BIODIVERSITAS
Volume 25, Number 1, January 2024
Pages: 392-403

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250146

Biodiversity of hydrolytic enzymes-producing soil bacteria from a Durian Park, Jombang, Indonesia: Beneficial prospect for sustainable agriculture

NI'MATUZAHROH[1,2,3,♥], MOCH. AFFANDI[1,2], AGUS SUPRIYANTO[1,2], BERLIAN RUSTANTINA[1], LAILY AINUN JAIYAH[1], AISYAH RAHMAWATI[1], HESTI NURHAYATI[1], SILVIA KURNIA SARI[1], ANA MARIATUL KHIFTIYAH[1], DAMAN HURI[4]

[1]Department of Biology, Faculty of Science and Technology, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Unair, Mulyorejo, Surabaya 60115, East Java, Indonesia. Tel./fax: +62-315-936501, ♥email: nimatuzahroh@fst.unair.ac.id

[2]University Center of Excellence - Research Center for Bio-Molecule Engineering, Universitas Airlangga. Jl. Mulyorejo Surabaya, Surabaya 60115, East Java, Indonesia

[3]Faculty of Advanced Technology and Multidiscipline, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Unair, Mulyorejo, Surabaya 60115, East Java, Indonesia

[4]De Durian Park. Jl. Cemorosewu, Segunung, Wonosalam, Jombang 61476, East Java, Indonesia

Manuscript received: 3 August 2023. Revision accepted: 31 January 2024.

Abstract. *Ni'matuzahroh, Affandi M, Supriyanto A, Rustantina B, Jaiyah LA, Rahmawati A, Nurhayati H, Sari SK, Khiftiyah AM, Huri D. 2024. Biodiversity of hydrolytic enzymes-producing soil bacteria from a Durian Park, Jombang, Indonesia: Beneficial prospect for sustainable agriculture. Biodiversitas 25: 392-403.* Biofertilizer is an alternative to chemical fertilizer that can enhance plant growth but is specific to certain plants and soils. Various biofertilizers have been produced, but the specific biofertilizers to enhance durian growth have not been widely available. This study aims to determine the potential of hydrolytic enzyme-producing bacteria, one of the microbial components of biofertilizers, in the De Durian Park (DDP) area, Wonosalam, Jombang District, Indonesia. The bacteria were isolated using the selective medium plating technique. The obtained isolates were screened for their ability to produce cellulase, amylase, protease, and lipase. The clear zone around the colony indicated the ability of the isolates to produce hydrolytic enzymes, and then the clear zone index was obtained. The best three isolates for each enzyme production were characterized macroscopically, microscopically, and biochemically, then identified using 16S rRNA gene sequence analysis. Fifty-two isolates were successfully obtained from the soil of DDP. The best three cellulolytic bacteria had indices ranging from 1.83 to 2.14, categorized as moderate-strong, identified as *Bacillus anthracis*, *B. pacificus*, and *B. cereus*. The amylolytic bacteria ranged from 1.25 to 1.35, categorized as moderate, and identified as *Bacillus anthracis*, *B. paranthracis*, and *B. paramycoides*. The proteolytic bacteria ranged from 1.33 to 1.50, categorized as moderate, identified as *B. pumilus*, *B. paramycoides*, and *B. cereus*. The lipolytic bacteria ranged from 1.94 to 3.87, categorized as moderate-strong, and were identified as *Enterobacter sichuanensis*, *B. pacificus*, and *E. asburiae*. These cellulolytic, amylolytic, proteolytic, and lipolytic bacteria have the potency as candidates for a biofertilizer formula.

Keywords: Bacteria, biodiversity, De Durian Park Wonosalam Jombang, hydrolytic enzyme, soil fertility

INTRODUCTION

Wonosalam, Jombang, is the largest durian (*Durio zibethinus* Murr.) producing area in East Java (Chusnah et al. 2020). Situated on the slopes of Mount Anjasmoro (Wenny 2020), this region is home to the De Durian Park (DDP), a Small and Medium Enterprise (SME) managing durian plantations to make Wonosalam a national durian center. The DDP focuses on planting and intensively developing various local durian varieties, making it crucial to pay attention to the growth of durian in the area so that durian plants can grow optimally.

While chemical fertilizers have traditionally been used to enhance plant growth, their excessive usage can harm the environment (Mondal et al. 2017). Studies indicate that only 30-50% of chemical fertilizers are absorbed by plants, leading to nutrient loss (Wang et al. 2018; Acharya et al. 2021). To ensure the sustainability of durian productivity in DDP, efforts to reduce reliance on chemical fertilizers are essential.

As an alternative, the use of biofertilizers has been encouraged, in line with the Regulation of the Indonesian Minister of Agriculture No. 01 of 2019, which defines biofertilizers as microbes that directly or indirectly provide nutrients to plants, break down organic matter, and improve fertilizer efficiency, fertility, and soil health. Although various biofertilizers have been produced, those specifically formulated for durian are not widely available.

The effectiveness of biofertilizers depends on several factors, such as microbial viability under field conditions, plant genotypes, interactions with plant-host microbiomes, and environmental conditions (Pirttillä et al. 2021). Biofertilizers are specific for certain plants and certain soils, so this is a limitation of biofertilizers (Mahmud et al. 2021). Therefore, to hinder the limitations of these biofertilizers, exploration of the diversity of microbes that have the potential to be used as biofertilizers on

agricultural land is encouraged with the aim of developing fertilizers consisting of indigenous microbes to support plant growth. Research conducted by Verma and Chowdhury (2023) showed that N-fixing microbes isolated from sugarcane set samples in Chhattisgarh, India, were proven to be able to increase the growth of sugarcane plants when applied to sugarcane fields in Chattisgarh, India. The research indicates that the development of a biofertilizer for durians utilizing indigenous bacteria from durian plantations can be carried out to obtain an effective biofertilizer for durians.

Developing a biofertilizer for durian started by exploring the diversity of bacteria in durian plantations that can increase plant growth. One of the potential soil bacteria important for plant fertility, which is also a component of the biofertilizer, is organic matter-degrading bacteria. Soil microbes exhibit high diversity and play crucial roles in the soil nutrient cycle (Tang et al. 2020 a), particularly organic matter-degrading bacteria (Malik et al. 2020). The durian plantation soils harbor potential organic matter-degrading bacteria candidates for durian biofertilizer formulas. Organic matter-degrading bacteria indirectly help provide a source of carbon and nutrients for the survival of other heterotrophic bacteria, such as nitrogen-fixing bacteria and phosphate-solubilizing bacteria.

Bacteria degrade organic matter by producing extracellular enzymes (Gómez et al. 2020), including hydrolytic enzymes (Krishna and Mohan 2017). Previous studies have demonstrated the diversity of hydrolytic enzyme-producing bacteria isolated from various soils, including agricultural and forest soils. Cellulose-producing bacteria were isolated from agricultural soil (Shamshitov et al. 2023), while amylase and protease-producing bacteria were found in the soil of the Nasinuan community forest (Luang-In et al. 2019a, b). Additionally, lipolytic bacteria were identified in coffee plantations (Ervina et al. 2020) and in the Liwa botanical garden (Royanti et al. 2023). Despite these findings, there remains a lack of reports on the diversity of hydrolytic enzyme-producing bacteria in the soil of durian plantations, especially in Wonosalam, Jombang, Indonesia. The current reports on durian plantation soil bacteria from Indonesia have highlighted the ability of bacteria to produce phytohormones, dissolve phosphates, and inhibit pathogen growth (Fitri 2018).

Durian plantation soil is rich in organic matter due to durian tree residues or parts of the plants that fall into the ground. Since plant cell walls are made of cellulose, hemicellulose, mannan, a glycoprotein in small amounts, phenolic acid, mineral, and lignin, while seeds were packed with macronutrients such as starch, protein, and lipid (Masturi et al. 2020; Holland et al. 2020), the degradation of those organic matter must occur there and be assisted by microbes. The soil of DDP would be a good source of organic matter degrading bacteria to develop biofertilizers specific for durian. The bacterial isolation was performed to obtain bacteria capable of breaking down complex compounds, namely cellulose, starch, protein, and lipid, in the soil into simpler compounds beneficial for plant growth. Bacteria exhibiting such abilities are known for their hydrolytic activities; therefore, this research focused on isolating hydrolytic enzyme-producing bacteria. This research will not only focus on the diversity of hydrolytic enzyme-producing bacteria in durian plantation soil but also highlight the potential of these bacteria as biofertilizer candidates for durian. Utilizing biofertilizers is vital to achieve sustainable agricultural practices in durian plantations.

MATERIALS AND METHODS

Procedures

Collection of soil samples

Soil samples were collected from DDP, a durian plantation in Wonosalam, Jombang, East Java, Indonesia. Soil samples were collected using a purposive method from three different locations: the first location (112°38'981" S - 7.7°08'55" E), the second location (112°38'975" S - 7.7°10'27" E), and the third location (112°38'941" S - 7.7°10'25" E). At each location, three soil sampling points were designated. Next, 500 g of soil were collected for each point, and the three samples were combined to create a composite sample; a soil drill collected samples from 0-20 cm (Saputri et al. 2021). The collected samples were placed in an ice box to maintain the soil temperature during the transport from the DDP to the laboratory. While waiting for the bacterial isolation process, the collected soil samples are stored in the refrigerator for less than 24 hours at 2°C to maintain stable soil conditions.

Isolation of hydrolytic enzyme-producing bacteria

The soil samples were suspended in sterile distilled water (1:9 w/v) and diluted up to 10^{-8}. From the three highest dilutions, 1 mL of the suspension was inoculated into a Petri dish containing enriched Bushnell-Haas (BH) agar medium. The composition of the Bushnell-Haas agar (BHA) medium was as follows (g/L): K_2HPO_4 (1), KH_2PO_4 (1), NH_4NO_3 (1), $MgSO_4.7H_2O$ (0.2), $FeCl_3.6H_2O$ (0.05), and $CaCl_2$ (0.02) (Nazar et al. 2018). The medium was further enriched with 1% carboxymethyl cellulose (CMC), 1% starch, and 2% skimmed milk for isolating cellulase, amylase, and protease-producing bacteria, respectively. Additionally, the medium was enriched with 1.5 mL of olive oil, 1% rhodamine B, and tween 20 for isolating lipase-producing bacteria. The Petri dishes were then incubated for 24 hours at 37°C; the bacteria obtained from the incubation were further purified for the hydrolytic activity screening stage.

Screening of the isolates' ability to produce cellulase, amylase, protease, and lipase:

Moreover, to determine the ability of each isolate to produce cellulase, amylase, protease, and lipase, each isolate was tested for enzymatic activity by inoculating each bacterium on a BHA medium enriched with the same composition used in the isolation process. The Petri dishes were incubated at 30-33°C for 24 hours. The hydrolytic activities were determined by the presence of a clear zone around the bacterial colonies, which could be observed after dripping with Congo red for cellulolytic activity and

iodine for amylolytic activity. The clear zone was observed immediately without adding any reagent for proteolytic activity, and the lipolytic activity was detected by observing a clear halo under UV light. The hydrolytic index was obtained by measuring bacterial colonies' and clear zones' diameters. Measurements were made using a vernier caliper to ensure the accuracy of the numbers obtained. The hydrolytic index represents the potency of the tested bacteria to produce extracellular hydrolytic enzymes. It is expressed as the ratio of the clear zone's diameter to the colony's diameter (Choi et al. 2005). The top three bacteria demonstrating the highest cellulolytic, amylolytic, proteolytic, and lipolytic activities were potential.

Characterization of potential bacteria

The potential bacteria were subjected to macroscopic, microscopic, and biochemical characterization. The macroscopic characteristics included the shape, color, elevation, and optical properties of the bacterial colony grown on a Nutrient Agar (NA) medium. The microscopic characteristics observed encompassed the bacteria shapes, the Gram staining reaction, and the presence of spores in bacterial cells. Biochemical characterization was performed using the Microbact identification kit GNB 12A (Oxoid, United Kingdom).

Identification of potential bacteria

All potential isolates were subjected to identification through 16S rRNA gene sequence analysis. The total DNA of the bacterial isolates was extracted using the DNA Presto™ Mini gDNA Bacteria Kit (Geneaid, Taiwan). The 16S rRNA gene was amplified through the polymerase chain reaction (PCR) method using GoTaq® Green Master Mix (Promega, USA) with the universal primers 27F ('5-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (De Lillo et al. 2006). The PCR reaction was performed in a 50 µL reaction volume, comprising 25 µL of PCR mix, 2 µL of each primer (Forward and Reverse), 5 µL of the sample, and the volume completed to 16 µL using nuclease-free water. The thermocycling conditions were as follows: initial denaturation at 94°C for 3 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds; annealing at 55°C for 30 seconds; extension at 72°C for 39 seconds, and a final extension cycle at 72°C for 10 minutes, followed by holding the program at 4°C. The PCR products were separated and visualized on a 2% agarose gel using a UV transilluminator (Major Science, Taiwan), and a camera captured the gel image.

The DNA sequencing was analyzed by Macrogen Inc. using their ABI 3730xl genetic analyzer (Applied Biosystems, USA). The obtained nucleotide sequences of each isolate were checked for quality and trimmed using the Codon Code Aligner software (Codon Code Corporation). Nucleotide sequences were compared with the database from the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST) program (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The program displayed several bacterial isolates with sequence similarities to the three selected isolates, which helped determine their phylogenetic relationships. Only sequences showing the highest identity and maximum coverage were selected, and the obtained and selected sequences were aligned using Clustal X2 software. A phylogenetic tree was constructed using MEGA 6 software, employing the Neighborhood-joining method based on the Poisson model with Nearest Neighbor Interchange and a Bootstrap test of phylogeny. The bootstrap was set to test 1000 replicates to enhance the reliability of the phylogeny.

RESULTS AND DISCUSSION

Isolation of hydrolytic enzyme-producing bacteria

The isolation process involved using selective media containing complex compounds such as cellulose, starch, proteins, and lipids as carbon sources to be degraded by bacteria. Moreover, 52 bacteria were successfully isolated from the soil sampling points across all locations (Table 1), comprised of 16 isolates of cellulolytic bacteria (SL), 13 isolates of amylolytic bacteria (AL), 9 isolates of proteolytic bacteria (PL), and 14 isolates of lipolytic bacteria (LL). The successful isolation of cellulolytic, amylolytic, proteolytic, and lipolytic bacteria from each sampling location indicated the presence of diverse soil bacteria in the De Durian Park (DDP) area.

Screening of the isolates' ability to produce cellulase, amylase, protease, and lipase

All isolates were quantitatively tested for their hydrolytic activity (cellulolytic, amylolytic, proteolytic, and lipolytic) by comparing their hydrolytic indices (Table 2). The isolates were selected based on their ability to degrade the organic compounds in each selective medium. This ability was demonstrated by a clear zone around the bacterial colony (Figure 1).

Table 1. Hydrolytic enzyme-producing bacteria isolated from soil in De Durian Park Wonosalam, Jombang, Indonesia

Location	Isolate code	Amount
1st location	SL1.1, SL1.2, SL1.3, SL1.4, SL1.6, SL1.7, SL1.8, AL1.1, AL1.2, AL1.5, PL1.1, PL1.2, PL1.3, LL1.1, LL1.2, LL1.4, LL1.5.	17 isolates
2nd location	SL2.3, SL2.4, SL2.5, SL2.6, SL2.7, AL2.1, AL2.2, AL2.3, AL2.4, PL2.1, PL2.2, PL2.3, PL2.4, PL2.5, LL2.1, LL2.2, LL2.3, LL2.4.	18 isolates
3rd location	SL3.2, SL3.3, SL3.4, SL3.5, AL3.1, AL3.3, AL3.7, AL3.9, AL3.10, AL3.11, PL3.1, LL3.2, LL3.3, LL3.4, LL3.5, LL3.6, LL3.7.	17 isolates

Table 2. The index value of hydrolytic enzyme-producing bacteria

Isolate code	Hydrolytic Index	Isolate code	Hydrolytic Index	Isolate code	Hydrolytic Index	Isolate code	Hydrolytic Index
SL1.1	1.17±0.19	SL3.3	1.16±0.02	AL3.9	1.01±0.00	LL1.2	0.67±0.48
SL1.2	1.83±0.67	SL3.4	1.21±0.27	AL3.10	1.08±0.05	LL1.4	0.99±0.03
SL1.3	1.18±0.01	SL3.5	1.03±0.01	AL3.11	1.32±0.07	LL1.5	1.25±0.04
SL1.4	1.17±0.20	AL1.1	1.07±0.05	PL1.1	1.49±0.04	LL2.1	1.45±0.37
SL1.6	1.38±0.02	AL1.2	1.26±0.06	PL1.2	1.32±0.03	LL2.2	0.59±0.35
SL1.7	1.96±0.08	AL1.5	1.26±0.01	PL1.3	1.01±0.22	LL2.3	3.87±0.31
SL1.8	1.40±0.20	AL2.1	1.16±0.13	PL2.1	1.10±0.64	LL2.4	2.18±1.01
SL2.3	1.41±0.33	AL2.2	1.15±0.09	PL2.2	1.05±0.19	LL3.2	0.43±0.34
SL2.4	1.23±0.05	AL2.3	1.35±0.11	PL2.3	1.03±0.34	LL3.3	0.74±0.40
SL2.5	1.04±0.01	AL2.4	1.10±0.00	PL2.4	1.33±0.31	LL3.4	0.71±0.36
SL2.6	1.01±0.00	AL3.1	1.20±0.03	PL2.5	1.48±0.48	LL3.5	1.94±0.42
SL2.7	2.14±0.33	AL3.3	1.16±0.25	PL3.1	1.50±0.19	LL3.6	0.55±0.41
SL3.2	1.02±0.00	AL3.7	1.25±0.00	LL1.1	1.04±0.00	LL3.7	0.58±0.35

Note: SL: Cellulolytic bacteria; AL: Amylolytic bacteria; PL: Proteolytic bacteria; LL: Lipolytic bacteria

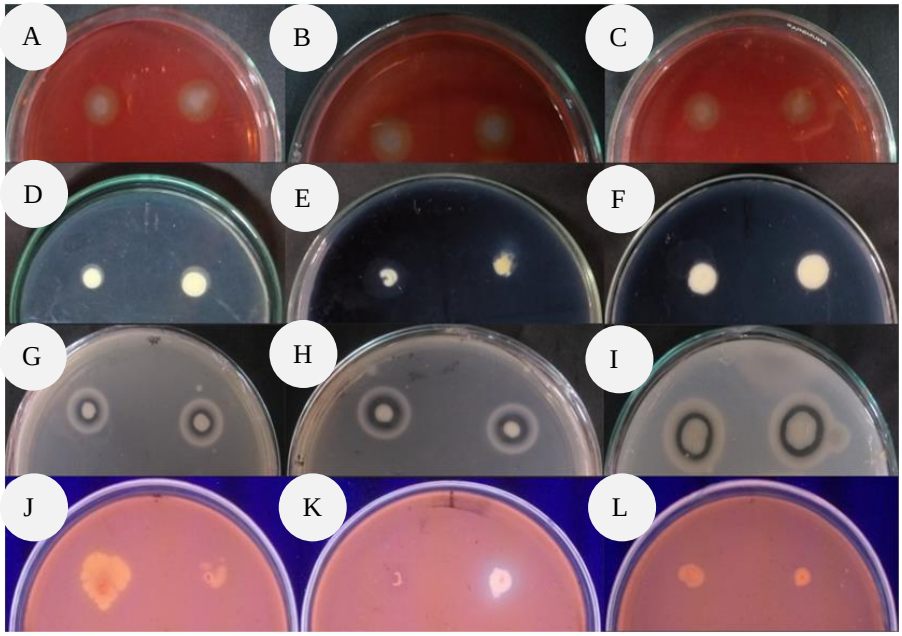


Figure 1. The clear zones around colonies of selected bacteria from the soil at De Durian Park, Jombang, on various selective media, show the isolates' ability to produce hydrolytic enzymes; The cellulolytic bacteria: (a) SL1.7, (b) SL1.2, (c) SL2.7; The amylolytic bacteria: (d) AL1.2, (e) AL2.3, (f) AL3.11; The proteolytic bacteria: (g) PL2.4, (h) PL2.5, (i) PL3.1; The lipolytic bacteria: (j) LL3.5, (k) LL2.3, and (l) LL2.4

A medium containing 1% carboxymethyl cellulose (CMC) was used for the cellulase activity test; the clear zone formed in the CMC medium indicated that the isolate could degrade the cellulose present. Three of the 16 isolates showed the highest indices, indicating significant hydrolytic cellulase enzyme production, namely SL1.2, SL1.7, and SL2.7. The amylolytic enzyme activity test was conducted using a medium containing 1% starch. The clear zone formed in the starch medium indicated that the isolate could degrade the starch present in the medium. The three bacterial isolates that exhibited the highest indices of amylolytic activities were AL1.2, AL2.3, and AL3.11. A medium containing 1% skimmed milk was used for the proteolytic activity test. The clear zone formed in the skimmed milk medium indicated that the isolate could degrade the protein in the medium. Among the nine isolates tested, PL2.5, P.L1.1 and PL3.1 exhibited the highest indices for producing hydrolytic protease enzymes. The clear zone formed in the lipolytic medium indicated that the isolate could degrade the lipids present in the medium. Among the 14 isolates, LL2.3, LL2.4, and LL3.5 demonstrated the highest indices for producing hydrolytic lipase enzymes. Three isolates that show the highest hydrolytic index of cellulase, amylase, protease and lipase enzymes will be used for the next stage. However, because isolate PL1.1 does not grow easily, this isolate will be replaced by isolate PL2.4 which has a higher index than the other remaining isolates.

This research classified the categories of extracellular enzymatic reactions into four types based on their index

value, following the criteria proposed by Choi et al. (2005). They are categorized as follows: Strong reaction (if the index is ≥2), Moderate reaction (if the index is approximately 2-1), Weak reaction (if the index is ≤1), and No reaction (if there is no reaction at all). Three isolates, namely SL2.7, LL2.3, and LL2.4, demonstrated strong categories in producing hydrolytic enzymes, while 41 isolates exhibited moderate categories, and 8 isolates showed weak categories.

Characterization of potential bacteria

The twelve selected bacteria that exhibited potential in producing hydrolytic enzymes were subsequently characterized macroscopically and microscopically to determine the characteristics of each isolate. The macroscopic and microscopic characters of the selected bacterial isolates are presented in Table 3.

The bacterial isolates displayed variations in the macroscopic characteristics of the colony. The majority of isolates exhibited circular colonies with white color, flat elevation, and opaque optical properties (Figure 2). The results of the macroscopic characterization indicate a diverse bacterial population colonizing the soil in the durian plantation at the De Durian Park (DDP) area.

Table 3. Characteristics of bacterial isolates from the soil of De Durian Park, Wonosalam Jombang, Indonesia

Isolate code	Macroscopic Characteristic				Microscopic Characteristic		
	Shape	Color	Elevation	Optical	Gram	Spore	Shape
SL1.2	Irregular	White	Flat	Opaque	+ (Purple)	Yes	Rod
SL1.7	Circular	Cream	Flat	Opaque	+ (Purple)	Yes	Rod
SL2.7	Circular	White	Flat	Opaque	+ (Purple)	Yes	Rod
AL1.2	Circular	White	Flat	Opaque	+ (Purple)	Yes	Rod
AL2.3	Circular	Light Yellow	Flat	Opaque	+ (Purple)	Yes	Rod
AL3.11	Circular	Yellow	Flat	Opaque	+ (Purple)	Yes	Rod
PL2.4	Circular	White	Raised	Opaque	+ (Purple)	Yes	Rod
PL2.5	Circular	White	Raised	Opaque	+ (Purple)	Yes	Rod
PL3.1	Irregular	White	Raised	Opaque	+ (Purple)	Yes	Rod
LL2.3	Circular	Cream	Flat	Opaque	- (Red)	No.	Rod
LL2.4	Irregular	Cream	Convex	Opaque	+ (Purple)	Yes	Rod
LL3.5	Circular	Cream	Flat	Opaque	- (Red)	No	Rod

Note: SL: Cellulolytic bacteria; AL: Amylolytic bacteria; PL: Proteolytic bacteria; and LL: Lipolytic bacteria

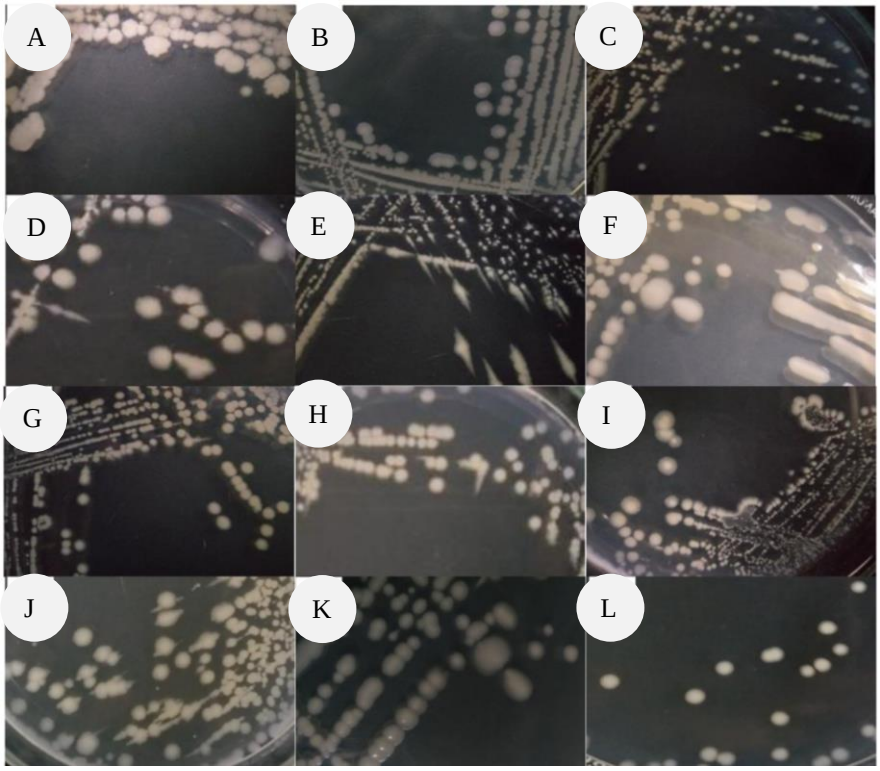


Figure 2. Macroscopic characteristics of hydrolytic enzyme-producing soil bacteria from De Durian Park, Jombang; The cellulolytic bacterial colonies: (a) SL1.2, (b) SL1.7, (c) SL2.7; The amylolytic bacterial colonies: (d) AL2.3, (e) AL3.11, (f) AL1.2; The proteolytic bacterial colonies: (g) PL2.4, (h) PL2.5, (i) PL3.1; The lipolytic bacterial colonies: (j) LL3.5, (k) LL2.4, and (l) LL2.3

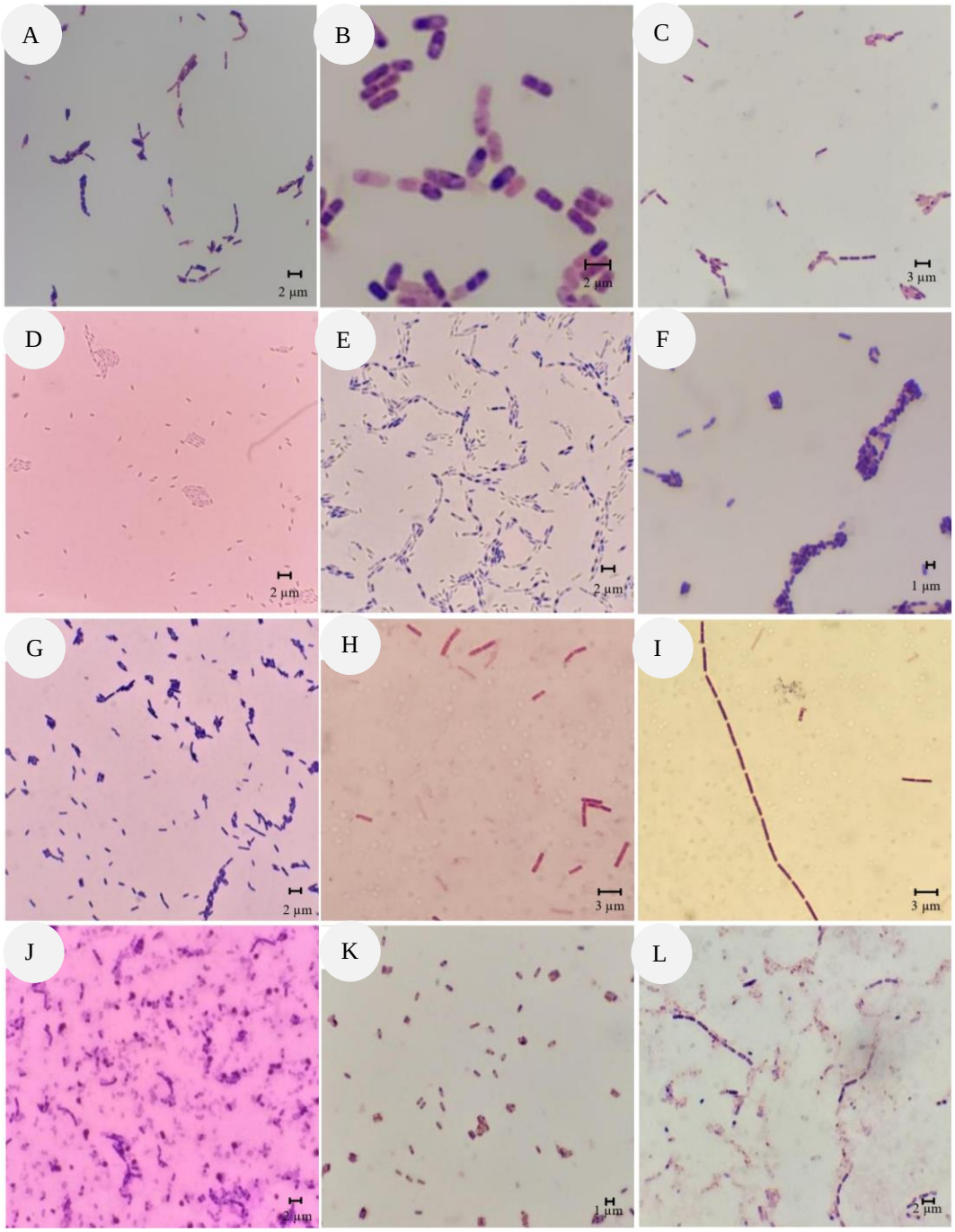


Figure 3. Microscopic characteristics of hydrolytic enzyme-producing soil bacteria from De Durian Park, Jombang; The cellulolytic bacterial colonies: (a) SL1.7, (b) SL1.2, (c) SL2.7; The amylolytic bacterial colonies: (d) AL2.3, (e) AL3.11, (f) AL1.2; The proteolytic bacterial colonies: (g) PL2.4, (h) PL2.5, (i) PL3.1; The lipolytic bacterial colonies: (j) LL2.4, (k) LL3.5, and (l) LL2.3

All the selected bacteria exhibited a diversity of colony morphology, a characteristic often used in bacterial isolation. Colony morphology aids researchers in differentiating bacteria as each bacterium displays unique characteristics in morphology when grown in specific media. In addition to macroscopic characterization, microscopic characterization was also conducted. The 12 bacterial isolates obtained in this study showed varying Gram staining and endospore characteristics (Figure 3). Microscopic characteristics of bacterial cells can be used to identify their genus. In Bergey's Manual and Determinative of Bacteriology, cell shape, Gram staining, and the presence of endospores are essential characteristics to determine as they are unique to certain groups of bacteria. The diversity of bacterial isolates obtained in this study indicates the presence of diverse soil bacteria in durian plantations at the De Durian Park (DDP). Almost all selected bacterial isolates were endospore-forming Gram-positive rods, demonstrating their survival ability in extreme environments (Chukwuma et al. 2023).

Table 4. Biochemical characteristics of enzyme-producing bacteria isolated from soil in De Durian Park, Wonosalam, Jombang, Indonesia

Test	Result											
	SL1.2	**SL1.7**	**SL2.7**	**AL3.11**	**AL1.2**	**AL2.3**	**PL2.5**	**PL2.4**	**PL3.1**	**LL2.4**	**LL2.3**	**LL3.5**
Motility test	-	+	+	+	-	+	-	+	-	+	+	+
Aerobic growth	+	+	+	+	+	+	+	+	+	+	+	+
Urease	-	-	-	+	-	-	-	-	-	+	-	+
Lysine	-	-	-	-	-	-	-	-	-	+	+	+
VP	+	+	+	-	+	+	+	+	+	+	+	+
Citrate utilization	-	-	-	-	-	-	-	-	-	+	-	+
ONPG	+	-	-	+	-	-	-	+	-	-	+	+
Indole	-	-	-	-	-	-	-	-	-	-	-	-
Oxidase production	+	+	+	+	+	+	+	+	+	+	+	+
Glucose	-	-	-	-	-	-	-	-	-	-	-	+
Mannitol	-	-	-	-	-	-	-	-	-	-	-	+
Ornithine	-	+	+	-	-	-	+	-	-	+	+	+
Xylose	-	-	-	-	-	-	-	-	-	-	-	+
TDA	-	-	-	-	-	-	-	-	-	-	-	-

Note: +: present; -: absent

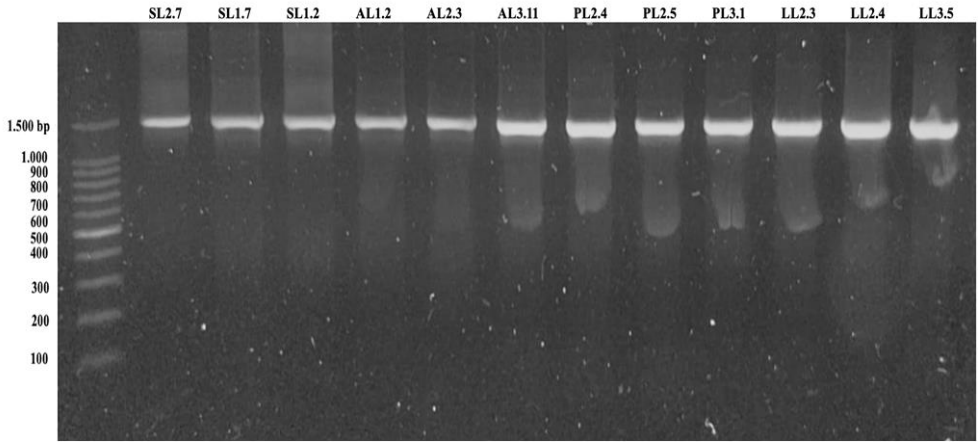


Figure 4. Electrophoregram of 16S rRNA gene in 0.8% agarose gel. M= 1kb DNA Ladder (bp); 16S rRNA gene of the PL2.3, PL2.5, PL3.1, SL2.7, SL1.7, SL1.2, LL2.3, LL2.4, LL3.5, AL1.2, AL2.3, AL3.11 isolates (1500 bp)

Besides conducting macroscopic and microscopic characterization, biochemical tests were performed on the 12 isolates; the results of the biochemical tests are presented in Table 4. All the selected bacterial isolates displayed various biochemical characteristics. Most of the isolated bacteria were motile and showed aerobic growth. According to Istiqomah et al. (2017), soil depths up to 20 cm generally have aerobic soil conditions; this depth can also enhance soil microorganism activity and increase soil nutrient availability (Chang et al. 2016).

Identification of potential bacteria

In this study, the 12 selected bacteria were identified. This stage was important to know the diversity of species present in the durian soil plantations and track the potential of these bacterial species concerning the degradation of organic compounds. The quality of the DNA used in this stage was conducted using electrophoresis, the results of which are shown in Figure 5. The agarose gel electrophoregram in Figure 4 showed a band glaring at about 1500 bp, indicating that the 16S rRNA genes of the all-selected bacteria had been successfully amplified.

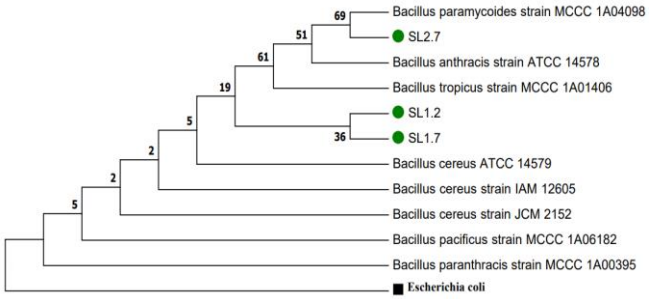
The amplified DNA was then sequenced to determine the sequence of nucleotide bases (Lokapirnasari et al. 2017). The quality-checked and trimmed nucleotide base sequences were then used for Basic Local Alignment Search Tool (BLAST) analysis. The BLAST results revealed the homology between the 16S rRNA gene sequences of the selected bacteria and the reference bacteria in the NCBI database. Based on the 16S rRNA sequences, the selected bacteria exhibited similarities with various strains of bacteria, with the majority belonging to the *Bacillus* genera. The identification results are presented in Table 5.

Moreover, most of the isolates were identified as members of the *Bacillus* genera, and some displayed the potential to produce the same hydrolytic enzyme, these isolates may share interesting kinship among strains. Furthermore, a phylogenetic analysis of the three isolates was conducted to determine their kinship among species

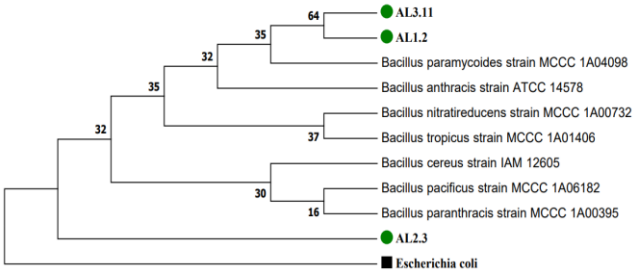
based on the 16S rRNA gene sequence. Figure 5 illustrates the phylogenetic tree, including all selected bacterial isolates and bacterial isolates that exhibited high percent similarity as indicated by the BLAST results. The position of bacterial isolates in the phylogenetic tree based on 16S rRNA gene sequence analysis are shown in Figure 5.

The phylogenetic tree in Figure 5 was constructed using the Neighborhood-joining method with 1000 repetitions to test the model's data set in the phylogenetic tree (Dharmayanti 2011). The bootstrap values, indicated as numbers on the branches of the phylogenetic tree, represent the reliability of the model data set (Wiesemüller and Rothe 2006); the higher bootstrap values indicate a more robust data set for analysis. The genetic distance presented in the phylogenetic tree provides valuable information about the classification of populations based on their evolutionary process (Dharmayanti 2011).

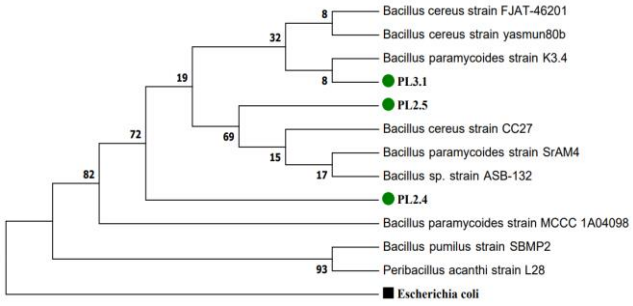
(a) Isolates SL 1.2, SL 1.7, and SL 2.7



(b) Isolates AL1.2, AL2.3, and AL3.11



(c) Isolates PL2.4, PL2.5, and PL3.1



(d) Isolates LL2.3, LL2.4, and LL3.5

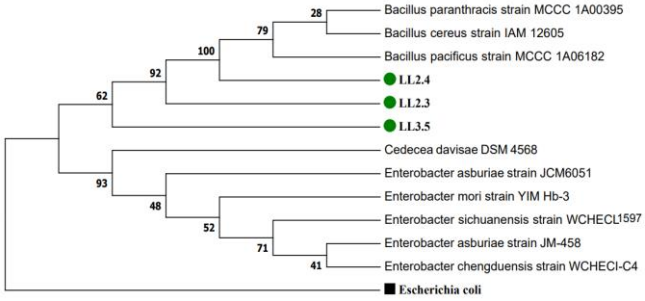


Figure 5. Phylogenetic tree of the best hydrolytic enzyme-producing bacteria from De Durian Park Jombang constructed using Neighborhood-joining with 1000× bootstraps

Table 5. Identification of bacterial species isolated from soil in De Durian Park Jombang using 16S rRNA genes with BLAST method

Isolate code	Species	Query cover	E value	Percent identity	Accession length
SL2.7	*Bacillus anthracis* strain ATCC 14578 16S ribosomal RNA, partial sequence	100%	0	99.43%	1306
SL1.7	*Bacillus pacificus* strain MCCC 1A06182 16S ribosomal RNA, partial sequence	100%	0	99.21%	1509
SL1.2	*Bacillus cereus* strain IAM 12605 16S ribosomal RNA, partial sequence	100%	0	99.63%	1486
AL1.2	*Bacillus anthracis* strain ATCC 14578 16S ribosomal RNA, partial sequence	100%	0	97.82%	1357
AL2.3	*Bacillus paranthracis* strain MCCC 1A00395 16S ribosomal RNA, partial sequence	100%	0	99.84%	1509
AL3.11	*Bacillus paramycoides* strain MCCC 1A04098 16S ribosomal RNA gene, partial sequence	100%	0	99.53%	1509
PL2.4	*Bacillus pumilus* strain SBMP2 16S ribosomal RNA, partial sequence	99%	4E-22	95.78%	1456
PL2.5	*Bacillus paramycoides* strain SrAM4 16S ribosomal RNA gene, partial sequence	99%	0	98.05%	1414
PL3.1	*Bacillus cereus* strain yasmun80b 16S ribosomal RNA gene, partial sequence	100%	0	99.76%	1412
LL2.3	*Enterobacter sichuanensis* strain WCHECL 1597 16S ribosomal RNA, partial sequence	100%	0	97.82%	1528
LL2.4	*Bacillus pacificus* strain MCCC 1A06182 16S ribosomal RNA, partial sequence	100%	0	99.69%	1509
LL3.5	*Enterobacter asburiae* strain JCM6051 16S ribosomal RNA, partial sequence	100%	0	98.17%	1422

Discussion

De Durian Park (DDP) in Wonosalam, Jombang, comprises a vast plantation area with varying altitudes. The soil in the plantation contains a diverse community of decomposer bacteria, including cellulolytic, amylolytic, proteolytic, and lipolytic bacteria, which can break down cellulose, starch, protein, and lipids in the soil. The study successfully identified the three most promising bacteria for each enzyme production category: cellulase, amylase, protease, and lipase.

Cellulolytic bacteria are crucial in decomposing organic materials in the soil, such as plant debris. These bacteria produce extracellular enzymes, including endoglucanase, exoglucanase, and β-glucosidase, which facilitate the breakdown of cellulose (Saraswati et al. 2007). The appearance of halo zones around bacterial colonies on selective media containing carboxymethyl cellulose (CMC) confirms their capability to produce enzymes that degrade cellulose.

Among the selected isolates, SL2.7, SL1.7, and SL1.2, which exhibited the highest index values, have the similarities of macroscopic and microscopic characteristics to the genus *Bacillus*, following Bergey's Manual of Determinative Bacteriology (Holt et al. 1994). Furthermore, based on the index value, SL1.2 and SL1.7 were categorized as moderate, while SL2.7 was classified as strong in enzyme production by Choi et al. (2005).

Isolate SL2.7 exhibited a 99.43% similarity with *Bacillus anthracis* strain ATCC 14578, which is known to produce cellulase, according to Duza and Mastan (2015). Furthermore, SL2.7 demonstrated a close kinship with *Bacillus paramycoides* strain MCC1A04098, a bacterial species capable of producing extracellular enzymes that degrade amylum. Ullah et al. (2021) also discovered that *B. paramycoides* strain MCC1A04098, found in Pakistan on a former brick kiln soil, produces both amylase and the indole acetic acid (IAA) hormone. Additionally, *B. paramycoides* produced cellulase, amylase, urease, proteinase, caseinase, and xylanase (Çağlayan, 2021; Hallol et al. 2022; Tiwari et al. 2022).

SL1.7 showed a similarity of 99.21% with *B. pacificus* strain MCCC 1A06182, previously found in the rhizosphere soil of cumin plants in India (Devi et al. 2022). The species found in India can hydrolyze starch and solubilize P, K, and Zn (Devi et al. 2022). Moreover, isolate SL1.7 displayed the closest kinship with SL1.2. As for SL1.2, it exhibited a 99.63% similarity with *B. cereus* strain IAM 12605. Sebastian et al. (2021) reported that *B. cereus* possesses several abilities, including phosphate solubilization, siderophore and ammonia production, and cellulase secretion. These research findings align with the statement of Maravi and Kumar (2020), emphasizing that *Bacillus* sp. is among the most efficient cellulolytic bacteria due to its capacity to secrete numerous extracellular enzymes than other genera. Therefore, the genus *Bacillus* sp. exhibits diverse extracellular enzyme production capabilities, with some strains capable of simultaneously producing multiple types of extracellular enzymes.

Based on the amylolytic bacteria isolation, the three best isolates fall into the moderate category in this research, as per Choi et al. (2005). Amylolytic bacteria can produce the amylase enzyme, responsible for the main breakdown of the glycoside chains in starch and related polysaccharide molecules to produce simpler sugar molecules (Kaur et al. 2019). One of the sources of starch in durian plantation soil comes from plant debris, such as durian seed (Agustina et al. 2017).

Isolate AL1.2 had a 97.82% similarity with *B. anthracis* strain ATCC 14578. *B. anthracis* can produce hydrolytic enzymes, such as amylase, lipase, and protease (Andleeb et al. 2022). According to Agbodjato et al. (2015), *B. anthracis* is a bacterium capable of hydrolyzing starch and one of the Plant Growth Promoting Rhizobacteria (PGPR) bacteria. Additionally, *B. anthracis* has potency in siderophore production and phosphate solubilization (Andleeb et al. 2022). In the phylogenetic tree of the amylolytic bacteria group (Figure 5), isolates AL1.2 and AL3.11 are on the same branch, indicating a closer relationship between the two isolates compared to other bacterial strains in the phylogenetic tree.

AL2.3 isolate has a 99.84% resemblance to *Bacillus paranthracis* strain MCCC 1A00395. *Bacillus paranthracis* bacteria has the ability in phosphate solubilization, proteolytic, lipolytic, and amylolytic activities, and they can produce siderophore and indole acetic acid (IAA) (Andleeb et al. 2022; Naseer et al. 2022). The branches of the AL2.3 isolate separate it from other *Bacillus* strains in the phylogenetic tree, showing the relationship between this isolate and the strains in the phylogeny tree.

AL3.11 isolate has a 99.53% similarity with *Bacillus paramycoides* strain MCCC 1A04098. According to Nisa et al. (2021), *Bacillus paramycoides* bacteria found in Ngebel Lake was also known to produce amylase. Microorganisms can produce amylase enzymes, especially from the *Bacillus* sp. (Bhattacharjee et al. 2019); this is also consistent with the results of this study (Table 2), where the bacteria with the highest index in amylolytic activities that were successfully isolated from DDP plantation soil belonged to the *Bacillus* sp., namely *B. anthracis*, *B. paranthracis*, and *B. paramycoides*.

Proteolytic bacteria possess protease enzymes, crucial in breaking down long-chain protein molecules into shorter fragments. In the durian plantation soil, plant cell walls, and durian shells are the protein sources (Holland et al. 2020; Masturi et al. 2020). These enzymes are commonly found in bacteria such as *Bacillus* sp. (Nursyirwani et al. 2021). This aligns with the results of isolates from the plantation soil in De Durian Park, Wonosalam, which share similarities with the species *Bacillus* sp., Isolates PL2.4, PL2.5, and PL3.1 demonstrated the highest proteolytic index and classified as moderate (Choi et al. 2005).

PL3.1 isolate has a 99.76% similarity with *Bacillus cereus* strain yasmun80b. Research conducted by Asha and Palaniswamy (2018) reported that *B. cereus* originating from organic waste soil in India has a proteolytic activity. This isolate has the closest kinship with *B. paramycoides* strain K3.4 (Figure 5). *B. paramycoides* bacteria can

produce different enzymes simultaneously, such as cellulases, protease, and amylase (Sarwan and Bose 2022).

The isolate PL2.5 in the phylogenetic tree is related to the branches of *B. cereus* CC27, *B. paramycoides* SrAM4, and *Bacillus* sp. ASB-132, even PL2.5 isolate, has 98.05% similarity with *B. paramycoides* strain SrAM4 (Figure 5).. Hortillosa et al. (2021) stated that *B. cereus* CC27 showed protease, cellulase and amylase activities. Many studies show that members of the genus *Bacillus* can produce protease enzymes. Alnahdi (2012) researched *Bacillus* sp. No. 2 EHN from the Red Sea coast in Jeddah, Saudi Arabia, which could produce protease enzyme reaching 243 U/mL. This aligns with the research conducted by Sevinc and Demirkan (2011) on *Bacillus* sp. N-40 from Turkey's soil, which showed a maximum protease activity of 224 U/mL.

In this study, the PL2.4 isolate has a 95.78% similarity with *B. pumilus* strain SBMP2. This result are also in line with the research of Sangeetha et al. (2008) which also showed that *B. pumilus* SG2 can produce protease. Based on the phylogenetic tree in Figure 5, This isolate has a branch separating it from PL3.1 and PL2.5.

Moreover, lipolytic bacteria possess lipase enzymes (Opara and Anumudu 2020). These soil bacteria play a significant role in degrading animal carcasses and plant debris containing lipid substrates. In the durian plantation, the protein sources were the lipids from plant debris, such as durian seed gum (Amid et al. 2012) and durian shell (Masturi et al. 2020). In this study, the selected bacteria exhibited a clear zone index that was quite large compared to other isolates. Isolates LL2.3 and LL2.4 were classified as having a strong index, while isolate LL3.5 was classified as having a moderate index (Choi et al. 2005).

Isolate LL2.3 has a 97.82% similarity with the species *Enterobacter sichuanensis* strain WCHECL 1597. The LL2.3 isolate is the *E. sichuanensis* WCHECL strain, which the same strain as bacterial isolates found in adjuevan samples in fish that have the ability to produce biogenic amines (nitrogen compounds) (Abre et al. 2022). This research will complete the data on the potential of *Enterobacter sichuanensis* strain WCHECL 1597 in producing lipase.

Isolate LL2.4 has a similarity of 99.69% with *Bacillus pacificus* strain MCCC 1A06182; furthermore, LL2.4 has the same species as the cellulolytic bacteria with isolate code SL1.7. These results align with the statement of Royanti et al. (2023) that *Bacillus* sp. is one of the bacteria with various benefits, such as being a biocontrol agent, biodetergent, and biodegradation agent. Therefore, there is a possibility that the isolates obtained have the ability to produce several hydrolytic enzymes.

The LL3.5 isolate has a similarity of 98.17% with the species *Enterobacter asburiae* strain JCM6051. According to Ghosh (2023), *E. asburiae* bacteria have protease, amylase, and lipase enzymes. However, LL3.5 in this study has similarities with the species *E. asburiae* strain JCM6051, indicating differences in strain compared to the statement found by Ghosh (2023). The isolates in this study shared the same strain as the research by Swati and Singh (2022) found in Uttarakhand Industrial Waste, which can bioremediate wastewater. Therefore, this research complements the data on the potential of *E. asburiae* in producing lipase.

Moreover, this research obtained hydrolytic enzyme-producing bacteria that can degrade organic matter in the durian plantation soil, DDP Wonosalam Jombang, successfully. These bacteria were isolated from around the durian plantations; hopefully the indigenous bacteria that have been isolated are those potentially to be developed. Hence, they can be returned to the durian plantation soil as a biofertilizer formulation. Biofertilizer was selected as an alternative to chemical fertilizers so that durian plants have optimal vegetative and generative growth to realize sustainable agriculture. These 12 selected bacteria were chosen due to the investigation results, which were supported by several other research references described previously. Those references revealed that the genera *Bacillus* sp. and *Enterobacter* sp. have a very good and great potential in producing extracellular enzymes capable of degrading organic compounds.

This research is an early stage for developing biofertilizers, especially for DDP durian plantations. Further research is needed to evaluate further the bacterial isolates' ability to increase plant growth. Although the enzymatic activity values of each bacteria have not yet been obtained, this hydrolytic index value data can already be used to provide information that the bacterial isolates obtained can be used as biofertilizer formulas. The study by Tang et al. (2020 b), who applied a biofertilizer containing *Serratia nematodiphila* C46 on corn plants, proved to affect plant height and stem diameter on corn after 56 days of seedling; the ability of *S. nematodiphila* C46 bacteria was evaluated using the hydrolysis index. In addition, hydrolytic bacteria, which have a clear zone index value, produce enzymes with good activity even with different amounts. This is aligned by Gusmawartati et al. (2017) that the bacterial isolate GSI-2 with the smallest cellulolytic index value, namely 1, had an enzyme activity of 0.0178 units/mL; also isolate GSI-1 with the largest index, namely 2.36, had an enzymatic activity of 0.1012 units/mL. These results indicate that the greater the index value, the better the bacteria's enzymatic activity. This study's results corroborated with several studies showing that bacterial isolates from the soil of the De Durian Park plantation have the potential to be developed as bacteria based biofertilizer in other durian plantation areas.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to Universitas Airlangga, Surabaya, Indonesia, for funding the research through the "*Penelitian Unggulan Fakultas* (PUF) 2022" scheme with contract number l432/UN3.l.8/PT/2022. We thank DDP management for allowing us to conduct research at DDP, all DDP staff for helping us take samples, as well as Fitriana Martiani, S.Si, for helping us during the research in the microbiology laboratory.

REFERENCES

Abre MG, Kedjebo DKB, Attchelouwa CK, Ehouman GO, Kouakou-Kouame CA, N'guessan FK. 2022. Molecular identification of non-lactic acid bacteria isolated on MRS medium and associated to the production of biogenic amines in adjuevan, a fermented fish of Côte d'Ivoire. World J Adv Res Rev 15 (01): 626-634. DOI: 10.30574/wjarr.2022.15.1.0666.

Acharya M, Amansa JA, Yichao Y, Joan MB, Jung AL, Roshani SA. 2021. Soil microbial diversity in organic and non-organic pasture system. PeerJ 9: e11184. DOI: 10.7717/peerj.11184.

Agbodjato NA, Noumavo PA, Baba-Moussa F, Salami HA, Sina H, Sezan A, Bankole H, Adjanohoun A, Baba-Moussa L. 2015. Characterization of potential plant growth promoting rhizobacteria isolated from maize (*Zea mays* L.) in Central and Northern Benin (West Africa). Appl Environ Soil Sci 2015: 1-9. DOI: 10.1155/2015/901656.

Agustina A, Sari RN, Kuntari. 2017. Utilization of pectin from durian (*Durio zibethinus*) seeds in adsorption of methyl violet dye. Proceeding The 2nd International Seminar on Chemical Education, September 12-13, 2017.

Alnahdi HS. 2012. Isolation and screening of extracellular proteases produced by new isolated *Bacillus* sp. J Appl Pharm Sci 2 (9): 071-074. DOI: 10.7324/JAPS.2012.2915.

Amid BT, Mirhosseini H, Kostadinović S. 2012. Chemical composition and molecular structure of polysaccharide-protein biopolymer from *Durio zibethinus* seed: extraction and purification process. Chem Cent J 6 (1): 117. DOI: 10.1186/1752-153X-6-117.

Andleeb S, Shafique I, Naseer A, Abbasi WA, Ejaz S, Liaqat I, Ali S, Khan MF, Ahmed F, Ali NM. 2022. Molecular characterization of plant growth- promoting vermi-bacteria associated with *Eisenia fetida* gastrointestinal tract. PLoS ONE 17 (6): e0269946. DOI: 10.1371/journal.pone.0269946.

Asha B, Palaniswamy M. 2018. Optimization of alkaline protease production by *Bacillus cereus* FT 1 isolated from soil. J Appl Pharm Sci 8 (02): 119-127. DOI: 10.7324/JAPS.2018.8219.

Bhattacharjee I, Mazumdar D, Saha SP. 2019. Microbial amylases and their potential application in industries: A review. Structure 18: 19-20.

Çağlayan P. 2021. Determination of important enzymes and antimicrobial resistances of gram-positive haloalkaliphilic bacteria isolated from Salda Lake. J Fish Aquat Sci 38 (3): 375-382. DOI: 10.12714/egejfas.38.3.14.

Chang YC, Uphoff N, Eiji Y. 2016. A conceptual framework for eco-friendly paddy farming in Taiwan, based on experimentation with System of Rice Intensification (SRI) methodology. Paddy Water Environ 14: 169-183. DOI: 10.1007/s10333-015-0488s-9.

Choi YW, Hodgkiss IJ, Hyde KD. 2005. Enzyme production by endophytes of *Brucea javanica*. J Agric Sci Technol 1: 55-60.

Chukwuma OB, Rafatullah M, Kapoor RT, Tajarudin HA, Ismail N, Siddiqui MR, Alam M. 2023. Isolation and characterization of lignocellulolytic bacteria from municipal solid waste landfill for identification of potential hydrolytic enzyme. Fermentation 9 (3): 298. DOI: 10.3390/fermentation9030298.

Chusnah M. 2020. Keunggulan durian Bido dalam pengembangan agrowisata durian Wonosalam Jombang. Jurnal Ilmu-Ilmu Pertanian 2 (2): 103-111. DOI: 10.32764/agrosaintifika.v2i2.834. [Indonesian]

De Lillo, Ashley FP, Palmer RM, Munson MA, Kyriacou L, Weightman AJ, Wade WG. 2006. Novel subgingival bacterial phylotypes detected using multiple universal polymerase chain reaction primer sets. Oral Microbiol Immunol 21: 61-68. DOI: 10.1111/j.1399-302X.2005.00255.x

Devi D, Gupta SD, Mishra BK, Uma, Shesma MK. 2020. Isolation and characterization of endophytic rhizospheric phosphate solubilizing bacteria of cumin and their evaluation in vitro. Intl J Chem Sci 8: 1583-1593. DOI: 10.22271/chemi.2020.v8.i5v.10530.

Dharmayanti I. 2011. Filogenetika molekuler: Metode taksonomi organisme berdasarkan sejarah evolusi. Wartazoa 21 (1): 1-10. DOI: 10.14334/wartazoa.v30i1.2469. [Indonesian]

Duza, MB, Mastan SA. 2015. Optimization studies on cellulase production from *Bacillus anthracis* and *Ochrobactrum anthropic* (YZ1) isolated from soil. Intl J Appl Sci Biotechnol 3 (2): 272-284. DOI: 10.3126/ijasbt.v3i2.12616.

Ervina E, Ekowati CN, Sumardi S, Rosa E. 2020. Lipolytic-screening of *Bacillus* genera as biocontrol candidate in coffee plantation. J-BEKH 7 (1): 31-34. DOI: 10.23960/jbekh.v7i1.12.

Fitri R. 2018. Isolasi dan karakterisasi Plant Growth Promoting Rhizobacteria (PGPR) dari rizosfer tanaman durian (*Durio zibethinus* Murr.). [Thesis]. Universitas Islam Negeri Sultan Syarif Kasim Riau, Pekanbaru. [Indonesian]

Ghosh T. 2003. Different types of enzyme production abylity by halophilic bacteria isolated from Bay of Bengal Water. Eur Chem Bull 12 (1): 2859-2870.

Gómez EJ, Delgado JA, González JM. 2020. Persistence of microbial extracellular enzymes in soils under different temperatures and water availabilities. Ecol Evol 10 (18): 10167-10176. DOI: 10.1002/ece3.6677.

Gusmawartati, Agustian, Herviyanti, Jamsari. 2017. Isolation of cellulolytic bacteria from peat soils as decomposer of oil palm empty fruit bunch. J Trop Soil 22 (1): 47-53. DOI: 10.5400/jts.2017.22.1.43.

Hallol M, Helmy O, Shawky A-E, El-Batal A, Ramadan M. Optimization of alpha-amylase production by a local *Bacillus paramycoides* isolate and immobilization on chitosan-loaded barium ferrite nanoparticles. Fermentation 8 (5): 241. DOI: 10.3390/fermentation8050241.

Holland C, Ryden P, Edwards CH, Grundy MM. 2020. Plant cell walls: impact on nutrient bioaccessibility and digestibility. Foods 9 (2): 201. DOI: 10.3390/foods9020201.

Holt JG, Krieg NR, Sneath PHA, Staley JT and Williams ST. 1994. Bergey's Manual of Determinative Bacteriology, Ninth Edition. Williams and Wilkins, Baltimore, USA.

Hortillosa EM, Amar MJA, Nuñal SN, Pedroso FL, Ferriols VMEN. 2022. Effects of putative dietary probiotics from the gut of milkfish (*Chanos chanos*) on the growth performance and intestinal enzymatic activities of juvenile Nile tilapia (*Oreochromis niloticus*). Aquac Res 53 (1): 98-108. DOI: 10.1111/are.15556.

Istiqomah N, Indarto TN, Nugroho VA, Prayogo C. 2017. Ketersediaan nitrogen dan populasi bakteri tanah di bawah pengaruh pemupukan pada system of rice intensification. Jurnal Tanah dan Iklim 41 (2): 1-10. DOI: 10.33366/bs.v18i1.936. [Indonesian]

Kaur J, Gosal SK, Walia SS, Kaur J. 2019. Impact of green manure and consortium biofertilizer on amylolytic bacterial population and their activities in maize rhizospheric soil. Chem Sci Intl J 26 (4): 1-7. DOI: 10.9734/CSJI/2019/v26i430100.

Krishna MP, Mohan M. 2017. Litter decomposition in forest ecosystems: A review. Energy Ecol Environ 2: 236-249. DOI: 10.1007/s40974-017-0064-9.

Luang-In V, Yotchaisarn M, Saengha W, Udomwong P, Deeseenthum S, Maneewan K. 2019a. Isolation and identification of amylase-producing bacteria from soil in nasinuan community forest, Maha Sarakham, Thailand. Biomed Pharmacol J 12 (3): 1061-1068. DOI: 10.13005/bpj/1735.

Luang-In V, Yotchaisarn M, Saengha W, Udomwong P, Deeseenthum S, Maneewan K. 2019b. Protease-producing bacteria from soil in Nasinuan Community Forest, Mahasarakham Province, Thailand. Biomed Pharmacol J 12 (2): 587-595. DOI: 10.13005/bpj/1678.

Mahmud AA, Upadhyay SK, Srivastava AK, Bhojiya AA. 2021. Biofertilizers: A Nexus between soil fertility and crop productivity under abiotic stress. Curr Res Environ Sustain 3: 100063. DOI: 10.1016/j.crsust.2021.100063.

Malik AA, Martiny JBH, Brodie EL, Martiny AC, Treseder KK, Allison SD. 2020. Defining trait-based microbial strategies with consequences for soil carbon cycling under climate change. ISME J 14 (1): 1-9. DOI: 10.1038/s41396-019-0510-0.

Maravi P, Kumar A. 2021. Cellulase: distribution, production, characterization and industrial applications. Biotechnol J Intl 25 (3): 36-71. DOI: 10.9734/BJI/2021/v25i330143.

Masturi, Alighiri D, Edie SS, Drastisianti A, Khasanah U, Tanti KA, Maghfiroh RZ, Kirana KGC, Choirunnisa F. 2020. Identification of flavonoid compounds and total flavonoid content from biowaste of local durian shell (*Durio zibethinus*). J Phys: Conf Ser 1567 (4): 042084. DOI: 10.1088/1742-6596/1567/4/042084.

Mondal T, Datta JK, Mondal NK. 2017. Chemical fertilizer in conjunction with biofertilizer and vermicompost induced changes in morpho-physiological and bio-chemical traits of mustard crop. J Saudi Soc Agric Sci 16 (2): 135-144. DOI: 10.1016/j.jssas.2015.05.001.

Naseer A, Andleeb S, Basit A, Abbasi WA, Ejaz S, Ali S, Ali NM. 2022. Phylogenetic illustration of *Eisenia fetida* associated vermi-bacteria involved in heavy metals remediation and retaining plant growth

promoting traits. J Oleo Sci 71 (8): 1241-1252. DOI: 10.5650/jos.ess21366.
Nazar ARS, Salehi N, Salehi N, Karimi Y, Kermanshahi RK, Baheshti M. 2018. Assessing the biological inhibitors effect on crude oil wax appearance temperature reduction. J Pet Technol 8 (2): 70-85. DOI: 10.22078/jpst.2017.2442.1418.
Nisa IK, Prabaningtyas S, Lukiati B, Saptawati RT, Rodiansyah A. 2021. The potential of amylase enzyme activity against bacteria isolated from several lakes in East Java, Indonesia. Biodiversitas 22: 42-49. DOI: 10.13057/biodiv/d220106.
Nursyirwani N, Samiaji J, Tanjung A, Effendi I, Claudia KM. 2021. Growth and enzyme production of proteolytic bacteria from mangrove sediment. Earth Environ Sci 695: 1-6. DOI: 10.1088/1755-1315/695/1/012044.
Opara CN, Anumudu CK. 2020. Characterization and lipolytic activity of bacteria isolates from freshwater clam (*Mercenaria mercenaria*) in Bayelsa State, Nigeria. J Food Technol Sci 2 (2): 1-4. DOI: 10.47363/JFTNS/2020(2)109.
Pirttilä AM, Tabas HMP, Baruah N, Koskimäki JJ. 2021. Biofertilizers and biocontrol agents for agriculture: How to identify and develop new potent microbial strains and traits. Microorganisms 9 (4): 817. DOI: 10.3390/microorganisms9040817.
Royanti V, Handayani K, Ekowati CN, Sumardi. 2023. Isolasi dan karakterisasi *Bacillus* lipolitik dari Tanah Kebun Raya Liwa. Conf. Ser. 18. Seminar Nasional Biologi (Semabio), Bandung, 1 Januari 2023. [Indonesian]
Sangeetha R, Geetha A, Arulpandi I. 2008. Optimization of protease and lipase production by *Bacillus pumilus* SG 2 isolated from an industrial effluent. Internet J Microbiol 5 (2): 1-8. DOI: 10.5580/2126.
Saputri KE, Idiawati N, Sofiana MSJ. 2021. Isolasi dan karakteristik bakteri penambat nitrogen dari rizosfer mangrove di Kuala Singkawang. Jurnal Laut Khatulistiwa 4 (2): 17-21. DOI: 10.26418/lkuntan.v4i2.45316. [Indonesian]
Saraswati R, Husen E, Simanungkalit RDM. 2007. Metode analisis biologi tanah. Balai besar penelitian dan pengembangan sumberdaya lahan pertanian, Bogor. https://repository.pertanian.go.id/server/api/core/bitstreams/63eecc44-8bb1-4b39-b71f-d24523bf0635/content. [Indonesian]
Sarwan J, Bose KJC. 2022. Isolation, screening and characterisation of *Bacillus paramycoides* for producing cellulases, proteases and amylases from fabric industry. Res Square 2022. DOI: 10.21203/rs.3.rs-1700944/v2.
Shamshitov A, Decorosi F, Viti C, Fornasier F, Kadžienė G, Supronienė S. 2023. Characterisation of cellulolytic bacteria isolated from agricultural soil in Central Lithuania. Sustainability 15 (1): 598. DOI: 10.3390/su15010598.
Sebastian AM, Umesh M, Priyanka K, Preethi. 2021. Isolation of plant growth-promoting *Bacillus cereus* from soil and its use as a microbial inoculant. Arab J Sci Eng 46: 151-161. DOI: 10.1007/s13369-020-04895-8.
Sevinc N, Demirkan E. 2011. Production of protease by *Bacillus* sp. N-40 isolated from soil and its enzymatic properties. J Biol Environ Sci 5 (14): 95-103.
Swati, Singh P. 2022. Bioremediation of hazardous azo dye methyl red by a newly isolated *Enterobacter asburiae* strain JCM6051 from industrial effluent of Uttarakhand regions. J Appl Biol Biotechnol 10 (2): 64-72. DOI: 10.7324/JABB.2022.10s206.
Tang A, Haruna AO, Majid NMA, Jalloh MB. 2020a. Potential PGPR properties of cellulolytic, nitrogen-fixing, phosphate-solubilizing bacteria in rehabilitated tropical forest soil. Microorganisms 8: 1-22. DOI: 10.3390/microorganisms 8030442.
Tang A, Haruna AO, Majid NMA, Jalloh MB. 2020b. Effects of selected functional bacteria on maize growth and nutrient use efficiency. Microorganisms 8 (6): 854. DOI: 10.3390/microorganisms8060854.
Tiwari S, Singh R, Yadav J, Gaur R, Singh A, Yadav JS, Pandey PK, Yadav SK, Prajapati J, Helena P, Dewangan J, Jamal F. 2022. Three-step purification and characterization of organic solvent-tolerant and alkali-thermo-tolerant xylanase from *Bacillus paramycoides* T4 [MN370035]. Catalysts 12 (7): 749. DOI: 10.3390/catal12070749.
Ullah I, Khan MS, Khan SS, Ahmad W, Zheng L, Shah SUA, Ullah M, Iqbal A. 2021. Identification and characterization of thermophilic amylase producing bacterial isolates from the brick kiln soil. Saudi J Biol Sci 28 (1): 970-979. DOI: 10.1016/j.sjbs.2020.11.017.
Verma NP, Chowdhury T. 2023. Development of indigenous nitrogen fixing bio-fertilizer for fertilizer saving and increasing productivity of sugarcane crop. Pharm Innov 12 (7): 1834-1840.
Wang Y, Zhu Y, Zhang S, Wang, Y. 2018. What could promote farmers to replace chemical fertilizers with organic fertilizers. J Cleaner Prod 199: 882-890. DOI: 10.1016/j.jclepro.2018.07.222.
Wenny V. 2020. Ekowisata berbasis masyarakat untuk mendukung pembangunan berkelanjutan: Studi kasus ekowisata Wonosalam Kabupaten Jombang Jawa Timur. In: Hapsari CM, Hastomo A (eds.). Prosiding Seminar Nasional Magister Ilmu Lingkungan Sekolah Pascasarjana-Universitas Diponegoro Tahun 2020. Universitas Diponegoro, Semarang, 2 Desember 2020. [Indonesian]
Wiesemüller B, Rothe H. 2006. Interpretation of bootstrap values in phylogenetic analysis. Anthropologischer Anzeiger 64 (2): 161-165. DOI: 10.1127/anthranz/64/2006/161.