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POLYHYDROXYALKANOATES PRODUCTION FROM *RALSTONIA PICKETTII* BACTERIA: STRUCTURAL AND MECHANICAL STUDIES

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Abstract. *Bacterial Polyhydroxyalkanoates (PHAs) are a remarkably versatile category of biodegradable polymers with a variety of applications in the packaging, agricultural, biomedical, and pharmaceutical fields. In the present study, bacterial PHAs films are characterized by Fourier transform infrared (FTIR), Scanning electron microscope (SEM), Gas chromatography-mass spectroscopy (GC-MS), Differential scanning calorimetry (DSC), and Universal testing machine (UTM). It was found that almost 20% (w/w) of PHAs was produced from Ralstonia pickettii, and the five major types of the produced polymer were validated via FTIR analysis, i.e., 1046-1185 cm^{-1} (C-O stretching), 1723 cm^{-1} (C=O stretching), 2974-2926 cm^{-1} (CH_3 and CH_2 stretching) and 3450 cm^{-1} (OH stretching). The GC-MS chromatogram generated two main peaks, i.e., 2-butenoic acid methyl ester and 4-hexenoic acid methyl ester, at retention times of 4.62 min and 5.79 min, respectively. The main compounds of 2-butenoic acid methyl ester and 4-hexenoic acid methyl ester had percentage areas of 28% and 43%, respectively. Based GCMS analysis shows two monomer PHAs ie. 2-butenoic acid methyl ester and 4-hexenoic acid methyl ester, correspondingly. The blending of PHAs R. pickettii and PEG-400 positively decreases thermal properties and tensile strength and increases elongation at break.*

Keywords: *R. pickettii, polyhydroxyalkanoates, PEG-400, tensile strength, elongation at break*

1. Introduction

Petroleum-based plastics were first developed in 1940. Nowadays, these products have caused severe degradation to ecosystems [1]. Due to the high crystallinity and molecular weight, surface area, and poor morphology of synthetic plastics, it is difficult for enzymes and water to penetrate and diffuse within the polymer network, thus limiting biodegradation processes [2-3]. synthetic plastic's detrimental effects have led to the death of marine mammals as well as some species of seabirds [4]. Consequently, the use of natural polymers is vital in facilitating enzyme

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accessibility to structure degradation of the biopolymer. Various natural sources such as starch, pectin, and carrageenan have been utilized in the production of composite plastics, including microbial-derived polymers such as cellulose, polylactic acid, and polyhydroxyalkanoates (PHAs) [5-6]. In particular, PHA is a linear polyester accumulated in the form of intracellular granules that can be utilized for a mixture of synthetic plastics or other applications in the field of pharmaceutical, agriculture, biofuel, drugs, and chromatography [7–8].

Polyhydroxyalkanoate is a polyester synthesized by several original and recombinant bacteria, eukaryotic, and microalga [7]. Hence, different sources of microbes and production conditions will result in various kinds of HAPs [9]. Currently, the new route of biosynthesis from bacteria has remained unexploited, except the eight routes. The first route has been studied, involving three key enzymes of *β*-ketothiolase, NADPH-dependent acetoacetyl-CoA reductase, and PHA synthase, and is codified by the genes phaB, and phaC, respectively. These three genes are combined in *Ralstonia eutropha* on an operon that is relatively constant during PHA production [10-11].

The investigation is continuing to find a variety of PHAs-producing sources, particularly those derived from microorganisms. Meanwhile, *R. pickettii* is a species of *Ralstonia* with the ability to synthesize PHA, although no previous study reported this bacteria as a proficient source. Microorganisms could produce secondary metabolites depending on several factors, including enzyme complexity and metabolite availability. The carbon source determines the PHA types that are provided in the various forms of copolyester, homo-polyester, polyester, or ter-polyester [12]. The metabolism of PHA occurs at different levels, namely first is the activation of gene expressions through specific environmental labels such as food deficiency; second is the activation of the artificial PHA enzymes by specific cell components or metabolic media; third is the inhibition of metabolic enzymes from the competitive paths that enrich the mediators required for PHA synthesis; or fourth the mixture of all these pathways [13].

Bacterial-produced PHA has significantly higher potential value for industrial applications because of its high crystallinity and elongation at break properties. However, some limitations exist, such as high brittleness and low thermal stability [14]. One alternative to improve the thermal properties is to mix with other types of polymers such as cellulose, starch, β-propiolactone, polyvinyl acetate, polypropylene, polylactic acid, and polyethylene glycol. These polymers have been used for the modification of the physical and mechanical properties of PHA [14–16]. Previous studies [15] and [17] also reported the influence of PEG400 in changing the physical and mechanical properties of poly (3-hydroxybutyrate) [15,17]. It was found that PEG400 reduced the tensile strength and increased the elongation of pure PHB at the break. Therefore, PEG400 was used in this study to modify the PHA *R. pickettii*, also, the essential properties of the pure and

composite forms were evaluated by the related instruments, such as the identification of functional groups in PHA using Fourier transform-infrared (FT-IR), the analysis of monomers with gas chromatography-mass spectrometry (GC-MS), and morphology characterization using scanning electron microscopy (SEM). The thermal and mechanical properties were also assessed with differential scanning calorimetry (DSC) and the universal testing machine (UTM).

2. Methods

2.1. Materials

R. pickettii NBRC 102503 strains were purchased from the NBRC (NITE Biological Resource Center), Kisarazu-shi. Chiba, Japan. Other materials used include Glucose, Nutrient agar (Himedia), Nutrient Broth (Himedia), Methanol (Merck, ≥99%), and *n*-hexane (Merck, ≥99%). All chemical reagents used were of analytical grades and purchased from local suppliers.

2.2. Culture conditions

The media used to prepare the inoculum was a nutrient broth containing 5 $g.L^{-1}$ peptones and 3 $g.L^{-1}$ beef extracts. The PHA was synthesized using 5% glucose as the carbon source. The pH of the media was adjusted to 7.0 using NaOH or HCl, the culture was incubated for 30 h at 30°C in a rotary shaker at 150 rpm [17, 19].

2.3. PHA Extraction

The biomass pellet was separated from the substrates by centrifugation at 3000 rpm, 15°C, and for 30 minutes. The pellets were then washed with ethanol and distilled water separately to eliminate undesirable substances. All generated cells were freeze-dried at -89 °C. After lyophilization, 0.5 g of cells were resuspended in 50 mL of chloroform: methanol (2:1 v/v) in glass flasks and incubated at 60 °C for 5 h under vigorous shaking [20]. To remove the cellular debris, the samples were filtered under vacuum using a Millipore filter with 0.5 mm pores and a 47 mm diameter. The solubilized polymer in the chloroform: methanol solution was then placed in a rotary evaporator to evaporate the solvent. The PHA granules were washed using n-hexane to remove the fats. After extraction, the polymers were measured using a gravimetric technique to determine the PHA production in $g.L^{-1}$.

2.4. Preparation of PHA Films

The preparation of PHA films was carried out by varying the plasticizer (PEG400) concentration at 10%, 20%, 30%, and 40% (w/w). The bioplastic films were made by dissolving 0.27 g of PHA in chloroform (20 mL), then the solution was consistently stirred for 1h at 50°C using an upright cooler. After one hour, PEG400 was added to 0.03 g of the PHA chloroform

solution to produce a concentration of 10%, then all mixed solution was stirred again for 15 min. After the solution was thoroughly mixed, it was placed into a glass and stored at room temperature until the chloroform evaporated and produced a film. The same procedure was performed for the other plasticizer concentrations of 10%, 20%, 30%, and 40% (w/w). Pure PHA film was prepared without plasticizer as a reference.

2.5. Physico-chemical Characterization

Here, PHA samples were characterized by FTIR spectroscopy (SHIMADZU) between 4000 cm^{-1} and 400 cm^{-1} [21, 22]. A 10 mg of PHA sample was dissolved in chloroform and dropped on a KBr pellet, then it was analyzed by infrared spectroscopy. Film morphology was examined using SEM (Zeiss EVO MA-10). A thin layer of film was mounted on the holder of aluminum specimens by double-sided tape. It was coated with gold/palladium, with a thickness of about 30 nm, the film surface was then recorded at the desired magnification [23,24]. DSC was used to analyze the thermal properties of PHA and PHA/PEG400 in concentrations of 10%, 20%, 30%, and 40% including the melting (melting point, T_m), and glass transition temperature (T_g), as well as enthalpy changes on the samples during the process. The samples were weighed up to approximately 20 mg and placed into the 40 µL crucible. The analysis was performed by heating the sample from 30°C up to 200°C with a flow rate of about 10°C/min. Liquid nitrogen was used for the cooling process with a flow velocity of 50 mL/min. Measurement of tensile strength and elongation was carried out using a Universal Testing Machine (UTM) with the brand Strograph VG10-E. The test method is referred to as ASTM D 882-92. A sample of the shaped sheet was cut with a length of 45 mm and a width of 10 mm. The investigation was performed with a speed of 10 mm/min. Both tensile strength of PHA and composite PHA/PEG400 was calculated with the equation:

$$\tau = \frac{F_{max}}{A} \quad \text{..... (1)}$$

Where τ: tensile strength (MPa); Fmax: maximum strength load (Kgf); A: area of the cross-section (mm^2). Meanwhile, elongation was calculated with the equation (Jang, 1994):

$$\varepsilon = \frac{d - a}{a} \text{ x } 100\% \quad \text{... ... ... (2)}$$

Where ε: elongation (%); d: length at break (mm); a: length at first (mm).

2.6. Gas Chromatography-Mass Spectrometry (GC-MS)

PHAs were prepared by the degradation and acetylation processes before conducting GC-MS analysis (GC Agilent HP6890). Subsequently, PHAs were dissolved in a mixture of chloroform/methanol solution of 3 mL; 2:1, which had previously been preheated. About 300 mg

of periodate acid was added to the mixture of tetrahydrofuran/water (3 mL; 8:1) and was stirred for 1h at room temperature (25°C). After one hour, the reaction was halted by adding 10 mL of water and was re-stirred for only a few minutes. The solution was extracted with 15 mL of *n*-hexane using three times repetitions with a vortex. All *n*-hexane extracts were combined and were evaporated afterward with a rotary evaporator. 1 mL of PHAs-degraded solution obtained from the dissolved chloroform was pipetted into another test tube. It was added with 0.85 mL of methanol containing approximately 0.15 mL of concentrated sulfuric acid. The mixture was subsequently heated at 80°C for 2h, then cooled, and 1 mL of distilled water was added. All mixture was then executed with a vortex for 1 minute until the organic layer and the water were technically separated. A total of 1 mL of the organic layer at the bottom was injected into the GC-MS. The GC-MS conditions are as follows Table 1.

Table 1. GC-MS analysis conditions

Parameter	GC-MS
Column	HP-5ms (5%-Phenyl)-methylpolysiloxane, L of 30m and D of 250 µm
Career gas	Helium
Heating rate	10°C/min
Injection mode	Split Ratio: 5:1 - Flow split: 3.7001 mL/min
Injection volume	1 µL
Initial T	80°C for 2 min
Final T	280°C
Final time	24 min
Column flux	0.74 mL/min (10 psi)

3. Results and Discussion

3.1. Production and Extraction of PHA

The production of PHA was performed in the phase of the stationary cell, following the growth curves. In this condition, the cells accumulate carbon reserves used in energy metabolism and regeneration. *R. pickettii* performs as a PHA reshuffle that comes into the energy cycle with the help of PHA depolymerase (PhaZRpiT1) [23, 24]. Therefore, the extraction in this study was complete in the stationary phase at 21st and 27th hours. It began with the isolation of biomass using centrifugation at 5000 rpm for 10 minutes and lyophilization at -98˚C, for producing dry biomass of 0 .56 g/L. The biomass concentration of PHA-producing cells varies greatly due to various factors which affect the fermentation process. However, a different result was found in *Azotobacter beijerinckii* WDN-01 of 4.7 g/L and *Cuprividus necator* of 0.15 g/L [25, 26]. The differences in the numbers of harvested biomass were considerably influenced by several factors, such as the type of isolates either original or recombinant, the source of carbon or nitrogen, fermented pH, C/N ratio, and fermentation method including batch or fed-batch. The obtained lyophilic cells were refluxed using a solvent mixture of chloroform: methanol (2:1) for ±5 hours

at 50 ˚C. The reflux was aimed at breaking down the cell membrane for even distribution of cell and PHA granules compartment into the solvent. The separation process used some filter papers, culminating in the production of filtrates which were added with *n*-hexane to precipitate the PHA. Based on the results, the yield of PHA *R. pickettii* was 20% (w/w) and influenced by numerous factors, such as the type of strains as well as the carbon sources. Meanwhile, *Cupriavidus necator* H16 produced a yield ranging from 3.9% to 40.7% using acetic, butyrate, lactate, and propionate acid as carbon sources [27]. *Pseudomonas putida* also produced a yield between 8.93% and 1.75% [28], while sludge bacteria yielded 61.40% PHA, with non-volatile fatty acids (non-VFAs) [29]. All harvested PHAs at this stage were further characterized according to their functional groups, thermal and morphology properties, and the blending with polyethylene glycol 400 (PEG400).

3.2. Fourier Transform-Infrared (FT-IR)

FT-IR analysis was used to determine the main functional groups of PHA produced by *R. pickettii*. The FTIR spectra before and after the purification process as well as the form of composite PHA/PEG400 are shown in Figure 1. The highly visible peaks that appear different in PHAs were found at the peak of C=O, on the band of 1657 cm^{-1} and 1723 cm^{-1} before and after purification, respectively. This could be explained by the fact that before purification, it was still components of protein, carbohydrates, and fats in the polymer which caused overlapping peaks. Furthermore, the peak of OH before purification was sufficiently comprehensive. This might occur due to the contribution of OH, which is derived from fat. In contrast to the case after the purification, the peak of free OH was no longer found in wider bands [28–30]. The spectrum results of the FTIR on the crude PHA samples indicated the presence of 5 major peaks, at the wavenumbers of 1046-1185 cm^{-1}, 1723 cm^{-1}, 2926 cm^{-1}, 2974 cm^{-1}, and 3450 cm^{-1}. All discovered peaks correlated with pure PHA, at the wavenumbers of 1054 cm^{-1}, 1726 cm^{-1}, 2933 cm^{-1} 2984 cm^{-1}, and 3440 cm^{-1} [31–33].

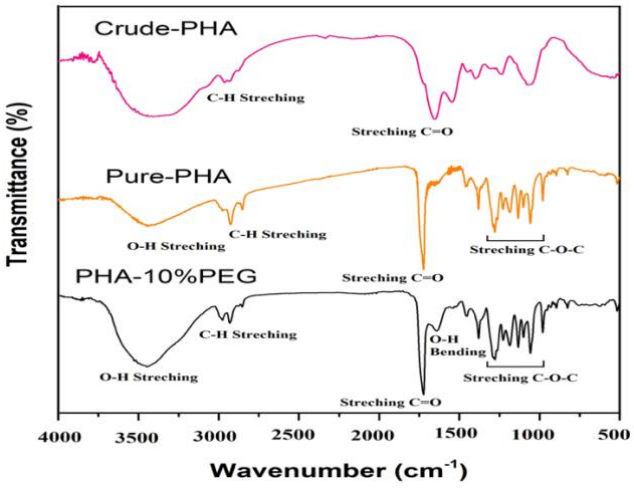

Figure 1. FTIR spectrum of PHA and PHA/PEG-400

The functional group characteristics of R. pickettii were confirmed on commercial PHA. The wavenumbers in the commercial FT-IR PHB spectrum were as follows: 1054 cm-^{1} indicated the vibrational binding of C-O, 1726 cm^{-1} for C=O in ester, 2933 cm^{-1} and 2984 cm-^{1} from C-H, as well as 3440 cm^{-1} for O-H [16]. PHA *Bacillus cereus* has the same characteristics of spectra as *R. pickettii*. The wavenumber at 1000-1300 cm^{-1} was produced from the vibrational binding of C-O-C, 1675-1735 cm^{-1} was for carbonyl ester, 1530 cm^{-1} represents C-H, while 2930 cm^{-1} and 3272 cm^{-1} were for OH [32, 34, 35]. Similarly, the PHA produced from *Pseudomonas guezennei* showed the structure of a typical ester with intense absorption peaks, which is by the stretching tape of the carbonyl ester (C=O) at 1723 cm^{-1} and the three intense ribbons between 2951 and 2961 cm^{-1} indicating a stretching asymmetric and symmetric mode of CH_3 and CH_2 [36]. Stretching tape of C=O at the spectral range of 1740-1720 cm-^{1} is the vibrational movement of the carbonyl groups of amorphous and crystal. In this study, the wave number 1723 cm^{-1} demonstrates the C=O stretching band in PHBHHx [31, 32, 35]. For the interaction of PHA/PEG400, it was confirmed that O-H stretching at 3400 cm^{-1} became wider, including OH bending, at 1641 cm^{-1}.

3.3. Gas Chromatography-Mass Spectrometry (GC-MS)

To determine the monomer constituents, GC-MS analysis was used. Based on the results, the PHA produced was composed of the copolymer of P(HB-*co*-HHx), namely polyhydroxy butyrate copolymer hydroxyhexanoate. The initial treatment before injection was the process of degradation and esterification of the polymer. The degradation process was conducted by dissolving PHA in chloroform and reacting it with a periodate acid, which was dissolved in tetrahydrofuran as a source of H^+ at room temperature. The reaction was terminated by pouring some additional water, hence, both the aqueous and organic phases comprising fragments of PHB or HHx were obtained. Afterward, fragments formed in a carboxylic acid were subjected to the esterification process using methanol and a sulfuric acid catalyst.

The esterification products were further analyzed using GC-MS, based on the results, two significant peaks were found at the retention time of 4.62 min and 5.79 min, respectively. These include peaks from 2-butenoic acid methyl and 4-hexenoic acid methyl ester. The others were the peaks of minor components, such as the fatty acids of hexadecanoic and tetradecanoic acid. These are groups of fatty acids extracted altogether with polyhydroxyalkanoates and other components. The percentage area produced by the major acid was 28% and 43%, while for the minor ones, the average was below 5%. Figures 2 and 3 show a spectrum pattern of the mass acid compounds of 2-butenoic acid methyl ester and 4-hexenoic acid methyl ester. The peak of the molecular ions at *m/z* 100 correlated with the relative mass of 2-butenoic acid methyl ester (Figure 2), while the peak of the molecules at m/z 128 was by the 4-hexenoic acid methyl ester (Figure 3).

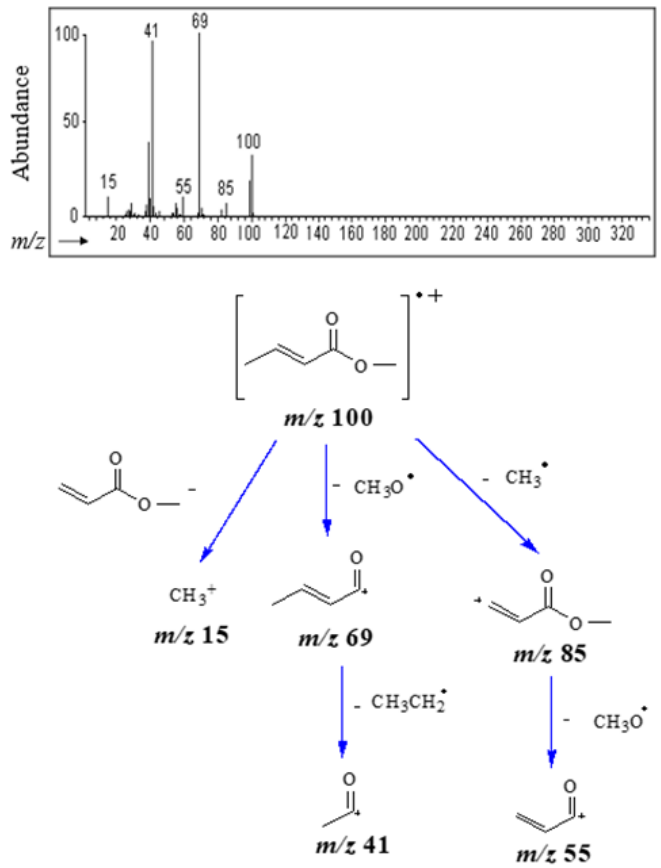

Figure 2. Mass Spectrum and Fragmentation pattern in 2-Butenoic acid methyl ester.

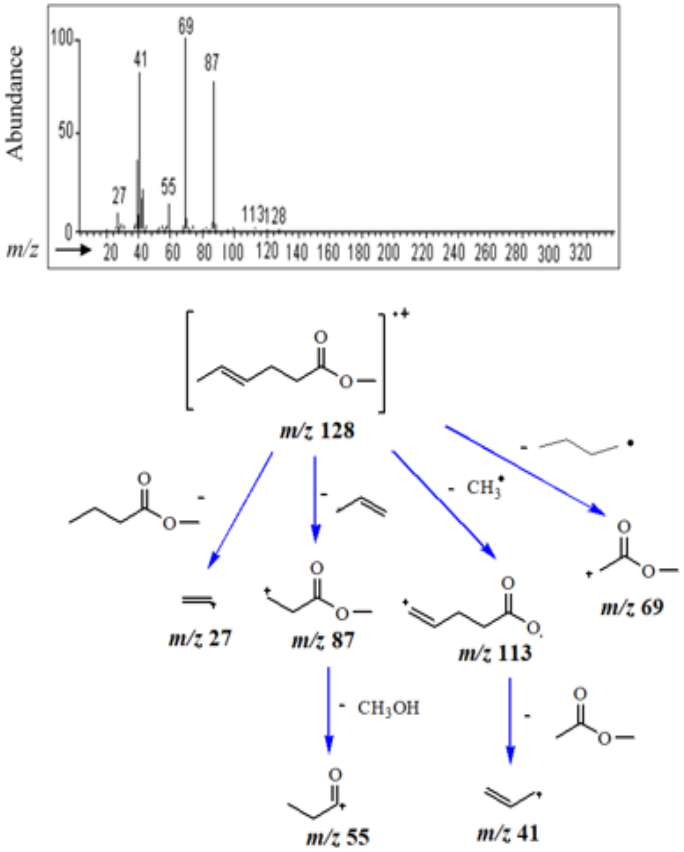

Figure 3. Mass Spectrum and Fragmentation pattern in 4-hexenoic acid methyl ester

Figure 2 shows the release of the consecutive methoxy and methyl radicals, as well as the molecules of acrylic acid methyl ester from the molecular ions producing cations at m/z 69 or base peak, 85, and 15. The fragment cation with m/z 69 released the methoxy radicals, culminating in a cation of m/z 55. Meanwhile, m/z 41 was produced from the ethyl radical release in the fragment cation of m/z 69. Figure 3 shows the peak of molecular ions at m/z 128, which is in line with the relative mass acid compounds of 4-hexenoic acid methyl ester. The consecutive release of butyl and methyl radicals, including molecules of propane and butyric acid methyl ester from the molecular ions, produced several cations at m/z 69 or base peak, 113, 87, and 27. The fragment cation with m/z 113 subsequently discharged the acid molecules of acetic methyl ester, leading to the fragment cation of m/z 41. The m/z 55 was produced from the methanol molecule discharge at the fragment cation of m/z 87. Based on GC-MS analysis, the PHA polymer consists of the monomer 2-butenoic acid and 4-hexenoic acid methyl ester, while alkene or crotonic monomers were identified as thermodynamic products. This process is a dehydration reaction producing alkenes as the most stable structure in acidic conditions. The reaction begins with hydroxyl-oxygen protonation that allows hydrogen at the alpha position to stabilize structures in the form of alkenes. Besides, crotonic formation generally occurs in acidic conditions [37] and through thermal degradation *through the 6-membered ring* [38].

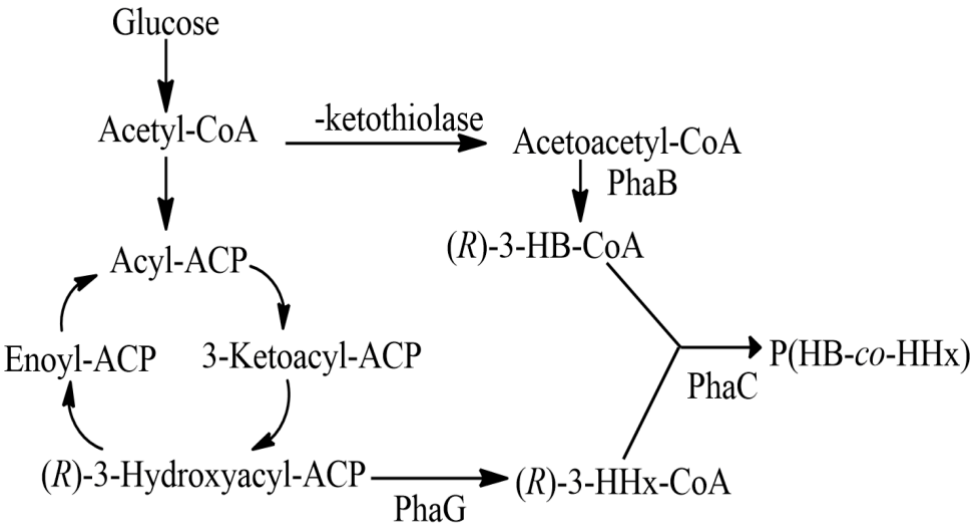

Figure 4. Biosynthesis of P(HB-*co*-HHx) from *P. putida* GPp104 [39]

PHA produced from *Ralstonia pickettii* as a strain test was composed of the copolymer P(HB-*co*-HHx) based on the analysis of GC-MS. This type of copolymer has also been reported in *Ralstonia eutropha* [40]. Copolymer P(HB-co-HHx) is in the recombinant group of *Aeromonas hydrophila*, *Pseudomonas putida*, and *Aeromonas hydrophila* [41]. The varying monomer structure is due to the different metabolic pathways contained within each microorganism. Each

monomer produced either from the same carbon source will pass through a different path. PHA homopolymers undergo a single pathway, while heteropolymers use a combined pathway. The investigated PHB-co-HHx biosynthesis through recombinant *P. putida* GPp104 is shown in Figure 4 [39].

Figure 4 shows the synthesis of P(HB-*co*-HHx), the precursor obtained from two different pathways. Firstly, HB-CoA was supplied from acetyl-CoA and then catalyzed by β-ketotiolase and acetoacetyl-CoA reductase. Secondly, HHx-CoA was supplied from the HHx-ACP intermediates of fatty acids de novo biosynthesis catalyzed by PhaG. The activity of the PhaG was then encoded by its genes to supply HHx-CoA. Afterward, PhaC (PHA synthase) polymerizes HB-CoA and HHx-CoA into P(HB-*co*-HHx) [42]. The second path differs in the supply of (R)-3-Hydroxyacyl-CoA derived from the Enoyl-CoA, and catalyzed by PhaC. Enoyl-CoA is considered an intermediate product of β-oxidation of fatty acids [43]. The second pathway was studied through *P. putida* GPp104 and *A. hydrophila* 4AK4 [39]. Besides, the catalytic mechanism in PHA depolymerase occurs in *Penicillium funiculosum*. In this model, Ser39 was responsible for the nucleophile attacks on the carbonyl carbon of the PHB chain, thereby forming covalent intermediates of acyl-enzymes followed by hydrolysis of water molecules [44]. Nucleophilicity from the hydroxyl group of Ser39 was enhanced by the system of hydrogen bonds of His155-Asp121. The genetic analysis also discovered certain catalytic triads comprising S139, D214, and H273 on the enzyme of PhaZRpiT1 (PHA depolymerase of *Ralstonia pickettii*), which degraded PHA [26]. The excess amount of PHA depolymerase indicates that the optimum time of PHA extraction from *R. pickettii* is in the middle of the stationary phase. At the end of this phase, the activity and the synthesis of PHA depolymerase increase to fulfill the carbon intake in the system of energy metabolism.

3.4. Morphology of PHA and PHA/PEG400

The SEM of pure PHA and PHA/PEG400 at 10%, 20%, 30%, and 40% are shown in Figure 5(a-e). Morphological observations showed that the PHA and PHA/PEG films had a porous structure. The addition of PEG-400 tends to maintain the pore morphology of the PHA films. This is consistent with a previous study that stated that the addition of PEG plasticizer in PHBHV films can increase the porosity of the films by approximately 6 - 7% [45]. The pores formed on the PHA and PHA/PEG films indicate that this polymer has a low density. In addition, the polymer pores in the film of this study can serve as access for the enzymes of decomposing microorganisms to reach the interior of the polymer. The physical condition of this polymer indicates that PHA is an environmentally friendly green polymer [15].

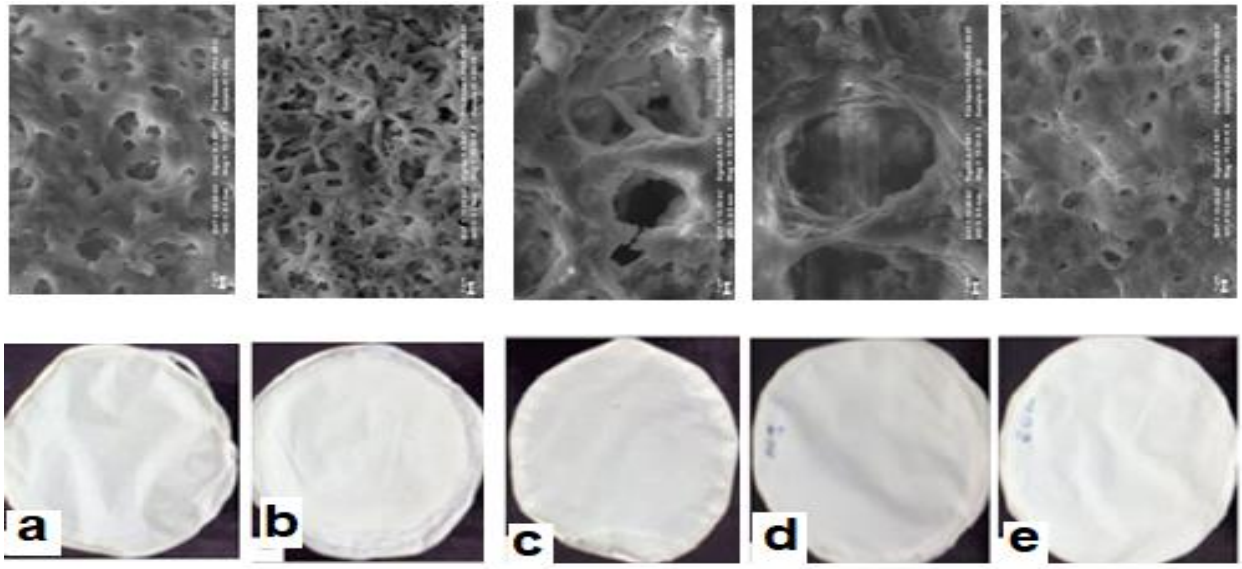

Figure 5. Morphology of PHA a). pure; b). PHA/PEG 10%; c). PHA/PEG 20%; d). PHA/PEG 30%; and e) PHA/PEG 40%

3.5. Thermal Properties of PHA and PHA/PEG400

The thermal property, specifically the transition temperature, is the point where the polymers experience changes in their nature or form due to either the elevation or decline in the temperature. The analyzed parameters in PHA comprise the temperature of glass intermediate Tg (glass transition) and the melting point Tm. The transition temperature of glass was not detected because the measurement process was initiated at room temperature and depended on the sensitivity of the tools used, hence, changes in the smallest enthalpy when the alterations occurred on samples from the glass into thermoplastic were not captured [46]. Meanwhile, the transition temperature of polymer glass was detected at 2-5^{o}C [15]. Tg is closely related to the mobility of the polymer chain segments, a low value implies low polymer crystallinity [46]. The profile of the DSC curve of the pure PHA and PHA/PEG400 at 10%, 20%, 30%, and 40% are shown in Table 2.

Table 2. The Melting point of PHA and PHA/PEG400

No	Sample	T_{m1} (°C)	T_{m2} (°C)
1	PHA	174	-
2	PHA/PEG 10%	150	168
3	PHA/PEG 20%	160	-
4	PHA/PEG 30%	140	153
5	PHA/PEG 40%	153	163

Table 2 shows that pure PHA has a Tm at 174˚C, and a lower value was found in a mixture of PHA and PEG400. This result is similar to the commercial PHA Tm, namely 173˚C and 177 ˚C reported by [12] and [17], respectively. Table 2 shows the decrease in Tm values in the PHA/PEG400 mixture. The values for the 10, 20, 30, and 40% PEG400 samples were 168˚C, 165 ˚C, 158˚C, and 165˚C, respectively. According to a previous study, the presence of plasticizers, in

general, can reduce the Tm of the native polymer [22], [47]. This is because PEG400 destroys the interpolymer arrangement and polymer crystallinity, thereby reducing the energy required to melt the polymer [12,15,44].

3.6. Tensile Strength and Elongation at Break

The physical properties of the crude and pure polymers can be identified through the tests of tensile strength and elongation at break. This is because a single or mixed polymer provides different properties of tensile strength and elongation at break based on the nature of the bonding and the type of monomers involved [48]. The results showed that tensile strength decreases in line with the elevation in the concentration of plasticizers and elongation at break. A precise mixture of PEG might cause the mobility of the PHA polymers to increase and enhance the viscoelastic of polymers when admitting external forces which in turn, raises the elongation value accordingly [12]. However, PEG might also reduce the bond energy against the chains of PHA polymer, which culminates in a decrease in tensile strength. The results are shown in Table 3.

Table 3. Tensile strength and Elongation at Break of PHA and PHA/PEG

Blend (%)	Tensile strength (MPa)	Elongation at break (%)
PHA/PEG100/0	0.16 ± 0.02^{a}	1.68 ± 0.11^{a}
PHA/PEG 90/10	0.13 ± 0.01^{b}	4.62 ± 0.22^{b}
PHA/PEG 80/20	0.11 ± 0.01^{c}	4.90 ± 0.21^{b}
PHA/PEG 70/30	0.10 ± 0.04^{d}	5.50 ± 0.24^{c}
PHA/PEG 60/40	0.08 ± 0.01^{e}	7.78 ± 0.15^{d}

Data are mean ± standard deviation (n = 2). Values with different superscripts within columns are significantly different ($p < 0.05$).

Table 3 shows that the tensile strength gradually reduced, while the elongation at break increased after the PHA was added with PEG400. Plasticizers can reduce cohesion interaction in the PHA molecules by forming hydrogen bonds between carbonyl and hydroxyl groups [17,47]. These intermolecular interactions presumably increase chain flexibility and elongation at break [15]. In addition, the decrease in tensile strength value was caused by the increasing distance between the polymer chains culminating in an inhibited chain motion which affects the strength of the PHA chain intermolecular bonds [5,46]. The same decrease in tensile strength was reported for the P(HB-co-HV) *Rhizobium meliloti* composite with glycerol, polyethylene glycol, and polyvinyl acetate (PVAc). The presence of the three plasticizers decreased the tensile strength four times and increased the elongation at break 12 times [48]. A further decrease in tensile strength was also found in the mixture of PHB and PLA [5]. The decrease or increase in tensile strength and elongation at break in this study and other reports indicate that plasticizers affect the mechanical properties of PHA or other composited polymers [22,45].

4. Conclusions

Polyhydroxyalkanoates were successfully synthesized from *Ralstonia pickettii* which contains polyhydroxy butyrate copolymer hydroxyhexanoate (PHB-co-HHx) based on GC-MS analysis results. PEG400 plasticizer showed no morphological changes in PHA but contributed to a decrease the thermal properties of the film. In addition, the plasticizer PEG400 had an essential role in enhancing the mechanical properties of the produced films compared to pure PHA film. PHA-PEG 400 base composite showed promising results. The decrease in thermal properties illustrates that this composite supports the development program of biodegradable plastics.

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