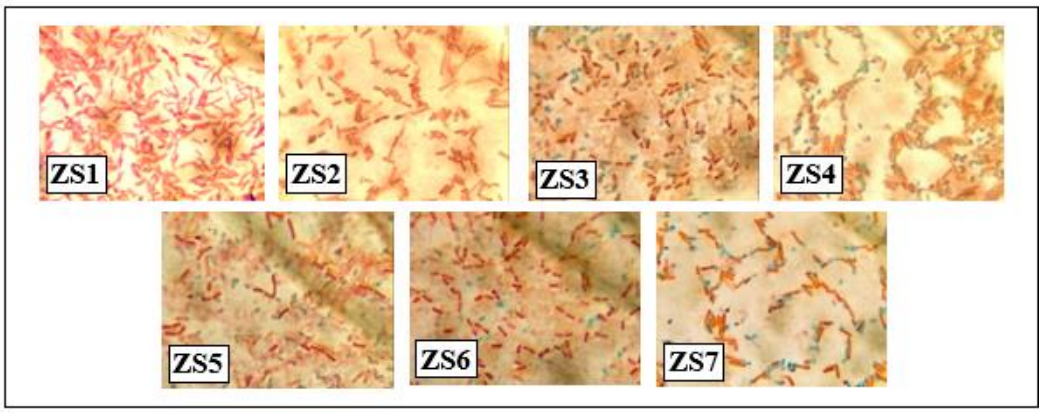


Figure 2 Results of observations of spores of seven thermophilic bacterial isolates at 1000× magnification

3.3. Enzymatic Activity and Enzymatic Index of Thermophilic Bacteria

The results of the enzymatic activity test show that all isolates gave a positive reaction to the catalase enzyme test. The protease enzyme test showed positive results in six thermophilic bacterial isolates (ZS1, ZS2, ZS3, ZS4, ZS5, and ZS6). The amylase enzyme test showed positive results in six thermophilic bacterial isolates (ZS1, ZS2, ZS3, ZS4, ZS6, and ZS7). The cellulase enzyme test results showed positive results in four thermophilic bacterial isolates (ZS1, ZS5, ZS6 and ZS7) (Table 2).

Table 2 Enzymatic activity test results of thermophilic bacterial isolates from surface compost in the pre-decomposition phase

Isolate Code	Enzymatic Activity			
	Catalase	Protease	Amylase	Cellulase
ZS1	+	+	+	+
ZS2	+	+	+	-
ZS3	+	+	+	-
ZS4	+	+	+	-
ZS5	+	+	-	+
ZS6	+	+	+	+
ZS7	+	-	+	+

Description: + = positive (forms a clear zone) ;- = negative (does not form a clear zone)

The highest EI protease value was shown in isolate ZS 4, which was 6.1, and the lowest EI value was shown in isolate ZS5, which was 0.78. In the amylase enzyme test, the highest EI value was shown in isolate ZS2, which was 4.37 and the lowest EI value was shown in isolate ZS3, which was 0.46. In the cellulase enzyme test, the highest EI value was shown in isolate ZS1, which was 6.5 and the lowest EI value was shown in isolate ZS5, which was 1.3 (Table 3).

Tabel 3 Clear zone area, colony area, and enzyme assay EI value of thermophilic bacterial isolates from surface compost in the pre-decomposition phase

Enzyme Test Type	Isolate Code	ATz (cm^2)	ATc (cm^2)	Enzymatic Index	Category
Protease	ZS1	0.76	0.3	1.7	Low
	ZS2	0.5	0.1	4	High
	ZS3	0.33	0.1	2.3	High
	ZS4	0.93	0.13	6.1	Very high
	ZS5	1	0.56	0.78	Low
	ZS6	0.23	0.1	1.3	Low
	ZS7	-	-	-	-
Amylase	ZS1	1.4	0.63	1.2	Low
	ZS2	0.86	0.16	4.37	High
	ZS3	0.63	0.43	0.46	Low
	ZS4	1.93	0.46	3.19	High
	ZS5	-	-	-	-
	ZS6	0.66	0.4	0.65	Low
	ZS7	0.86	0.3	1.86	Low
Cellulase	ZS1	1.13	0.15	6.5	Very high
	ZS2	-	-	-	-
	ZS3	-	-	-	-
	ZS4	-	-	-	-
	ZS5	0.46	0.2	1.3	Low
	ZS6	0.26	0.1	1.6	Low
	ZS7	0.66	0.13	4.07	High

Description:ATz = Average Total Clear Zone Area; ATc = Average Total Colony Area; - = Does not produce clear zone

3.3.1. Catalase Activity

Based on the results of the catalase enzyme test, positive results were obtained in all isolates characterized by the formation of bubbles after the isolate was added with 3% H_2O_2 (Figure 3). Previous research by Jaf et al.[21] found 19 isolates of thermophilic bacteria that had positive results in the catalase test. Bacteria produce the enzyme catalase as protection from the toxic effects of hydrogen peroxide produced at the end of aerobic metabolism by breaking it down into water and oxygen [14]. Catalase activity is an important indicator in composting and the rate of decomposition of organic matter. The catalase enzyme can protect microbes from damage and allows microbes to remain alive and active in the composting process. High catalase activity will accelerate the decomposition rate. This is because the catalase enzyme helps provide more conducive conditions for microbes to degrade organic matter into compost [22].

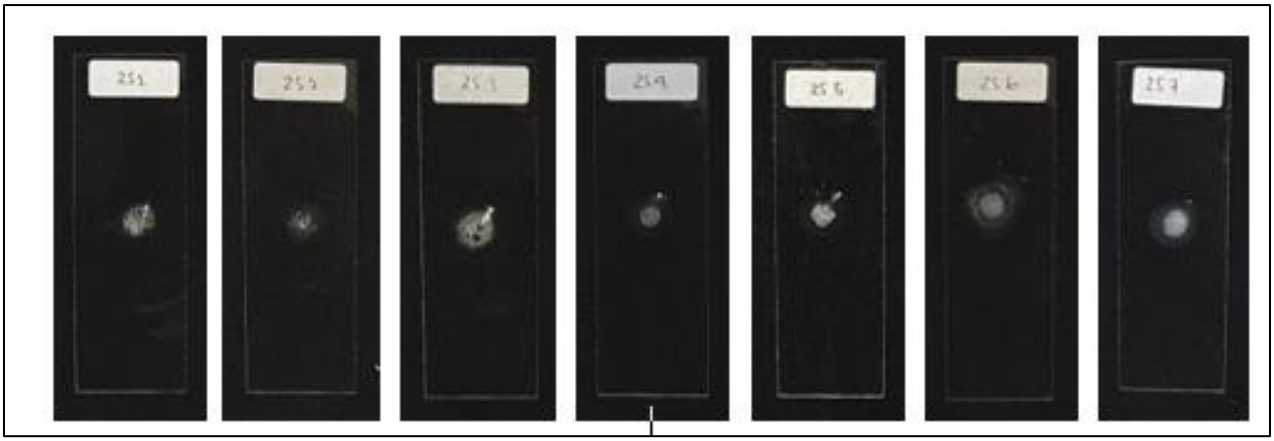
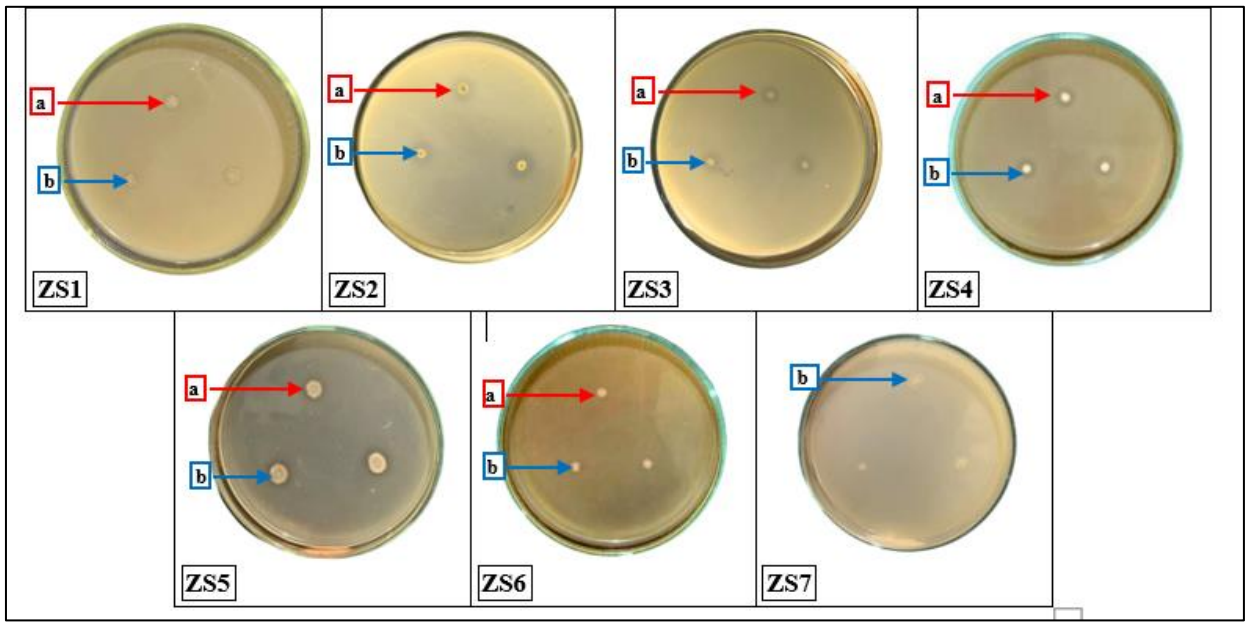


Figure 3 Catalase test results of seven thermophilic bacterial isolates

3.3.2. Protease Activity

Based on the results of the protease enzyme test, six isolates were obtained that were able to form a clear zone, namely ZS1, ZS2, ZS3, ZS4, ZS5, and ZS6 (Figure 4). The formation of clear zones around bacterial colonies on the surface of Skim Milk Agar media is a sign that there is a hydrolysis reaction of peptide bonds in casein contained in skim milk into simpler amino acid monomers by protease enzymes produced by bacterial isolates [23].



Description:a= clear zone; b= colony

Figure 4 Observation results of protease enzyme tests on seven thermophilic bacterial isolates

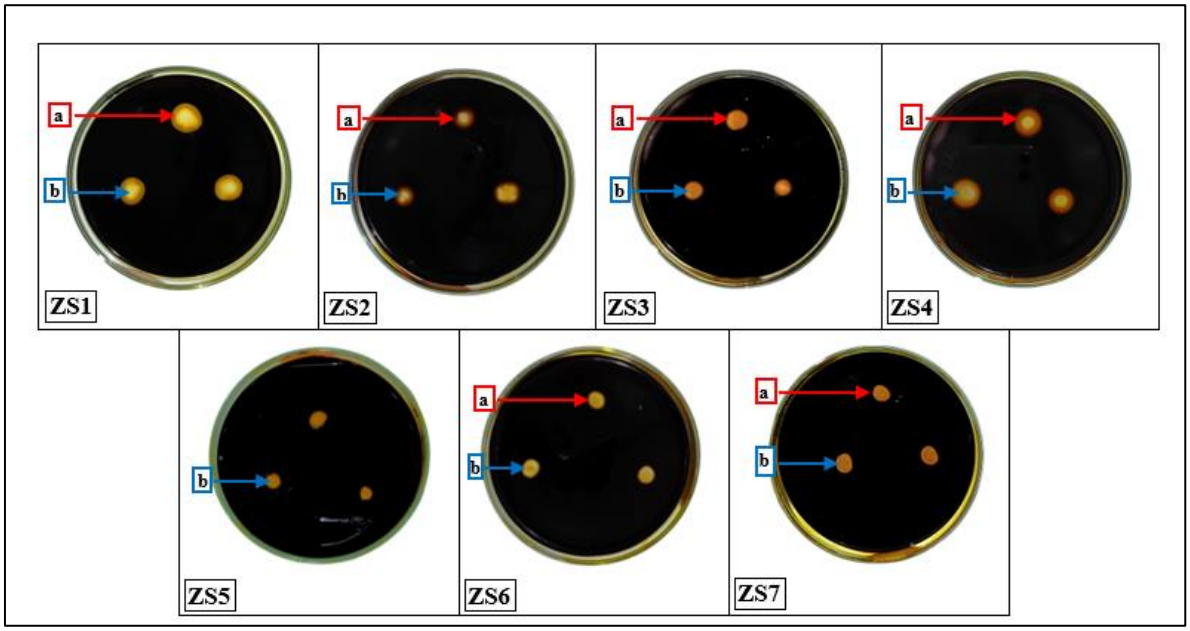
The results showed that the enzyme activity measured based on the highest protease enzymatic index value was shown by isolate ZS4, which amounted to 6.1 (Table 3). These results indicate that isolate ZS4 has a very high potential to produce protease enzymes. This ability indicates that isolate ZS4 has the best adaptation in degrading protein substrates in the pre-decomposition phase of surface compost, and allows that this test condition is optimal for isolate ZS4 to produce protease enzymes. Meanwhile, isolate ZS7 is known not to produce a clear zone. This is likely due to environmental factors not favorable for ZS7 isolates to produce protease enzymes. Optimal conditions for protease activity generally vary between species and even between strains. Factors such as media composition, temperature, pH, the presence of inhibitors, and inducers affect protease enzyme production [24].

Research conducted by Muqarramah et al. [9] reported that 18 of the 20 isolates of thermophilic bacteria that were successfully isolated showed the ability to produce protease enzymes with proteolytic indices ranging from 0.31 to 2.51.

This value is lower than the IE value of isolates ZS1, ZS2, ZS3, ZS4, ZS5, and ZS6 in this study, which is in the range of 0.78 to 6.1.

3.3.3. Amylase Activity

Based on the results of the amylase enzyme test, six isolates were able to form clear zones, namely isolates ZS1, ZS2, ZS3, ZS4, ZS6, and ZS7 (Figure 5). The formation of a clear zone around the bacterial colony indicates that there is a hydrolysis reaction of the starch polymer contained in the media into simpler monomers by the amylase enzyme produced by the bacterial isolate. The clear zone will be visible after the addition of iodine solution. The iodine solution will bind to the starch polymer contained in the media and produce a blue colony color [25].



Description:a= clear zone; b= colony

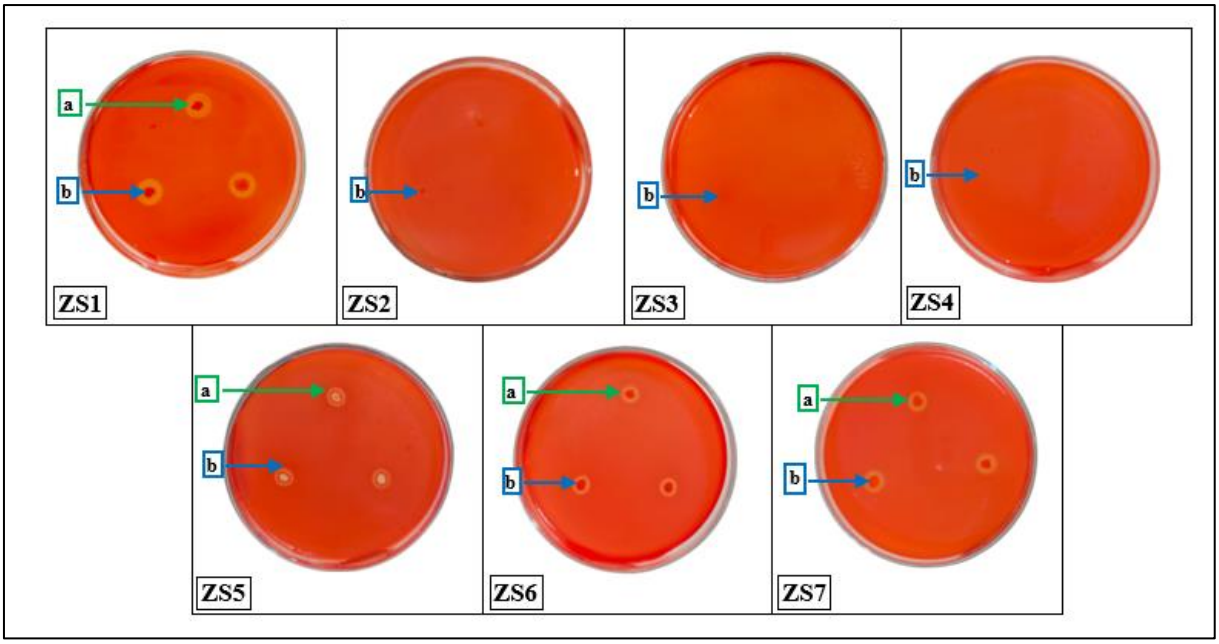
Figure 5 Observation results of amylase enzyme tests on seven thermophilic bacterial isolates

Isolate ZS2 showed the highest EI value of 5.6 (Table 3). This value indicates that isolate ZS2 has the best adaptation in degrading starch substrates in surface compost in the pre-decomposition phase, and allows these test conditions to be optimal conditions for isolate ZS2 to produce amylase. There was one isolate that did not produce a clear zone, namely ZS5. This result indicates that the ZS5 isolate is not able to produce amylase enzyme under these test conditions. Each isolate of thermophilic bacteria has its optimum environmental conditions for producing enzymes. Amylase enzyme activity by bacteria is influenced by incubation time, media pH, and incubation temperature [26].

Research conducted by Satrimafitrah et al. [27] reported that 30 isolates of thermophilic bacteria that were successfully isolated could produce amylase enzymes with IE values ranging from 0.5 to 4.04. This result shows a lower IE value compared to isolates ZS1, ZS2, ZS3, ZS4, ZS6, and ZS7 in this study, which can produce amylase enzymes with IE values ranging from 0.4-5.6.

3.3.4. Cellulase Activity

The results of the cellulase enzyme test showed that four isolates were able to produce clear zones, namely isolates ZS1, ZS5, ZS6, and ZS7 (Figure 6). The formation of a clear zone around the bacterial colony is a sign that there is a hydrolysis reaction of cellulose polymers from the substrate in the form of Carboxymethyl Cellulose (CMC) by the cellulase enzyme produced by the bacterial isolate [28]. The clear zone will be visible after the addition of Congo red solution. Congo red solution will interact strongly with the β-1,4 glycosidic bonds of polysaccharides so that it will color the media red [29]. Hydrolyzed cellulose polymers cannot bind to the Congo red solution, resulting in the formation of a clear zone. The clear zone will be seen more clearly by rinsing with NaCl 1 M, which will dissolve the Congo red that is not strongly bound [30].



Description:a= clear zone; b= colony

Figure 6 Observation results of amylase enzyme tests on seven thermophilic bacterial isolates

The highest EI value was shown by isolate ZS1, which amounted to 6.5 (Table 3). Isolate ZS1 has a large average clear zone area with a small average colony area, resulting in a high EI value. This makes ZS1 a very promising candidate for producing cellulase. Meanwhile, isolates ZS2, ZS3, and ZS4 did not show the formation of clear zones, which indicates the inability of the isolates to produce cellulase enzymes under these test conditions, such as temperature, pH, nutrients, type of substrate and others. Cellulase enzyme activity of thermophilic bacteria is strongly influenced by environmental factors, namely temperature, pH, and type of substrate [31].The results of previous research by Naresh et al. [32] obtained seven isolates of thermophilic bacteria that have the potential to produce cellulase enzymes. Two of them showed optimum activity at 45°C with IE values of 3.21 and 3.40, respectively. The other five isolates were optimum at 55°C with IE values ranging from 2.61-3.42. These values are lower than the IE values of isolates ZS1, ZS5, ZS6, and ZS7 in this study, which ranged from 1.3 to 6.5.

4. Conclusion

Seven isolates of thermophilic bacteria were obtained from surface compost samples in the pre-decomposition phase of PT Great Giant Pineapple Compost Plant with diverse macroscopic and microscopic characteristics. All seven isolates were able to produce catalase enzyme, isolates ZS1, ZS2, ZS3, ZS4, ZS5, and ZS6 were able to produce protease enzyme with the highest enzymatic index ZS4 (6.1), isolates ZS1, ZS2, ZS3, ZS4, ZS6, and ZS7 were able to produce amylase with the highest enzymatic index ZS2 (4.37), and isolates ZS1, ZS5, ZS6, and ZS7 were able to produce cellulase enzyme with the highest enzymatic index ZS1 (6.5).

Compliance with ethical standards

Acknowledgments

The authors would like to thank the Department of Research and Development Laboratory of PT. Great Giant Pineapple, for providing the facilities for this research.

Disclosure of conflict of interest

All authors have no conflict of interest.

References

[1] López MJ, Jurado MM, Lopez-Gonzalez JA, Estrella-Gonzalez MJ, Martinez-Gallardo MR, Toribio A, Suarez-Estrella F. Characterization of thermophilic lignocellulolytic microorganism in composting. Frontiers in Microbiology. 2021;12:1–13.

[2] Biyada S, Merzouki M, Demcenko T, Vasiliauskiene D, Ivanec-Goranina R, Urbonavicius J, et al. Microbial community dynamics in the mesophilic and thermophilic phases of textile waste composting identified through next-generation sequencing. Reports in Scientific Research. 2021;11(1):23624.

[3] Papale M, Romano I, Finore I, Giudice AL, Piccolo A, Cangemi S, et al. Prokaryotic diversity of the composting thermophilic phase: the case of ground coffee compost. Microorganisms. 2021;9(2):218.

[4] Rastogi M, Nandal M, Khosla B. Microbes as vital additives for solid waste composting. Heliyon. 2020;6(2):e03465.

[5] Masi C, Tebiso A, Kumar KVS. Isolation and characterization of potential multiple extracellular enzyme-producing bacteria from waste dumping area in Addis Ababa. Heliyon. 2023;9(2):e13200.

[6] Lai JC, Then YL, Hwang SS, Lee CS. Optimal aeration management strategy for a small-scale food waste composting. Carbon Resources Conversion. 2024;7(1):100190.

[7] Finore I, Feola A, Russo L, Cattaneo A, Donato PD, Nicolaus B, et al. Thermophilic bacteria and their thermozymes in composting process: a review. Chemical and Biological Technologies in Agriculture. 2023;10(7):1–22.

[8] Saidan SA, Jarboui R, Alsharari SS, Azab M. Characterization and identification of thermophilic bacteria isolated from different sites located in Al-Jouf Region, Saudi Arabia. Journal of Pure and Applied Microbiology. 2024;18(1):243–56.

[9] Muqarramah M, Agustien A, Alamsjah F. Isolation, screening and partial characterization of thermophilic bacteria producing protease from BukikGadang hot springs, Solok Regency. International Journal of Progressive Sciences and Technologies. 2023;40(1):101.

[10] Kolwzan B, Adamiak W, Ribak J. Sanitary Biology. Wroclaw: Wroclaw University of Technology; 2011.

[11] Tripathi N, Zubair M, Sapra A. Gram staining. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025.

[12] Dash C, Payyappilli RJ. KOH string and vancomycin susceptibility test as an alternative method to Gram staining. Journal of International and Dentistry. 2016;3(2):88–90.

[13] Bertuso PDC, Marangon CA, Nitschke M. Susceptibility of vegetative cells and endospores of Bacillus cereus to rhamnolipid biosurfactants and their potential application in dairy. Microorganisms. 2022;10(9):1860.

[14] Khatoon H, Anokhe A, Kalia V. Catalase test: biochemical protocol for bacterial identification. AgroCos e-Newsletter. 2022;3(1):53–55.

[15] Sumardi S, Qatrunada V, Ekowati CN, Farisi S, Arifiyanto A. Hydrolase enzyme activity in the screening of actinomycetes isolates as shrimp probiotic candidates. BIOMA: Journal of Biology and Biology Learning. 2021;6(1):24–36.

[16] Rosa E, Ekowati CN, Handayani TT, Ikhsanudin A, Apriliani F, Arifiyanto A. Characterization of entomopathogenic fungi as a natural biological control of American cockroaches (Periplaneta americana). Biodiversitas. 2020;21(11):5276–82.

[17] Guta M, Abebe G, Bacha K, Cools P. Screening and characterization of thermostable enzyme-producing bacteria from selected hot springs of Ethiopia. Microbiology Spectrum. 2024;12(3):1–14.

[18] Ortega-Villar R, Escalante A, Astudillo-Melgar F, Lizárraga-Mendiola L, Vázquez-Rodríguez GA, Hidalgo-Lara ME, et al. Isolation and characterization of thermophilic bacteria from a hot spring in the state of Hidalgo, Mexico, and geochemical analysis of the thermal water. Microorganisms. 2024;12(6):1066.

[19] Siliakus M, van der Oost J, Kengen SWM. Adaptations of archaeal and bacterial membranes to variations in temperature, pH and pressure. Extremophiles: Life Under Extreme Conditions. 2017;21(4):651–70.

[20] Basta M, Annamaraju P. Bacterial Spores. Treasure Island (FL): StatPearls Publishing; 2023.

[21] Jaf WHY, Erez ME, Ertas M. Determination of extracellular hydrolytic enzyme capabilities of some Anoxybacillus isolated from hot spring environments. Frontiers in Life Sciences and Related Technologies. 2022;3(2):56–61.

[22] Amini J, Hossaini H, Hossini H, Pirsaheb M. Effect of calcium oxide on enzymatic activities in co-composting of sewage sludge and municipal solid waste. Journal of Hazardous Materials Advances. 2025; 18:100695.

[23] Shaikh IA, Turakani B, Malpani J, Goudar SV, Mahnashi MH, Al-Serwi RH, et al. Extracellular protease production, optimization, and partial purification from Bacillus nakamurai PL4 and its applications. Journal of King Saud University-Science. 2023;35(1):102429.

[24] Afrin S, Tamanna T, Shahajadi UF, Bhowmik B, Jui AH, Miah MAS, et al. Characterization of protease-producing bacteria from garden soil and antagonistic activity against pathogenic bacteria. The Microbe. 2024; 4:100123.

[25] Elamary R, Salem WM. Optimizing and purifying extracellular amylase from soil bacteria to inhibit clinical biofilm-forming bacteria. PeerJ. 2020;8: e9562.

[26] Bouaita S, Sayad Z, Ziani D, Bouguerba R, Gomri MA. Study of the amylolytic activity of thermophilic bacteria isolated from an Algerian hot spring (Azzaba, Skikda). Biology and Life Sciences Forum. 2024;1(36):8.

[27] Satrimafitrah P, Razak AR, Hardi J, Puspitasari DJ, Yelenggete I. Thermostable amylase activity produced by thermophilic bacteria isolated from Pulu Hotspring, Central Sulawesi. Journal of Physics: Conference Series. 2020; 1434:012034.

[28] Bahatkar BP, Gahukar SJ, Akhare AA, Rathod DR, Charpe AM, Ingle YV. Isolation, screening, and identification of cellulose-degrading bacteria from different types of samples. The Pharma Innovation. 2022;11(12):2500–7.

[29] Castrillo ML, Bich GÁ, Amerio NS, Barengo MP, Rodriguez MD, Zapata PD, et al. A combination of solid Mandels medium, CMC, and Congo red technique for rapid, sensitive and reproducible screening of cellulase-producing fungi. Biogeneric Science and Research. 2020;4(1):1–6.

[30] Abdella MAA, Ibrahim GE. Application of statistical designs strategy to improve cellulase production using agro-waste residue by a novel isolate Bacillus licheniformis strain-MA1 and assessing the enzyme effect on apple juice quality. BMC Microbiology. 2024;24(1):511.

[31] Shyaula M, Regmi S, Khadka D, Poudel RC, Dhakal A, Koirala D, et al. Characterization of thermostable cellulase from Bacillus licheniformis PANG L isolated from the Himalayan soil. International Journal of Microbiology. 2023;2023:3615757.

[32] Naresh S, Kunasundari B, Gunny AAN, Teoh YP, Shuit SH, Ng QH, et al. Isolation and partial characterisation of thermophilic cellulolytic bacteria from North Malaysian tropical mangrove soil. Tropical Life Sciences Research. 2019;30(1):123–47.