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ORIGINAL RESEARCH PAPER

Physicochemical characterization of biodegradable polymer polyhydroxybutyrate from halophilic bacterium local strain *Halomonas elongata*

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ABSTRACT

BACKGROUND AND OBJECTIVES: Petroleum-based plastics produce tremendous amounts of plastic waste every year, which contributes to environmental problems. Biological polymers, such as polyhydroxybutyrate, have caught attention as an ecofriendly substitute to petroleum-based plastics. The present study focused on the production, enhancement, and characterization of polyhydroxybutyrate from the prospective local bacterium *Halomonas elongata*. This research aimed to develop an environmentally sustainable material for reducing the accumulation of plastic waste in the ecosystem.
METHODS: A local bacterial strain from Mud Crater Bledug Kuwu, Grobogan, Central Java, Indonesia, was isolated and identified as *Halomonas elongata*. Nile red staining method confirmed that this bacterium accumulated polyhydroxybutyrate. The effect of incubation time, sodium chloride concentration, nitrogen, and carbon sources were evaluated via gas chromatography to enhance its productivity. The functional groups of isolated polyhydroxybutyrate were analyzed using nuclear magnetic resonance and Fourier transform infrared spectroscopy. Morphology and composition were demonstrated by scanning electron microscopy and energy-dispersive x-ray spectroscopy. Thermogravimetric analysis, differential thermogravimetry, and differential thermal analysis were used to analyze thermal stability.
FINDINGS: *Halomonas elongata* produced polyhydroxybutyrate utilizing glucose as a carbon source, as evidenced by orange-fluorescence colonies under ultraviolet light. The optimum condition of polyhydroxybutyrate production was achieved when the bacterium was cultivated in a high medium containing 5 percent sodium chloride, 0.2 percent yeast extract, and 5 percent glucose (as measured by weight per volume) after 72 hours of incubation. The maximum polyhydroxybutyrate production in this medium reached 2.93 ± 0.03 gram per liter dry cell weight and 78 ± 1 percent polyhydroxybutyrate concentration. Structural elucidation studies revealed that the biopolymer produced by this bacterium was high-purity polyhydroxybutyrate, as proven by the presence of functional groups and proton resonance signals in the monomer structure. The isolated polyhydroxybutyrate consisted of 14 percent carbon and 86 percent oxygen. Thermal stability analysis showed that the isolated polyhydroxybutyrate had a maximum decomposition temperature of 270 degrees Celsius. Micrographically, the isolated polyhydroxybutyrate appeared as a sheet structure with interconnected fibers measuring 0.7–0.8 micromter in length. This finding also demonstrates that the isolated polyhydroxybutyrate has good thermal stability given that fibers linked each polyhydroxybutyrate molecule, which boosted the structure of polyhydroxybutyrate.
CONCLUSION: This study successfully synthesized polyhydroxybutyrate using a local strain of *Halomonas elongata*, with glucose as a carbon source. Physicochemical characterization revealed that polyhydroxybutyrate from this bacterium has a high thermal stability. The yield of polyhydroxybutyrate can be increased through the improvement of production parameters. This research emphasizes an important milestone toward the large-scale production of polyhydroxybutyrate for application as food packaging while reducing environmental issues.

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NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
56	10	1

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INTRODUCTION

Petroleum-based plastic refers to a synthetic polymer that comprises natural gas and crude oil, and it is distinguished by its high flexibility, strength, durability, and good thermal insulation (Febria *et al*., 2024). This unavoidable material is extensively used in various industries (Rodrigues *et al*., 2019), including electronics (Singh *et al*., 2020), construction (da Silva *et al*., 2021), transportation (Vieyra *et al*., 2022), medicine (Mohammadalipour *et al*., 2023), packaging (Manikandan *et al*., 2020), etc. Given its numerous applications, more than 260 million metric tons of this plastic is produced globally (Thompson *et al*., 2009). Despite its wide range of applications, this material is difficult to degrade naturally. Undecomposed plastic accumulates high volumes of plastic waste (Dalal *et al*., 2023), which poses a threat to human health (Seewoo *et al*., 2023) and the environment (Tian *et al*., 2022). According to the Organization for Economic Cooperation and Development Global Plastic Outlook, the world generated 353 million tons of plastic waste in 2019, which increased by more than twofold since 2000 (Amobonye *et al*., 2021). Several approaches, including recycling, incinerating, and landfilling (Evode *et al*., 2021), are used to address this issue. Only 14 percent (%) of waste is recyclable, and 14% are incinerable despite their emission of carbon dioxide (CO_2) and consumption of considerable energy. A total of 72% of wastes are released into the environment via landfilling, which results in decreased soil fertility (Zhang *et al*., 2020). An innovative approach to ecofriendly polymers, such as bioplastics, is necessary to reduce and replace petroleum-based plastics (Evode *et al*., 2021). Bioplastics include biodegradable polymers produced from sustainable materials, and they can potentially reduce plastic waste (Rosenboom *et al.,* 2022). Based on their sources, these polymers can be classified into products from biomass (polysaccharides, protein, and lignin) (Lim *et al.,* 2021), biotechnology (polylactide acid) (Bella *et al.,* 2021), and microorganism polyhydroxyalkanoates (PHA) (Adnan *et al*., 2022). PHA is a polyester that naturally decomposes and accumulates as a reserve compound in the form of intracellular granules in various bacteria (Tarrahi *et al*., 2020). Polyhydroxybutyrate (PHB) is the most extensively studied and characterized member of the PHA family (Das and Maiti, 2021). This compound plays an essential role in the long-term sustainability of bioplastics because it is fully biodegradable, hydrophobic, resistant to ultraviolet (UV) light, and oxygen permeable (Adnan *et al*., 2022). These characteristics have piqued the interest of governments and researchers because of the potential of PHB to replace conventional plastics, such as polyethylene (PE) and polypropylene (Chek *et al*., 2019). Under extreme conditions with limited amounts of phosphorus and magnesium but abundant carbon, many bacterial strains produce PHB (Rizki *et al*., 2023). Suitable nutrients, such as minimal media with appropriate carbon and nitrogen sources, are essential for bacteria to synthesize PHB. Simple sugars, such as glucose, are readily absorbed and converted by bacteria to boost the production of PHB, which is easier and faster to degrade (Munir and Jamil, 2018)., PHB can be utilized directly as a homopolymer for liposome stabilizers and UV filters in active-compound delivery systems for cosmetics (Pavelkova *et al.,* 2020). Meanwhile, indirectly, PHB is manufactured as a copolymer with polyhydroxyvalerate to generate poly (hydroxybutyrate-co-hydroxyvalerate), which is utilized as a leak-proof packaging material (Kulkarni *et al.,* 2011). In the agricultural sector, the copolymer poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) has been patented by Daminar Scientific as a biodegradable and easily compostable mulch (Samrot *et al.,* 2021). The copolymer of PHB with polyethylene glycol has shown an advantage in the release of sorafenib and doxorubicin for anticancer drug delivery (Babos *et al.,* 2020). Halophilic bacteria have been explored as potential homopolymer PHB producers due to their adaptability to high salt levels and ability to prevent contamination (Ji *et al.,* 2023). Indonesia has a unique mud crater located far from the sea and periodically spouts high saline water; it is located in Bledug Kuwu, Purwodadi, Grobogan, Central Java (Fazli and Hertadi, 2018). In 2018, a moderately halophilic bacterium from that region was successfully isolated and identified as *H. elongata* BK-AG25, which produces a compatible solute ectoine (Parwata *et al*., 2019). The present study focused on the production, enhancement, and characterization of PHB from the local strain *H. elongata* BK-AG25 as a potential producer of PHB. The current study aimed to increase the production of PHB with a high thermal stability from *H. elongata* BK-AG25 and conducted in the biochemistry laboratory, Chemistry Department, Faculty of Mathematics and Natural Sciences,

Bandung Institute of Technology in 2023.

MATERIALS AND METHODS

Qualitative assay

Qualitative assessment of PHB accumulation in *H. elongata* BK-AG25 was performed using a staining method. Bacterial isolates were cultured in accordance with a previous procedure. Then, the ability of this culture to synthesize PHB using glucose as a carbon source was determined through the addition of Nile red solutions following a previously described method. Positive results were demonstrated by colonies that glowed orange when exposed to UV light (Rizki *et al*., 2023).

Production and extraction of PHB

PHB production from *H. elongata* BK-AG25 was performed following a previous procedure; a high medium (HM) containing glucose as the sole carbon source was used (Rizki *et al*., 2023). Through the extraction method, PHB granules were obtained via the addition of chloroform: sodium dodecyl sulfate (SDS) solution (1:1) to dried pellet cells. The chloroform phase was added with four times the volume of cold methanol to precipitate PHB. The resulting PHB yield was calculated using Eq. 1 (Rizki *et al*., 2023).

$$PHB\ yield\left(\%\right)=\frac{weight\ PHB}{Dry\ cell\ weight\left(DCW\right)}\times 100\% \quad (1)$$

Increasing PHB production

PHB production was increased incubation time (24–120 hours (h)) and HM components, such as sodium chloride (NaCl) concentration, nitrogen sources, and carbon sources. In this experiment, the NaCl concentration was varied at 1–20 percent (%) weight/volume (w/v). The 5% carbon sources included palm-oil mill effluent (POME), glucose, and a combination of POME–glucose (1:1). The nitrogen sources were varied at 0.2% (w/v) concentration of yeast extract, ammonium sulfate, and a combination of yeast extract–ammonium sulfate (1:1). The quantity of PHB in dry cell weight (DCW) was determined using a gas chromatography–flame ionization detector (GC-FID) from Agilent Technologies-7820A (Juengert *et al*., 2018). The DCW was suspended in 1 milliliter (mL) chloroform and 1 mL methanolysis solution (containing sulfuric acid/methanol, 15:85), heated to 100 degrees Celsius (°C) for 2 h, and cooled to room temperature. The solution was added with 0.5 mL demineralized water and then vortexed for 1 minute (min) until homogenous. The chloroform phase containing methyl ester monomers was injected into the GC-FID (Variant 3400) (Mostafa *et al*., 2020).

PHB characterization

The physicochemical properties of the PHB produced by *H. elongata* BK-AG25 to were determined using analytical tool kits.

Functional groups

The functional groups of the PHB monomer were determined via the Fourier transform infrared (FTIR) investigation of the PHB structure. The PHB granules were mixed with potassium bromide (KBr) crystals to form a pellet. The pellet sample was observed using the FTIR instrument (from Shimadzu, Japan) at 400–4500/centimeter (cm) for 8 scans (Rizki *et al*., 2023).

Structural elucidation

The structure was further elucidated using proton nuclear magnetic resonance (^{1}H NMR) spectroscopy to validate the monomer structure and purity. The dried granules were dissolved in deuterochloroform ($CDCl_3$) and homogenized via vortexing for 10 min. The spectrum was recorded using an Agilent 500 Megahertz (MHz) NMR spectrometer with a second-generation DirectDrive console system, and tetramethyl silane was used as the internal standard.

Thermal stability

A thermogravimetric approach was applied to study the thermal stability of isolated PHB granules. These thermal stabilities of decomposition temperature, decomposition rate at maximum temperature, and phase transitions were assessed. Measurement was performed under a nitrogen atmosphere over a temperature range of 30 °C-600 °C (all from Hitachi Corporation in Japan) (Rizki *et al*., 2023).

Morphology and composition

Scanning electron microscopy (SEM) was performed to observe the morphology of isolated PHB granules (JEOL, Japan) at 20,000x magnification. Then, the composition was analyzed via energy dispersive X-ray spectroscopy (EDS) using an EDAX detector (Rizki *et al*., 2023).

RESULTS AND DISCUSSION

Qualitative assay

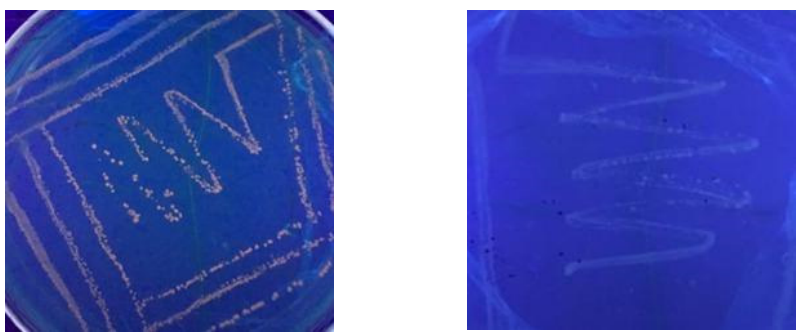
Qualitative assay involved the detection of PHB accumulated by *H. elongata* BK-AG25 via Nile red staining (Fig. 1). *Escherichia coli* TOP10 served as a negative control. Fig. 1a reveals that colonies of *H. elongata* BK-AG25 exhibited an orange fluorescence when exposed to UV light, which indicates the strong binding affinity of Nile red for ester groups on the PHB molecule (Morya *et al*., 2018). *Rhodococcus* sp. strain BSRT1-1, which can produce PHB utilizing glucose as a carbon source, also gave positive results and showed a high orange fluorescence intensity during Nile red staining (Trakunjae *et al*., 2021). These results prove the ability of *H. elongata* BK-AG25 to accumulate PHB.

PHB production

The PHB content was correlated to dried cells and mass of PHB granules (Fig. 2). *H. elongata* BK-AG25 reached 380 ± 50 milligram per liter (mg/L) of DCW, PHB granules was up to 82 ± 10 mg/L medium, and the PHB content amounted to 21.2 ± 1.6%. For the highest PHB production of this bacterium, PHB production variables, such as incubation period, NaCl concentration, carbon source variation, and nitrogen source variation, were optimized for further analysis.

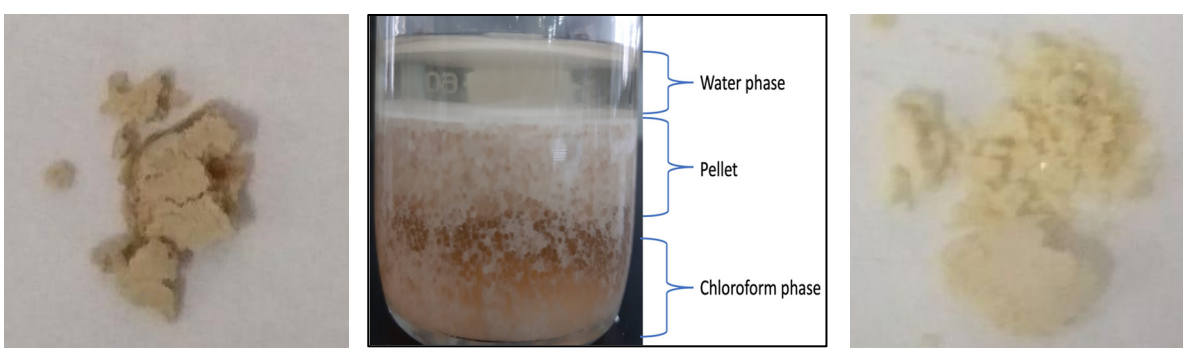
Improvement of PHB production

PHB production factors, such as incubation time and nutritional components, considerably affect the amount of PHB produced by bacteria. Incubation time was optimized to ascertain the maximal quantity of PHB that can be stored as an energy source before further degradation. Meanwhile, nutritional factors, such as carbon, nitrogen, and salt influence, bacterial cell growth and are directly proportional to PHB production.



(a) (b)

Fig. 1: Qualitative assay using Nile red staining. a) *H. elongata* BK-AG25 and b) *E. coli* TOP10 as a negative control



(a) (b) (c)

Fig. 2: PHB production. a) DCW, b) extraction process using SDS and chloroform, and c) PHB granules

Suitable incubation time

The optimum production time for the maximum production of PHB was determined by varying the incubation times to 24, 48, 72, 96, and 120 h. Fig. 3 depicts the effect of incubation time on DCW and PHB content. The slight decrease in DCW might have been caused by inadequate nutrition, which consequently affected the production of PHB after 48 h incubation. Afterward, 72 h incubation yielded the maximum PHB content of 76 ± 4%. The findings are consistent with those of previous studies on *Bacillus* sp. NII2, which also attained the maximum PHB content at 72 h incubation (Sirohi *et al*., 2021). Furthermore, several studies have shown that PHB synthesis by *Halomonas elongata* requires a 72-h incubation period (Mohanrasu *et al.,* 2020).

Optimum NaCl concentration

The NaCl concentration in the HM medium was varied between 1%, 5%, 10%, 15%, and 20% (w/v) to determine the optimal salt concentration for PHB production by *H. elongata* BK-AG25. Fig. 4 shows

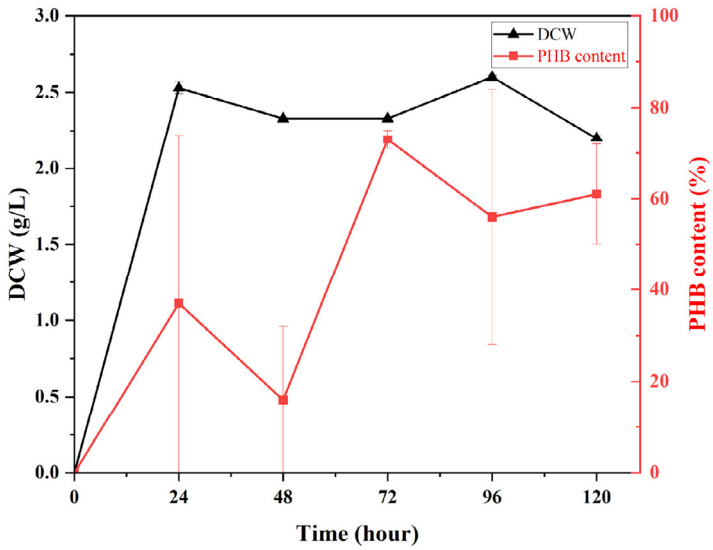


Fig. 3: Suitable incubation time for PHB production by *H. elongata* BK-AG25

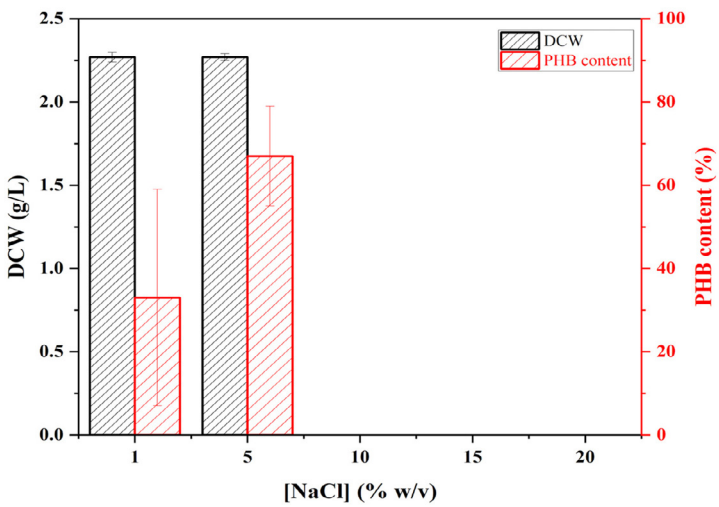


Fig. 4: Optimum NaCl concentration for PHB production by *H. elongata* BK-AG25

the effect of NaCl concentration on DCW and PHB contents. The highest PHB content was obtained at 5% (w/v) NaCl with 2.27 ± 0.02 gram per liter (g/L) DCW and 72 ± 6% PHB content. These data are consistent with those of a previous study that obtained the highest PHB content from *Salinivibrio* sp. at 5% (w/v) NaCl (Rizki *et al*., 2023). Thus, *H. elongata* BK-AG25 can also be considered a moderate halophilic bacterium.

Suitable nitrogen source

For the investigation of the influence of nitrogen sources on PHB synthesis by *H. elongata* BK-AG25, alterations were made on various nitrogen sources on the modified HM. With yeast extract as a nitrogen source, the highest DCW and PHB content were 4.07 ± 0.05 g/L and 63 ± 14%, respectively (Fig. 5). Yeast extract can stimulate cell development, which results in a high biomass. This finding is consistent with that of prior research on PHB production by *Erythrobacter aquimaris* (Mostafa *et al*., 2020). The use of ammonium sulfate caused no effect on bacterial growth and PHB accumulation, which suggests that the compound cannot be absorbed by bacterial cells. Notably, the combination of yeast extract and ammonium sulfate attained PHB concentration similar to that obtained using a single yeast extract, and the results were statistically indistinguishable. This synergistic effect was also observed in PHB production by *Salinivibrio* sp. (Rizki *et al*., 2023).

Suitable carbon source

The effect of carbon source on PHB production by *H. elongata* BK-AG25 was studied by varying the carbon sources, such as glucose, POME, and a combination of both. The PHB-producing bacterium metabolized POME and glucose as carbon sources to accumulate PHB. Fig. 6 shows the highest DCW and PHB content of 2.93 ± 0.03 g/L and 78 ± 1%, respectively, which were obtained by utilizing glucose as a carbon source. The data aligned with those of a previous study, which revealed that PHB production by *Haloarcula* sp. strain NRS20 using 10 g/L glucose as a carbon source can produce 2.397 mg/L PHB (Hagagy *et al*., 2022). Upon comparison of these carbon sources, glucose was preferred to promote PHB production. Glucose was probably more easily metabolized by PHB-producing bacteria than POME (Sun *et al*., 2020). POME is liquid palm-oil waste containing high glycerol concentrations (Fazli and Hertadi, 2018). According to PHB biosynthesis pathways, glycerol is an unfavorable carbon source for PHB production (Cui *et al*., 2015). In addition, excess glycerol concentration may inhibit cell growth because of its toxicity (Kim *et al*., 2022). These findings are consistent with our results. Based on the findings of a single-factor optimization, maximum production

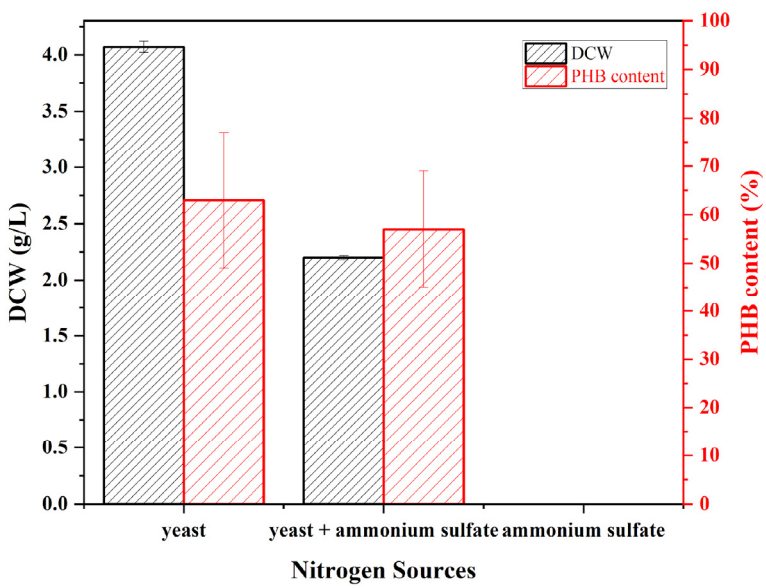


Fig. 5: Suitable nitrogen source for PHB production by *H. elongata* BK-AG25

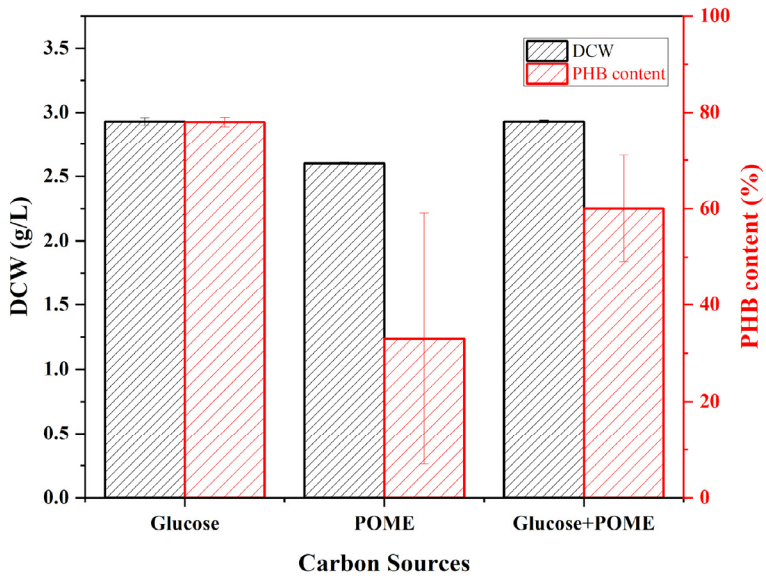


Fig. 6: Suitable carbon sources for PHB production by *H. elongata* BK-AG25

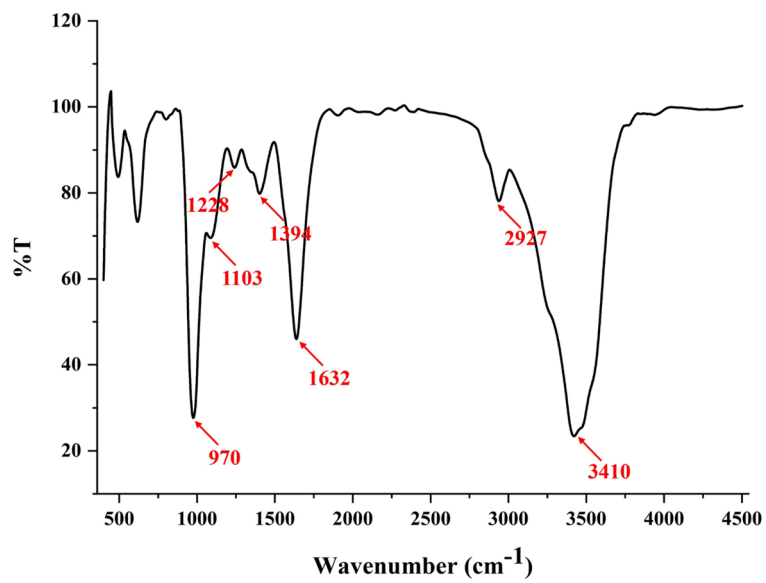


Fig. 7: FTIR spectra of isolated PHB

occurred within 72 h in a HM medium containing 5% NaCl, 0.2% yeast extract, and 5% glucose, which yielded 2.93 ± 0.03 g/L and 78 ± 1% PHB content.

Characterization of isolated PHB

Fourier transform infrared spectrometer (FT-IR) analysis

FTIR analysis was conducted on the functional groups of the isolated PHB (Fig. 7). PHB is an ester polymer formed through the condensation of alcohol and carboxylic acid groups. Comparable to that of PHB produced by *Providencia* sp., the band at 1632/cm confirmed the presence of Carbonyl group (C=O) stretching from the ester group (Kopperi *et al*., 2021). In addition, the absorption band at 970–1228/cm emphasized the presence of C-O ester compared with

that of PHB from *Rhodococcus* sp. BSRT1-1 at 1000–1300/cm (Trakunjae *et al*., 2021). The bands at 1394–2927/cm revealed the presence of C-H stretching vibration of the methyl groups. These results align with those of the commercialized standard PHB at 1394 and 2939/cm (Rizki *et al*., 2023). The broad and prominent absorption band at 3410/cm confirmed the stretching vibration of terminal -OH groups in the PHB molecule. Similar results were observed for PHB from *Bacillus* sp. RR02 at 3416/cm (Aluru, 2020). This data confirms that the functional groups of the sample isolated from *H. elongata* BK-AG25 consisted of PHB.

^{1}H NMR analysis

^{1}H NMR was used to corroborate the structure and purity of the isolated PHB (Fig. 8). The ^{1}H NMR spectrum of the isolated PHB revealed four H signals, namely, a doublet at 1.26 part per million (ppm), two double doublets at 2.46 and 2.59 ppm, and a quartet at 5.24 ppm. The peak at 1.26 ppm corresponded to -CH3 protons, those at 2.46 and 2.59 ppm to -CH2 protons, and that at 5.24 ppm to -CH protons. These data are in line with those of a previous study, which reported four signals in the ^{1}H NMR spectra of PHB from *Bacillus cereus* NDRMN001 at 1.67, 2.37, 2.71, and 5.28, which correspond to -CH3, -CH2, and -CH protons (Narayanan *et al*., 2020). Another previous study revealed that predominant peaks at 1.26 ppm corresponded to -CH3, followed by those at 2.44–2.63 and 5.29 ppm, which corresponded to -CH2 and -CH, respectively, in the PHB molecules produced by *Pseudomonas plecoglossicida* (Sabarinathan *et al*., 2018).

Thermal stability of isolated PHB

The thermal stability of isolated PHB granules was assessed via a thermogravimetric approach (Fig. 9). Fig. 9a depicts the thermogravimetric analysis (TGA) curve showing the presence of two weight-loss processes. The initial step resulted in 2.3% weight loss at 0−167 °C, which was due to the presence of evaporating solvents, such as chloroform, methanol, acetone, and water, during extraction and purification (Rizki *et al*., 2023; Pradhan *et al*., 2018). In the second step, 91% weight loss occurred rapidly at 168–270 °C, which suggests the maximum decomposition of PHB. In this case, decomposition possibly reduced

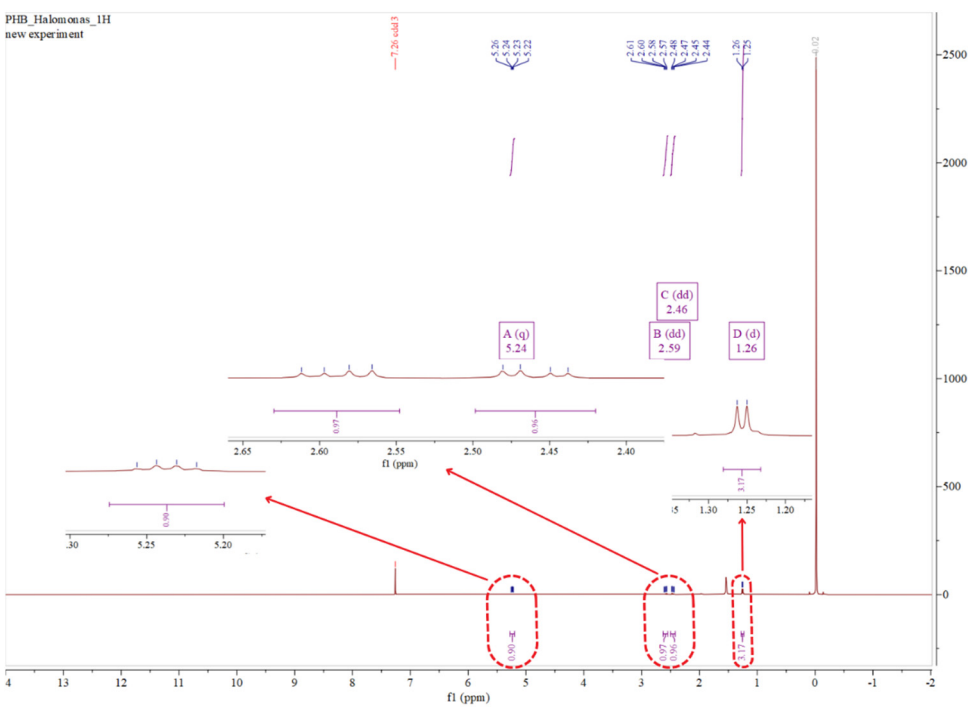


Fig. 8: NMR spectrum of isolated PHB

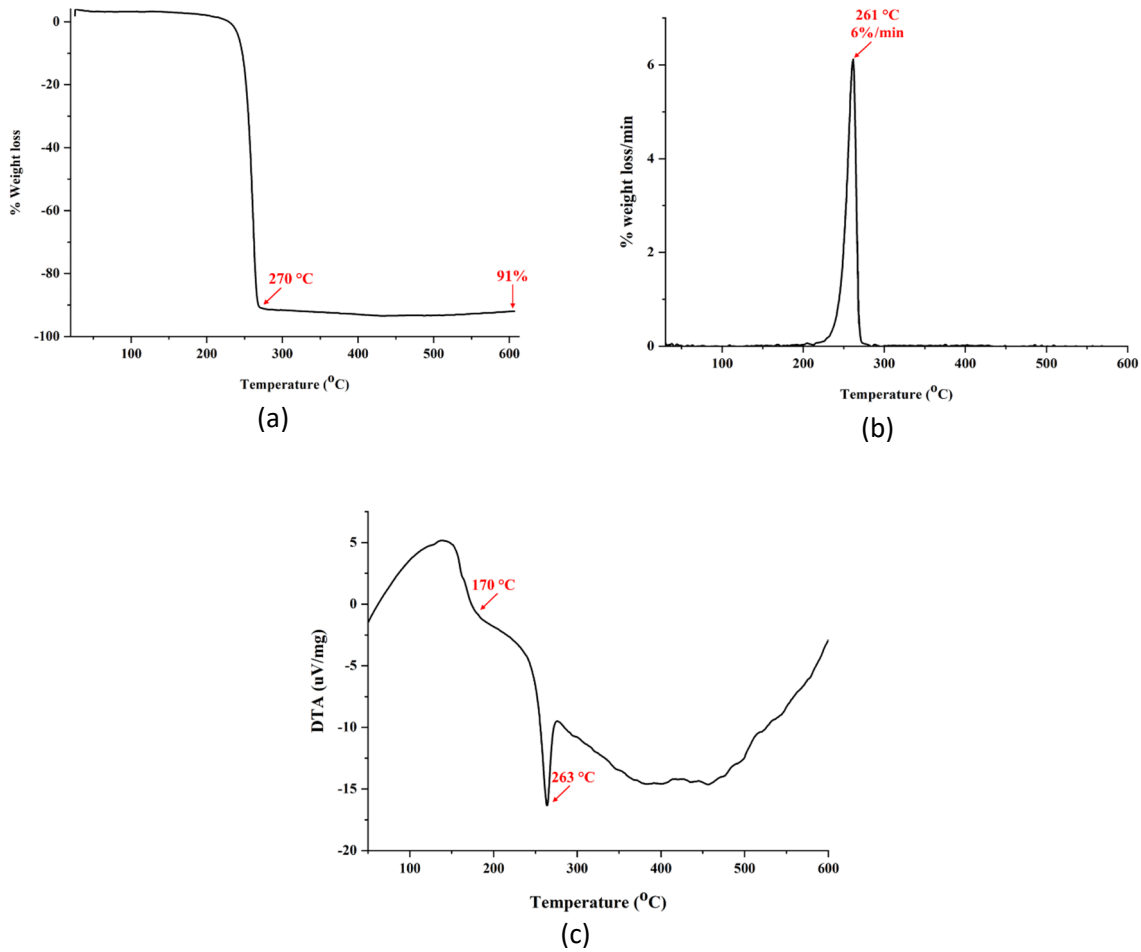


Fig. 9: Thermal stability of PHB. a) TGA, b) DTG, and c) DTA curves.

the molecular weight of PHB due to the polymer chain degradation that involved the breaking of ester bonds in the PHB molecule (Sirohi *et al*., 2021). Fig. 9a indicates that the highest decomposition of isolated PHB occurred at 270 °C. Previous studies reported that PHB from *Nostoc muscorum* NCCU-442 using glucose as a carbon source showed 80% weight loss at 256–284 °C (Ansari and Fatma, 2016); *Ralstonia eutropha* H-16 utilizing makgeolli lees enzymatic hydrolysate as a carbon source achieved maximum decomposition at 269.7 °C (Gang *et al*., 2019). Fig. 9b shows the differential thermogravimetry (DTG) curve determining the PHB decomposition rate at the maximum temperature. The highest decomposition of isolated PHB occurred at 261 °C with a 6%/min decomposition rate. Previous studies reported the decomposition of standard PHB at 236 °C at a rate of 30%/min. These findings indicate the higher thermal stability of isolated PHB than standard PHB (Pradhan *et al*., 2018). Fig. 9c reveals the differential thermal analysis (DTA) curve showing a small broad exothermic peak at 170 °C and a prominent exothermic peak at 263 °C. These data refer to PHB's thermal stability, which can be correlated to the phase transitions and combustion of hydrocarbon chains within the PHB molecule (Pradhan *et al*., 2018; Sirohi *et al*., 2021).

SEM and EDS of isolated PHB

SEM and EDS were used to study the morphology and composition of isolated PHB, respectively (Fig. 10). Micrographically, the isolated PHB possessed a sheet structure (Fig. 10a). Similarly, PHB from *Priestia*

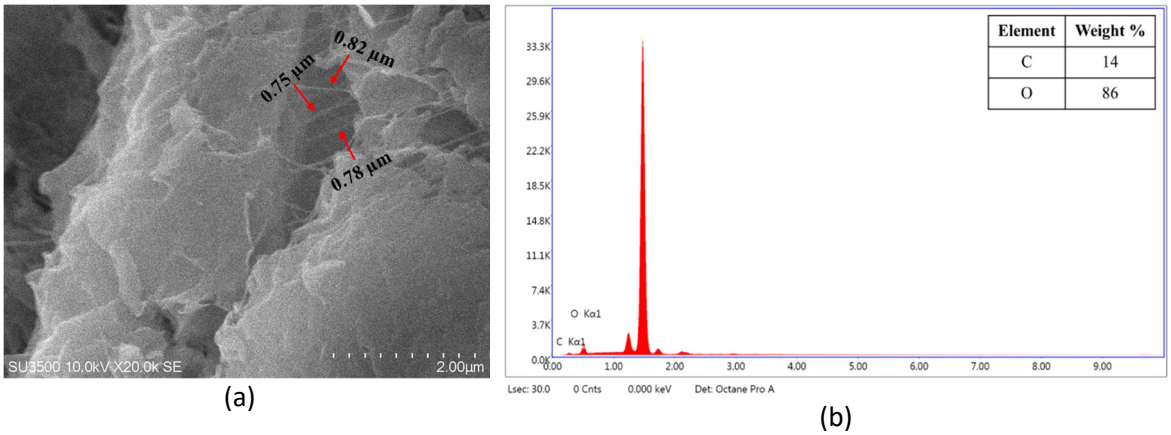


Fig. 10: Morphology and composition of PHB. a) SEM image and b) EDS spectrum

Table 1: Comparison of PHB concentration and thermal decomposition of *H. elongata* BK-AG25 and those of previous studies

No.	Bacterial strain	Carbon source	PHB content (%)	Decomposition temperature (°C)	Sources
1	*Halomonas salina*	Glucose	26.2	260.3	Hernández-Núñez *et al.*, 2019
2	*Bacillus megabacterium* strain B2	Cacao mucilage exudates (CMEs)	57	270.6	Quintero-Silva *et al.*, 2023
3	*Halomonas alkaliantarctica*	Cheese whey mother liquor	20.1	286.7	Mozejko-Ciesielska *et al.*, 2023
4	*Salinivibrio* sp.	Palm oil mill effluent	40	282.9	Rizki *et al.*, 2023
5	*Azotobacter vinelandii* OP	Grape residues	37.6	274.6	Andler *et al.*, 2023
6	Halomonas elongata BK-AG25	Glucose	78	270	The current study

flexa using glucose as a carbon source exhibited a brittle structure (Chathalingath *et al.*, 2023). Notably, fibers measuring 0.7–0.8 micrometer (µm) linked each sheet of isolated PHB, which possibly contributed to a robust PHB structure. These findings support the thermal data in Section 3.3, which show the good thermal stability of the isolated PHB. Thus, the isolated PHB had a high thermal stability. The EDS spectrum (Fig. 10b) revealed the presence of 14% carbon and 86% oxygen, and no other elements were detected. A high O content is related to the chain length and possibly contributes to thermal stability (Rizki *et al.*, 2023). PHB forms when alcohol condenses with carboxylate groups to form ester groups (McAdam *et al.*, 2020). The more -OH groups at the end of a polymer chain, the shorter the polymer chain. The results reveal the short chain length of the isolated PHB. Despite its short length, the fiber strengthened the polymer chain by forming numerous layers that resisted degradation. These findings support the thermal data presented in Section 3.3, which reveals the high thermal stability of the isolated PHB. Table

1 shows a comparison of PHB concentration and thermal decomposition of *H. elongata* BK-AG25 and the findings of previous studies.

Optimal conditions enable various bacteria to produce the highest levels of PHB utilizing different carbon sources (Table 1). Furthermore, the carbon source affects the thermal properties of the isolated PHB, which decomposes between 240 °C (Zhang *et al*., 2020) and 290 °C (Samorì *et al*., 2022). By contrast, plastics derived from petroleum-based products, including PE, decompose thermally between 200–300 °C (Ghosal and Nayak, 2022). Compared with PHB, these materials have nearly identical thermal stability. However, they are nondegradable and contribute to plastic waste accumulation in the environment. Therefore, PHB can be further developed as a sustainable material to address environmental issues and promote a circular economy.

CONCLUSION

In this study, PHB was successfully synthesized using a local strain bacterium (*H. elongata* BK-AG25) that utilized glucose as a carbon source. PHB accumulation by this bacterium was confirmed through Nile red staining, which yielded positive results as evidenced by the orange-fluorescence colonies under UV light. The maximum PHB concentration reached 78 ± 1% the HM containing 5% (w/v) NaCl, 5% (w/v) glucose, and 0.2% (w/v) yeast extract was used during 72 h of incubation. Functional group analysis via FTIR revealed that *H. elongata* BK-AG25 produced PHB, as demonstrated by the presence of C=O stretching of ester groups, C-H stretching vibration of methyl groups, and stretching vibration of terminal -OH groups at 1632, 1394–2927, and 3410/cm. Further analysis utilizing NMR was conducted to elucidate the structure and ensure the purity of the isolated PHB. The obtained NMR spectrum revealed the signals of a doublet for $-CH_3$ at 1.26 ppm, double doublet for $-CH_2$ at 2.46 and 2.59 ppm, and a quartet for -CH at 5.24 ppm. Based on the results of FTIR and NMR analyses, the biomaterial synthesized by *H. elongata* BK-AG25 was high-purity PHB. TGA, DTG, and DTA were performed to determine the thermal stability of the isolated PHB. The TGA curve revealed the presence of two weight-loss processes. The initial step revealed the evaporation of solvents used during the extraction and purification of the isolated PHB, such as chloroform, methanol, acetone, and water, which resulted in 2.3% weight loss at 0−167 °C. The second step revealed the decreased molecular weight of PHB due to polymer-chain degradation, which involved the breakage of ester bonds in the PHB molecule. This process resulted in a rapid weight loss (91%) at 168–270 °C, which suggests the maximum decomposition of PHB. In addition, DTG was performed to estimate the weight loss rate at maximum temperature. The highest decomposition of the isolated PHB occurred at 261 °C at a 6%/min decomposition rate. On the other hand, the DTA curve revealed a small broad exothermic peak at 170 °C, followed by a prominent exothermic peak at 263 °C. The three last methods confirmed the high thermal stability of the isolated PHB. In addition, the isolated PHB exhibited a sheet structure with 0.7-0.8 µm fibers connecting each layer of PHB molecules, which strengthened the PHB structure. These data support previous results showing the high thermal stability of isolated PHB. The EDS spectrum revealed that the isolated PHB comprised 86% oxygen and 14% carbon, with no additional elements. The high O content indicates that more -OH groups were attached to the end of the polymer chain. Based on these findings, the isolated PHB had a short chain length, which possibly contributed to its thermal stability. Despite the short chain length, the fiber reinforced the polymer chain through the generation of several degradation-resistant layers. The findings also confirm that the isolated PHB is highly thermally stable. Therefore, *H. elongata* BK-AG25 is a promising producer of PHB with high thermal stability. Given its unique physicochemical properties, PHB can be used as a biodegradable polymer to replace PE in food packaging applications. In addition, PHB can be used to promote a circular economy and reduce environmental problems.

AUTHOR CONTRIBUTIONS

W.O.S. Rizki conceptualized the study, designed the experiments, validated the results, and prepared the manuscript. S. Komariah performed the experiments and prepared the manuscript. D.G.T. Andini performed literature review and wrote and edited the manuscript. A.T. Simbara performed a literature review and wrote and edited the manuscript. E. Ratnaningsih performed literature review, validated the results, and edited the manuscript. R. Hertadi, the corresponding author, performed literature review and interpreted and validated the results.

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CONFLICT OF INTEREST

The authors declare that no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been completely observed by the authors.

ABBREVIATIONS

°C	Degree Celsius
µm	Micrometer
%	Percent
BK-AG25	*Halomonas elongata* strain
-CH	Methyl group
-CH2	Methyl group
-CH3	Methyl group
-OH	Hydroxyl
$(NH_4)_2SO_4$	Ammonium sulfate
^1H *NMR*	Proton nuclear magnetic resonance
C	Carbon
C-H	Carbon-hydrogen bond
C-O	Carbon-oxygen bond
C=O	Carbonyl group
$CaCl_2.2H_2O$	Calcium chloride dihydrate
CDCl3	Deuterated chloroform
CH_3Cl	Chloroform
CO_2	Carbon dioxide
cm	Centimeter
DCW	Dry cell weight
DD2	Second-generation direct drive console
DMF	N,N-dimethylformamide
DTA	Differential thermal analyzer
DTG	Differential thermogravimetric analysis
E. coli TOP10	*Escherichia coli* TOP10
FTIR	Fourier transform infrared spectrometer
g/L	Gram per liter
GC-FID	Gas chromatography with flame ionization detection.
h	Hour
H_2SO_4	Sulfate acid
H. elongata BK-AG25	*Halomonas elongata* BK-AG25

HM	High medium
KBr	Potassium bromide
KCl	Potassium chloride
KH_2PO_4	Potassium dihydrogen phosphate
LB	Luria Bertani
mA	Milliampere
MeOH	Methanol
mg/L	Milligram per Liter
$MgSO_4.7H_2O$	Magnesium (II) sulfate heptahydrate
MHz	Megahertz
min	Minute
mL	Milliliter
NaBr	Sodium bromide
NaCl	Sodium chloride
nm	Nanometer
O	Oxygen
PE	Polyethylene
PP	Polypropylene
PHA	Polyhydroxyalkanoates
PHB	Polyhydroxybutyrate
POME	Palm Oil Mill Effluent
ppm	Part per million
rpm	Revolutions per minute
SDS	Sodium dodecyl sulfate (surfactant for cell lysis)
SEM-EDS	Scanning Electron Microscopy and Energy Dispersive X-ray Spectroscopy
TGA	Thermogravimetric Analysis
UV	Ultraviolet
w/v	weight per volume

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