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RESEARCH ARTICLE

Isolation and screening of pectinase producers from fruit wastes

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Abstract - This study aimed to identify, screen and characterize pectinase-producing bacteria from fruit waste for potential use in the biotechnology sector. Three isolated bacterial strains were tested for their pectinase production using both commercial pectin and fruit waste substrates, specifically pineapple and banana peels, alongside an assessment of their growth characteristics. Among the three isolates, strain M1 exhibited the best performance in terms of growth and enzyme activity. When commercial citrus pectin was used as the substrate, M1 achieved the highest pectinase activity (8.15 U/mL), followed by B1 (7.423 U/mL) and P2 (7.035 U/mL). When fruit waste was used as the substrate, M1 again produced the highest amount of pectinase. Notably, pineapple peel yielded slightly higher pectinase activity for M1 (7.38 U/mL) compared to banana peel (7.032 U/mL), indicating that pineapple peel is a better inducer of pectinase enzyme production. In terms of growth dynamics, M1 also recorded the highest specific growth rate and the shortest doubling time across all substrates, reinforcing its potential as the most efficient pectinase producer. These findings suggest that strain M1, especially when cultivated on pineapple peel waste, holds significant promise for cost-effective and sustainable industrial pectinase production, contributing to the development of environmentally friendly bioprocesses.

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1. Introduction

Globally, an estimated 1.3 billion tonnes of food are wasted annually, with approximately 1,748 million tonnes attributed to fruit and vegetable waste [1, 2]. This constitutes a pressing environmental and economic issue. For instance, Malaysia alone generated 6.7 million tonnes of food waste in 2020, with fruit waste being a major contributor [3, 4]. The improper disposal of fruit waste, primarily through landfilling, contributes to environmental pollution and greenhouse gas emissions [5]. This growing issue underscores the urgent need for sustainable and innovative waste valorisation strategies that support both environmental protection and economic sustainability [6]. Pectinase enzymes, which break down pectin found in plant cell walls, are widely used in industries such as food processing, textiles, paper production, and pharmaceuticals [7]. Traditionally, these enzymes are produced using microbial strains cultivated on synthetic substrates, a costly and energy-intensive method [8]. Previous studies have focused on strains such as *Aspergillus niger*, which, while effective, often yield low enzyme productivity and require optimal, controlled fermentation conditions [9]. These limitations have prompted researchers to explore alternative sources and production methods that are more efficient and sustainable [10]. Recent research increasingly focuses on agricultural and food waste as cost-effective substrates for enzyme production [11]. fruit waste, including pineapple and banana peels, offers a rich source of pectin and microbial biodiversity [12]. It’s naturally occurring Microorganisms degrade pectin, making it an excellent source for isolating potent pectinase producers [13]. This approach not only reduces environmental burden but also lowers production costs, aligning with the principles of a circular economy and sustainable biotechnology [14].

Microbial sources, including bacteria and fungi, form the basis of industrial enzyme technology. These microorganisms play a vital role and provide benefits in various industries, including medical, pharmaceutical, food, and beverage sectors, through functions like refining and purification [7, 9]. pectinase enzymes have substantial applications in biotechnological processes that use pectin as a carbon source [14]. Pectin is a polysaccharide found in plant cell walls, mainly in the middle lamella of vegetables and fruits [12]. The breakdown of the pectin polymer is catalysed by a complex group of enzymes called pectinase [15]. These enzymes are utilised in numerous fields, including fruit juice preparation, textile processing, papermaking, wastewater treatment, degumming of plant bast fibres, wine clarification, oil processing, and the coffee and tea industries, among others [7]. This broad utility highlights the importance of optimising enzyme production to meet the increasing demand across various sectors [16]. Fruit wastes are an abundant and cost-effective source of pectin. Utilising fruit waste to produce pectinases not only reduces environmental pollution but also provides a cost-effective substrate for enzyme production, thus supporting a sustainable, circular economy model [12]. In this study, we propose a practical, environmentally friendly approach to enzyme production by isolating and screening pectinase-producing bacteria from fruit waste [17]. The current research focuses on characterising bacterial strains and evaluating their enzyme production capabilities using fruit waste as a substrate. This approach holds promise for developing cost-effective and scalable solutions for industrial pectinase production. Specifically, the objectives of this study are to isolate and screen the pectinase-producing bacteria from fruit wastes, and further to produce the pectinase enzyme using banana and pineapple peels as substrates. These findings may contribute to the development of eco-friendly, cost-efficient enzyme production systems for industrial use.

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2. Materials and Methods

2.1 Materials

All chemicals and reagents used in this study were analytical grade. The materials used consisted of banana and pineapple peels, which were collected from a local store near Universiti Malaysia Al-Sultan Abdullah (UMPSA), Gambang, Pahang, Malaysia and Rompin Integrated Pineapples Industries Sdn. Bhd. (RIPI), Malaysia, respectively.

2.2 Sample Collection and Preparation

Banana and pineapple peels were selected due to their high pectin content. Peels were collected from a pisang goreng stall near UMPSA Gambang, Pahang, and from Rompin Integrated Pineapples Industries Sdn. Bhd (RIPI). They were sorted by texture and firmness, oven-dried at 60°C for 24 hours, ground into a powder, and sieved to obtain fine particles. Powdered samples were analysed via FTIR for compositional analysis and stored in sealed containers for further use.

2.3 Pectinase-Producing Bacteria

Bacteria were isolated from pineapple slurry, banana slurry, rotten peel extract, and combined peel solutions using the method described by Geetha et al. [18]. Dilutions of 10^{-1} to 10^{-5} were plated on nutrient agar and incubated at 37°C for 24–48 hours. Colonies were purified by the streak plate technique. A selective agar containing yeast extract, ammonium sulphate, Na_2HPO_4, KH_2PO_4, and citric pectin (pH 4.0) was used [7, 12]. After incubation, iodine was added to detect clear zones. Colonies with large clear zones were identified as pectinase producers. Morphological identification was conducted by microscopic observation. Gram staining was used to classify isolates. Purple-stained cells were Gram-positive, and pink-stained cells were Gram-negative. Growth was assessed by measuring OD_{600} and dry cell weight (DCW) over time. OD_{600} indicates turbidity, while DCW gives a precise measure of biomass.

2.4 Pectinase Production from Fruit Wastes

A basal medium (g/L) was prepared based on the previous studies, containing in g/L, fruit waste (0.3), sucrose (10), KH_2PO_4 (0.1), $MgSO_4$ (0.25), $CaCl_2$ (0.1), $NaNO_3$ (2), K_2HPO_4 (0.5), KCl (0.5), and yeast extract (1) [18, 19]. The banana and pineapple peel powders (3% - w/v) were added into the basal medium before it was subjected to autoclave at 121°C for 15 min. The identified pectinase-producing bacteria (1% - v/v) were inoculated and incubated at 37°C, 150 rpm for 3 days. The sampling was conducted in triplicate at 6-hour intervals, and the samples were centrifuged at 10,000 rpm for 10 min. The supernatant was subjected to pectinase activity. Pellets were used for cell determination analysis.

2.5 Analytical Analysis

The pectin determination was done by using FTIR spectroscopy to determine esterified and free carboxyl groups in pectin to assess the degree of esterification (DE) [8]. Cell determination was performed by measuring the OD_{600} with a spectrophotometer to monitor bacterial growth [20], while DCW was measured by drying the pellet at 60–70 °C until a constant weight [21]. Crystal violet, iodine, ethanol, and safranin were sequentially applied for the determination of gram staining. Purple colour indicated Gram-positive; pink indicated Gram-negative [22]. Measuring Pectinase activity was determined by using the reducing sugar equivalents using the DNS method. The sample (0.5 mL) and 0.5 mL of 0.1 M acetate buffer with pectin were incubated at 40°C. After adding DNS and heating, absorbance was measured at 540 nm. One unit of enzyme activity was defined as 1 µmol of reducing sugar released per minute [19].

3. Results and Discussion

3.1 Fruit Wastes Composition

FTIR analysis was performed to detect the presence of pectin and its associated functional groups in banana and pineapple peel powders. The FTIR spectrum of pectin in fruit waste samples, including banana and pineapple peel, is illustrated in Figure 1. Based on Figure 1, the peaks at 3649.90 cm^{-1} indicate hydroxyl groups typical of cellulose and hemicellulose [1]. Peaks at 2919.59 cm^{-1} reflect C–H stretching vibrations, while 1622.22 cm^{-1} indicates C=O stretching from carboxylic or ester groups. The peaks at 1399.03 and 1073 cm^{-1} correspond to C-H bending modes of cellulose and lignin polymers. Similarly, the pineapple peel spectrum showed broad peaks in the 3,500–3,000 cm^{-1} range due to O-H and N-H vibrations and strong C–O stretching between 1,160 and 1,000 cm^{-1} [23]. This confirmed the presence of various functional groups, indicating that both fruit peels are rich in pectin, suitable for microbial enzyme production.

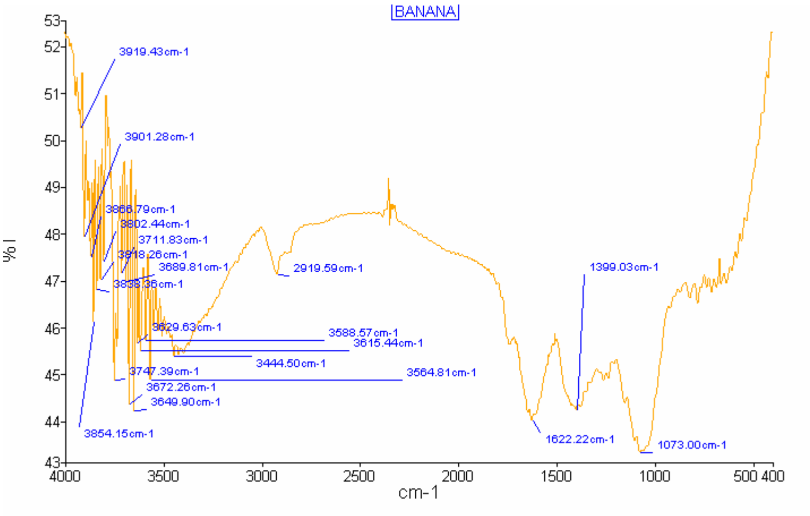
3.2 Isolation and Screening

3.2.1 Single culture

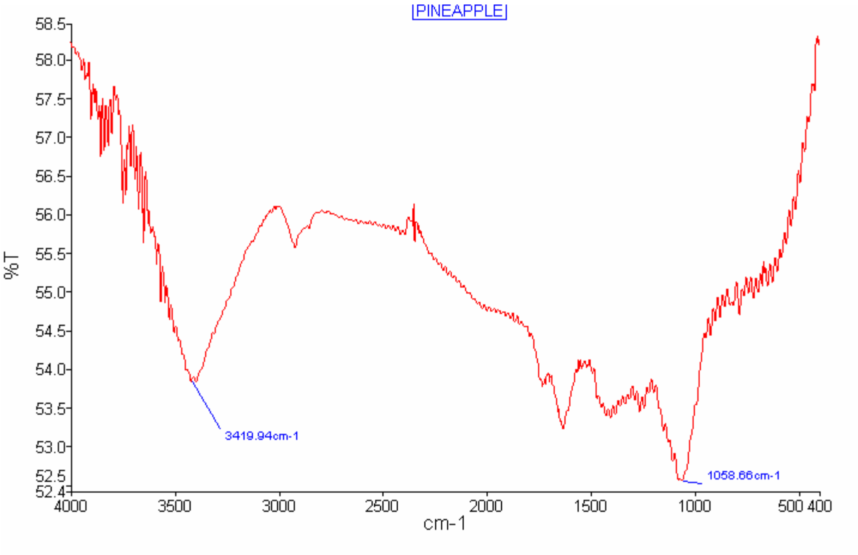
Multiple bacterial colonies were isolated from different sources: pineapple slurry, banana slurry, rotten peel extract, and a mixed peel solution. Colonies with distinctive morphologies were selected for subculturing to ensure isolate purity using the streak plate method. As a result, a total of six bacterial strains (B1, B2, M1, M2, P1 and P2) were isolated from the banana, mixed, and pineapple samples. These strains were further screened for pectinase activity.

3.2.2 Clearance zone

The isolates were screened for pectinase enzyme using a selective agar medium containing citrus pectin as substrate. A clear halo around the colonies indicated pectinase activity, as pectin was degraded [24]. As a result, three isolated strains were identified to successfully exhibit the potential of pectinase production. Among all isolates, three strains (M1, B1, and P2) were selected based on the presence and size of the clear zones, as illustrated in Figure 2.



(a) banana peels



(b) pineapple peels

Figure 1. FTIR spectrum of banana peels and pineapple peels

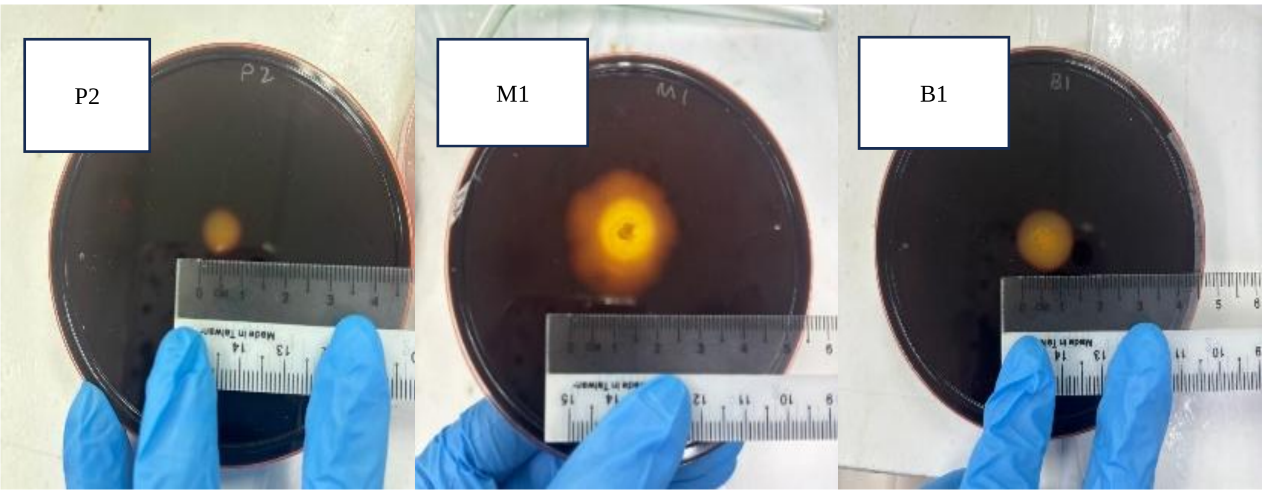


Figure 2. Clear zone determination by pectinase producing bacterial isolates

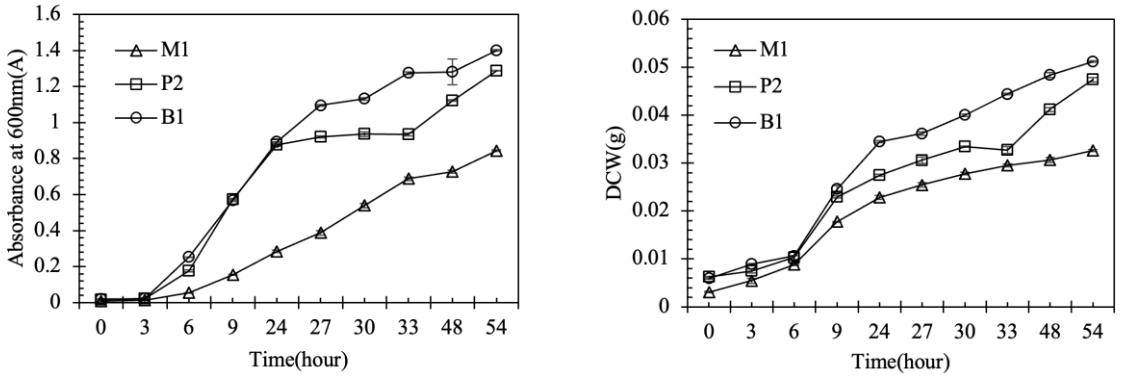
Based on the results obtained, these three pectinase-producing bacteria were subjected to morphological analysis. The results are summarised in Table 1. All three isolated strains, M1, B1 and P2, were found to exhibit rod-shaped and Gram-positive bacteria. Rod-shaped, Gram-positive isolates were presumed to belong to the Bacillus genus, which is well known for its ability to produce industrial enzymes [25, 26].

Table 1. Diameter of the screening zone generated around the bacterial colonies and characterisation of bacteria

Isolated strain	Diameter (cm)	Shape of bacteria	Gram Staining	Gram positive/negative
B1	1.5	Rod-shape	Purple	Gram positive
M1	2.6	Rod-shape	Purple	Gram positive
P2	1.0	Rod-shape	Purple	Gram positive

3.3 Pectinase Production Using Commercial Pectin

The isolated strains (B1, M1, and P2) were used to produce pectinase using commercial pectin as the primary substrate, and growth was monitored over time using optical density (OD600) and dry cell weight (DCW). The results obtained were summarised in Figure 3. Strain M1 showed the least and most subtle growth among the three strains. Its lag phase lasted until around 6 hours, followed by a modest log phase extending to about 33 hours. OD600 plateaued earlier and at a lower level than those of other strains, indicating a lower population density. In contrast, strain B1 showed the most vigorous growth in OD600 and DCW, followed by P2, and lastly M1. The classic bacterial growth phases (lag, log, and stationary) were clearly observed in both OD600 and DCW trends. The specific growth rate of M1 was 0.2472 hr^{-1}, which was higher than B1 (0.242 hr^{-1}) and P2 (0.2257 hr^{-1}). M1 also had the shortest doubling time at 2.80 hours, indicating rapid cell division and higher growth efficiency. Overall, strain B1 provides by far the most vigorous growth in cell density (OD600) and biomass accumulation (DCW); followed by P2, and lastly M1. The classic bacterial growth phases, which are lag, log, and stationary, are observable in both OD600 and DCW trends, with DCW providing a more direct measure of biomass. Pectinase activity of all three isolated strains (M1, B1 and P2) was illustrated in Figure 4.



(a) (b)

Figure 3. Growth profiling of the pectinase producing bacteria using commercial pectin as substrate. (a) OD_{600} (b) DCW

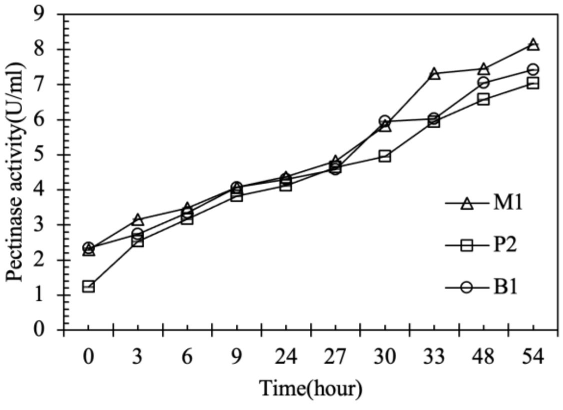


Figure 4. Pectinase activity of locally isolated pectinase-producing bacterial strains M1, P2, and B1 using commercial pectin

Based on the results obtained (Figure 4), strain M1 produced the highest pectinase activity at 54 hours, reaching 8.15 U/mL, while B1 and P2 had 7.423 and 7.035 U/mL, respectively. In comparison, the pectinase activity was lower than that reported by Alqahtani et al. [27]. The lower activity might be due to the crude enzyme preparation, which contains impurities such as proteins and cellular debris, reducing the observed activity. Based on the results obtained, M1 and B1 were selected for further employment in the production of pectinase using pineapple and banana peels as a feedstock.

3.4 Pectinase Production Using Pineapple Peel

Pineapple peel was used as an alternative pectin source to determine the potential of M1 and B1 for producing pectinase enzyme. The growth profiling of all three isolated strains was summarised in Figure 5. For strain M1, the lag phase lasted until approximately 6 hours, followed by a sharp log phase (6–30 hours) and then a stationary phase (30–54 hours). Strain B1 followed a similar profile. The lag phase lasted about 6 hours, with a log phase from 6 to 30 hours, then a plateau through 54 hours. DCW also increased gradually, peaking around 48 hours. M1 had a higher specific growth rate (0.5062hr^{-1}) and shorter doubling time (1.37 hours) than B1 (0.425 hr^{-1} and 1.63 hours, respectively), indicating faster and more efficient biomass production. According to Figure 6, M1 showed the highest pectinase activity using pineapple peel, reaching 7.38 U/mL at 54 hours, slightly higher than B1, which had 7.086 U/mL. These values are significantly higher than those reported in some literature, for instance, 1.473 U/mL for *Bacillus subtilis* at 20% concentration [28], likely due to strain and medium differences.

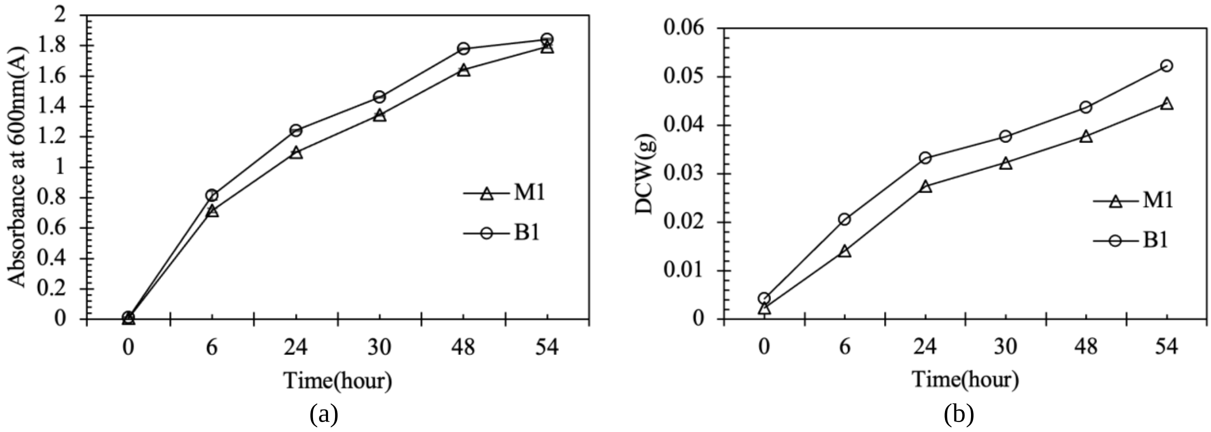


Figure 5. Growth profiling of locally isolated pectinase-producing bacterial strains M1 and B1 using pineapple peel. (a) OD600, (b) DCW

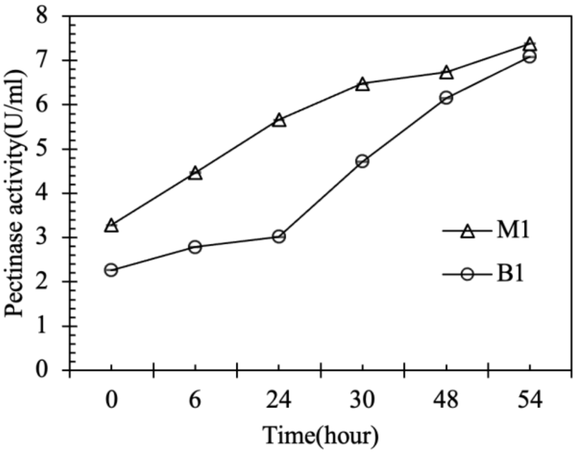


Figure 6. Pectinase activity of locally isolated pectinase-producing bacterial strains M1 and B1 using pineapple peel

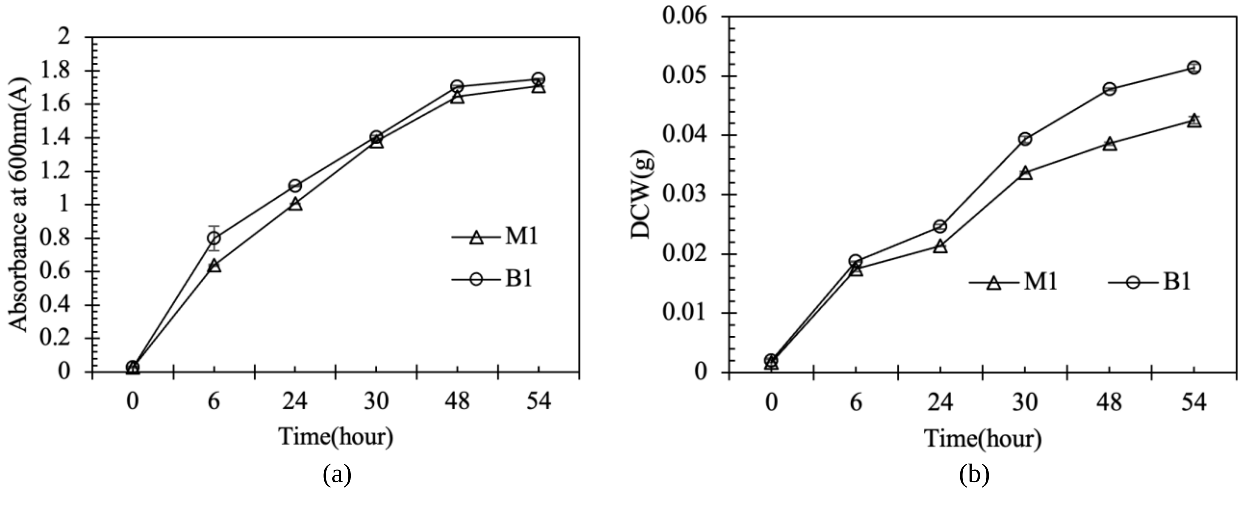


Figure 7. Growth profiling of locally isolated pectinase-producing bacterial strains M1 and B1 using banana peel (a) OD600 (b) DCW

3.5 Pectinase Production Using Banana Peel

3.5.1 Growth profiling

Figure 7 shows that for strain M1, a short lag phase (~6 hours) was followed by an extended log phase until 48 hours. The stationary phase began around 48 hours, with no death phase observed. The DCW curve (as illustrated in Figure 7b) mirrored this trend, with biomass rising sharply during the log phase and levelling off after 48 hours. Strain B1 showed similar growth stages but outperformed M1 in biomass yield. It had a higher specific growth rate (0.5538 hr^{-1}) and lower doubling time (1.25 hours) than M1 (0.5409 hr^{-1} and 1.28 hours), indicating faster proliferation and better adaptability to banana peel medium.

3.5.2 Pectinase Production

As shown in Figure 8, M1 produced 7.032 U/mL of pectinase at 54 hours using banana peel, while B1 yielded 6.342 U/mL. Based on the results obtained, the locally isolated pectinase-producing bacteria employed in this study produced pectinase activity comparable to that reported in previous studies [29, 30]. In comparison, as mentioned by Anab-Atulomah and Nwachukwu [30], pectinase activity obtained from bacteria isolated from solid market waste, which was identified as *B. subtilis,* exhibited 8.98 U/mL of pectinase using commercial pectin as a feedstock, while Abdollahzadeh et al. [29], have successfully optimised the production of pectinase up to 14.16 U/mL using a response surface methodology (RSM) approach. This indicates that this study has promising pectinase-producing bacteria for further optimisation studies.

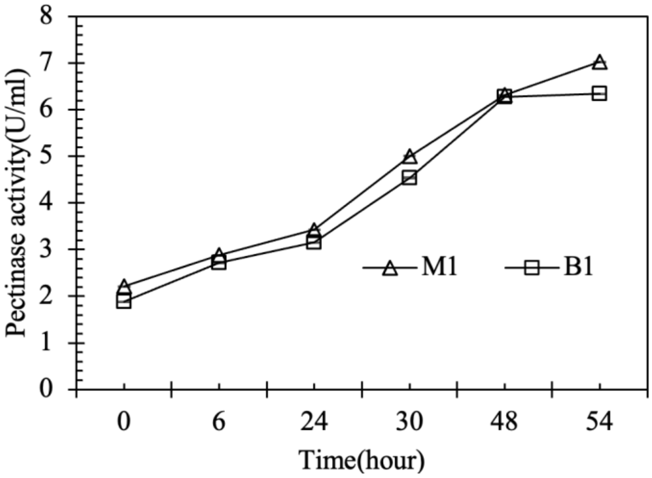


Figure 8. Pectinase activity of locally isolated pectinase-producing bacterial strains M1 and B1 using banana peel.

M1 showed the best performance in both specific growth rate and shortest doubling time in all three media tested compared to B1 and P2. In comparison between fruit waste substrates, pineapple peel induced slightly higher activity (M1 – 7.38 U/mL) than banana peel (M1 – 7.032 U/mL), which indicated that pineapple peel is a better inducer of the pectinase enzyme than banana peel by strain M1. The pineapple medium is suitable for M1 because of the acidic environment and the presence of bromelain, which breaks down proteins into smaller peptides and amino acids. These amino acids support bacterial growth and pectinase production [31]. Although B1 had the highest DCW in banana peel medium, M1 showed higher pectinase activity, indicating more efficient pectin utilisation. M1 is the most potent isolate due to the highest enzyme production and specific growth rate, as well as the shortest doubling time across all tested substrates. When compared with another study using avocado peel extract as the substrate, which reported a pectinase activity of 5.41 ± 0.14 U/mL, the pectinase activity produced by strain M1 using pineapple and banana peel substrates was significantly higher [24]. This highlights the effectiveness of pineapple and banana peels as substrates for increased enzyme production and reinforces the suitability of strain M1 for sustainable industrial applications.

4. Conclusions

The study isolated and evaluated three bacterial strains—M1, B1, and P2—for their potential in pectinase production using different substrates, including commercial citrus pectin and fruit wastes such as pineapple and banana peels. Among the isolates, strain M1 consistently demonstrated the highest specific growth rate and shortest doubling time across all tested media. For instance, in pineapple medium, M1 achieved a specific growth rate of 0.5062 hr^{-1} and a doubling time of 1.37 hours, outperforming B1, which had a specific growth rate of 0.425 hr^{-1} and a doubling time of 1.63 hours. In pectin medium, M1 also led with a growth rate of 0.2472 hr^{-1}, higher than B1 (0.242 hr^{-1}) and P2 (0.2257 hr^{-1}). In terms of pectinase activity, M1 again showed superior performance, reaching 8.15 U/mL in pectin media, followed by 7.38 U/mL using pineapple peel, and 7.032 U/mL using banana peel. These results confirm that strain M1 is the most effective among the tested isolates for both growth and enzyme production. Additionally, this study demonstrates that fruit waste substrates—particularly pineapple peel are viable, low-cost alternatives to commercial pectin, contributing to sustainable enzyme production. This aligns with circular economy principles by transforming agro-industrial waste into value-added products, such as biocatalysts for industrial use.

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Declaration of Competing Interest

The author declares no conflicts of interest.

CRediT Authorship Contribution Statement

L.X. Yi (Liew Xin Yi): Conceptualisation; Methodology; Investigation; Data curation; Formal analysis; Writing – original draft; Visualisation; Project administration.
S.A.A. Manaf (Shoriya Aruni Abdul Manaf): Validation; Writing – review & editing;
M.A. Jenol (Mohd Azwan Jenol): Supervision; Validation; Writing – review & editing; Resources; Writing – original draft; Visualisation; Project administration; Funding acquisition.

Availability of Data and Materials

The data supporting this study’s findings are available on request from the corresponding author.

Ethics Declarations

This study did not involve human participants or animals. Ethical approval was therefore not required.

Generative Artificial Intelligence Declarations

The authors claim that artificially intelligent-assisted technologies, such as generative AI, were not used to generate content, ideas, or theories. We have just utilised AI to enhance readability and refine the language. This was used with extreme human control and oversight. The authors take full responsibility for reviewing and approving the content.

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