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ORIGINAL ARTICLE

Biosynthesis of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) in *Bacillus aryabhattai* and cytotoxicity evaluation of PHBV/poly(ethylene glycol) blends

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Abstract

The study described poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) accumulation in *Bacillus aryabhattai* PHB10 for the first time and evaluated the polymer induced cytotoxicity in-vitro with PHBV/poly(ethylene glycol) (PEG) blends. The *B. aryabhattai* strain produced 2.8 g/L PHBV, equivalent to 71.15% of cell dry mass in a medium supplemented with propionic acid, after 48 h incubation. The optimum temperature and pH for the copolymer accumulation was 31 °C and 7, respectively. The gas chromatography–mass spectrometry and nuclear magnetic resonance analyses confirmed the polymer obtained as PHBV. The differential scanning calorimetry analysis revealed that the melting point of the material as 90 °C and its thermal stability up to 220 °C. The average molecular weight (Mn) and polydispersity index of the sample was estimated by gel permeation chromatography analysis and observed as 128.508 kDa and 2.82, respectively. The PHBV showed tensile strength of 10.3 MPa and elongation at break of 13.3%. The PHBV and their blends with PEG were tested for cytotoxicity on human keratinocytes (HaCaT cells) and the cells incubated with PHBV/PEG2kDa blends were 99% viable, whereas with the PHBV alone showed comparatively higher cytotoxicity. The significant improvement in the cell viability of PHBV/PEG2kDa blends indicates its potential as a candidate for skin graft applications.

Keywords *Bacillus aryabhattai* PHB10 · Cytotoxicity · Gas chromatography–mass spectrometry · HaCaT cells · Poly(ethylene glycol) · Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)

Introduction

Polyhydroxyalkanoates (PHA) are polyesters accumulated in microorganisms as intracellular carbon and energy storage material under unbalanced growth conditions (Andreeben et al. 2010). Owing to their biodegradability and physical properties similar to most of the synthetic plastics, PHAs are considered as a green substitute for petroleum-derived plastics. Poly-3-hydroxybutyrate (PHB) is the first discovered, most common and simplest form of PHA found in bacteria (Lemoigne 1926). The inferior physical properties of PHB such as brittleness, high crystallinity and instability during the melting stage hinder their wide-spread applications (Wang et al. 2013). The copolymer of 3-hydroxybutyrate (3-HB) and 3-hydroxyvalerate (3-HV), namely poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) is synthesized in bacteria especially when the growth medium contains organic acids (Chen et al. 2011). The superior properties of PHBV such as better thermal behavior, plasticity, toughness and biodegradability make them more attractive in the bioplastic market (Shang et al. 2004).

3-HV and 3-HB, the monomer units of PHBV are synthesized simultaneously in bacterial cytoplasm via two parallel pathways. The former one is synthesized by the condensation of two acetyl-coenzyme A molecules, and the later one is formed by the condensation of acetyl-coenzyme A and propionyl-coenzyme A molecules (Chen et al. 2011). Therefore, when propionic acid is supplemented in the culture medium constituted for PHB production, it acts as a direct precursor that triggers the formation of 3-HV, in addition to 3-HB. This leads to the copolymer accumulation in bacterial cells. PHBV production in genus *Bacillus* has been reported previously by supplementing the medium with organic acids

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(Kumar et al. 2006; Güngörmedi et al. 2014; Moorkoth and Nampoothiri 2016).

PHBV with varying molar ratios of 3-HV are of great importance in the field of biomedical engineering such as the fabrication of cardiovascular stents, drug delivery systems, surgical sutures, medical packaging, etc. (Avella et al. 2000; Riekes et al. 2013; Vilos et al. 2013; Wu et al. 2013; Smith and Lamprou 2014). Also, it has been reported that electrospun PHBV nanofibers are ideal for skin tissue engineering applications (Sundaramurthi et al. 2013). Nevertheless, reports are also available on further improvement of PHBV by blending with other polymers such as poly(butylene succinate), poly(ethylene glycol) (PEG), poly(lactic acid), etc. (Modi et al. 2013; Hu et al. 2017). Among these polymers, PEG lacks toxicity, antigenicity and consequently does not induce any immunogenicity on human cells. Hence PEG has been commonly applied in a wide range of therapeutic formulations, and it will be eliminated from the body through kidneys (Catoni et al. 2013). The blending of PHAs with PEG improves the surface hydrophilicity of the polymer; thereby increases the cell viability and it has been reported to be effective in improving the biocompatibility of PHBV (Shabna et al. 2014; Monnier et al. 2016). Therefore, PHBV-PEG blends can have high application potential in biomedical fields.

Bacillus aryabhattai was first reported from cryotubes used for collecting air from the upper stratosphere by Shivaji et al. (2009). Later, the bacterium was isolated from soil in many parts of the world (Lee et al. 2012; Pailan et al. 2015; Yan et al. 2016) and the PHB accumulating property of this strain was first studied by Van-Thuoc et al. (2012). The *B. aryabhattai* strain PHB10 used in the present study is also an environmental bacterial strain, and it has been reported previously for high levels of PHB accumulation in their cytoplasm (Pillai et al. 2017a). The genome of this strain harbours a short-chain-length PHA-specific polymer synthase gene (*phaC*) responsible for its PHB biosynthesis (Pillai et al. 2017b). In the present study, the PHBV accumulation property of this bacterium was addressed. In-vitro cytotoxicity studies were also conducted to evaluate the potential of these biopolymers and PEG blends thereof in perspective of their skin graft applications.

Materials and methods

Bacterial strain and culture conditions

The bacterial strain *B. aryabhattai* PHB10 used in this study was reported previously from the lab (Pillai et al. 2017a) and the strain is available at Microbial Type Culture Collection (MTCC Accession No. 12561). PHBV production experiment with this strain was conducted following the method described by Moorkoth and Nampoothiri (2016) with minor modifications. The basal medium (1.5 g of peptone, 1.5 g of yeast extract, 1 g of Na_2HPO_4 and 0.2 g of $MgSO_4.7H_2O$ per litre) supplemented with 20 g/L glucose and 10 mM propionic acid was used for the copolymer production. The pH of the medium was adjusted to 7.0 with 1 M NaOH solution. The medium was inoculated with 1% (v/v) seed culture of PHB10 and incubated in a rotary shaker at 31 °C and 180 rpm. For studying the effect of temperature and pH on polymer production, incubation temperatures between 28 and 40 °C and culture media of initial pH between 5 and 9 were used independently. The fermentation studies were conducted in 500 mL flasks with 200 mL culture medium for 48 h.

Extraction and quantification of the polymer

After the incubation, biomass was harvested by centrifugation at 5000 rpm for 3 min, lyophilized and cell dry mass (CDM) was calculated. The biomass was suspended in sodium hypochlorite solution (available chlorine 5% w/v) and incubated for one h at 45 °C for cell lysis (Shi et al. 1997). The suspended particles were collected by centrifugation at 5000 rpm for 3 min and the cell debris was removed by a series of washing with distilled water, acetone and absolute ethanol. The obtained solid mass was dissolved in boiling chloroform, filtered through glass wool and poured to a clean glass Petri plate. A PHA film was obtained after evaporation of the solvent.

The estimation of the accumulated polymer was carried out directly from the dried cell mass. The PHA in the CDM was converted to crotonic acid by sulphuric acid treatment and quantified spectrophotometrically (Law and Slepecky 1961). The polymer yield percentage (PHA%) was calculated by multiplying the PHA mass to CDM ratio by 100.

Characterization of the extracted polymer

Gas chromatography–mass spectrometry (GC–MS) analysis

The sample preparation for the GC–MS analysis followed the methanolysis method described by Juengert et al. (2018). A mixture of 10 mg of polymer sample, 1 mL chloroform and 1 mL acidified methanol (15% v/v H_2SO_4) was taken in a screw-capped glass bottle (20 mL capacity) with polytetrafluoroethylene stopper and heated at 100 °C for 2 h in an oil bath. After incubation, 1 mL chloroform containing an internal standard (0.2% v/v methyl benzoate) and 1 mL deionised water were added to the bottle for phase separation. The bottom organic phase was collected, dehydrated with anhydrous Na_2SO_4 and an aliquot of 1 µL was injected into the Shimadzu GC–MS QP2010S gas chromatograph, fitted with a Rxi-5Sil MS (30 m × 0.25 mm × 0.25 µm)

capillary column. The injection temperature was 280 °C and the Helium gas flow rate was set at 1 mL/min. The initial column temperature of 90 °C was maintained for 3 min, then increased to 190 °C at the rate of 7 °C/min, held for 5 min and then finally increased to 270 °C at the rate of 8 °C/min, and held for 5 min. After a solvent cut time of 3.6 min, mass spectra were recorded under scan mode in the range of 50–500 m/z. The peaks were compared to the mass spectral libraries (NIST 17 and Wiley) for identification of the compounds (Pillai et al. 2018).

Nuclear magnetic resonance (NMR) spectroscopy

The NMR spectrum of the polymer was recorded after suspending the samples in high purity deuterochloroform ($CDCl_3$) (Salgaonkar et al. 2013). ^{1}H NMR spectrum was obtained in model BrukerAvance^{II} 500 NMR spectrometer at 500 MHz and magnetic field strength of 11.7 T. ^{13}C NMR spectrum was recorded in model BrukerAvance^{III} 400 NMR spectrometer at 400 MHz and 9.4 T (Bruker Corporation, Massachusetts, USA).

Differential scanning calorimetry (DSC)

DSC analysis of PHA sample was carried out in a PerkinElmer DSC6000-Pyris Series instrument (PerkinElmer Inc., Massachusetts, USA) under a flowing nitrogen atmosphere at a heating rate of 10 °C per min (Gunaratne et al. 2004).

Thermogravimetric analysis (TGA)

TGA was carried out using a Perkin Elmer STA 6000 thermal analyzer instrument (PerkinElmer Inc., Massachusetts, USA) over a temperature range from 40 to 620 °C at a heating rate of 20 °C per min (Salgaonkar et al. 2013).

Gel permeation chromatography (GPC)

The molecular weight distribution and polydispersion index (PDI) of the polymer were determined by GPC using Waters HPLC system with 600 Series Pump and Waters Styragel HR series HR5E/4E/2/0.5 column equipped with a 7725 Rheodyne injector and refractive index 2414 detector (Waters Corporation, Massachusetts, USA) (Su 2013; Qi and Rehm 2001). Chloroform was used as the eluent (flow rate 1.0 mL/min) and polystyrene standards of molecular weight 1,865,000, 34,300 and 685 Da were used for relative calibration.

Tensile properties

The tensile characteristics of the polymer were measured in a universal testing machine (Make: Tinius Olsen, Model: 50ST) at room temperature with a 50 kN load cell at a fixed cross-head speed of 50 mm/min following the American Society for Testing and Materials standard (ASTM D882-12 2012) procedure. Average values from three independent tests were taken.

Formulation of polymer blends with polyethylene glycol

PHB and PHBV from the *B. aryabhattai* PHB10 along with a commercial-grade PHB (Sigma-Aldrich, Missouri, USA) were selected for blend preparation. The PHB polymer sample from the strain available in the lab prepared during our previous study was taken for this experiment (Pillai et al. 2017a). PEG of molecular weight 2 kDa and 8 kDa were blended individually with the polymer samples. The preparations followed a solvent casting technique by combining the polymer and PEG at a ratio of 4:1 (w/w) (Rodrigues et al. 2005). Briefly, 200 mg of PEG was dissolved in 50 mL chloroform in a sealed round bottom flask at 150 rpm and 50 °C, into which 800 mg of respective PHAs were added after the complete dissolution of PEG. The solution was allowed to cool to 25 °C after the polymers were completely dissolved and then poured to a clean glass Petri dish to cast the films. The film thickness was maintained approximately 0.05 mm by adjusting the concentration and the total volume of the polymer solution. Chloroform was evaporated overnight and was kept at room temperature for one week for complete evaporation of the solvent.

Evaluation of polymer induced cytotoxicity

Cytotoxicity of the polymer samples was evaluated according to Napathorn (2014) with minor modifications. The polymer films cut into circular discs of 0.4 mm diameter were used for the experiment after sterilization by immersion in 70% ethanol for 1 h and the subsequent UV irradiation (wavelength: 254 nm; intensity: 1.4 mW/cm^2, incubation period: 1 h). CellTiter 96 AQueous One Solution Cell Proliferation Assay System (Promega Corporation, Wisconsin, USA) which contains a novel tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt; MTS] and an electron coupling reagent (phenazine ethosulfate; PES) was used for measuring cytotoxicity (Mosmann 1983). The MTS tetrazolium compound (Owen's reagent) is bioreduced by mitochondrial succinate dehydrogenase enzyme into a colored formazan product soluble in the tissue culture medium. The cell viability was assessed indirectly

by measuring the mitochondrial succinate dehydrogenase enzyme activity. HaCaT cells were grown on Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 0.1 mM MEM non-essential amino acid and 1 mM sodium pyruvate. The cells were seeded at a cell density of 1×10^4 cells/well on flat bottomed 96-well standard microplates. The polymer samples were added to the wells and incubated for 24 h at 37 °C in a humidified 5% CO_2 incubator. Cells without polymer treatment were kept as control. After incubation, 20 µL of MTS solution was added to each well and the plates were incubated for 4 h in a CO_2 incubator. The absorbance was then measured with a microplate reader, at a wavelength of 490 nm and the cell viability was calculated as the percentage viability of cells with respect to the control experiment.

Statistical analysis

The experiments for PHA production and cytotoxicity evaluation were performed in triplicate and the mean values were taken. The values were subjected to Student's *t*-test and values $p \leq 0.05$ were taken as statistically significant (Parker 1979).

Results

Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) production in *Bacillus aryabhattai* PHB10

The strain PHB10 was tested for copolymer production in the presence of 10 mM propionic acid. The bacterium produced 3.9 (±0.26) g/L of biomass containing 2.8 (±0.31) g/L of PHBV after 48 h incubation. The effect of incubation temperature on copolymer production was evaluated at different temperatures and the results are presented in Fig. 1a. The optimum temperature for bacterial growth and PHBV accumulation was 31 °C with an yield of 71.15% of CDM. pH between 5 and 9 was also tested to study the effect of the initial pH of the culture medium on PHBV accumulation and the results are depicted in Fig. 1b. The optimum pH for the copolymer production was observed as 7 and the yield showed a decreasing trend towards either side of the pH value.

Identification and characterization of the polymer

The monomer composition of the PHBV was identified by GC–MS analysis. The results were compared with that of standard PHBV (Sigma-Aldrich, MO, USA) (Fig. 2a). The chromatogram of the tested PHBV (Fig. 2b) showed three major peaks with retention time 3.630 min, 19.224 min and 21.667 min. By comparing molecules in the GC–MS

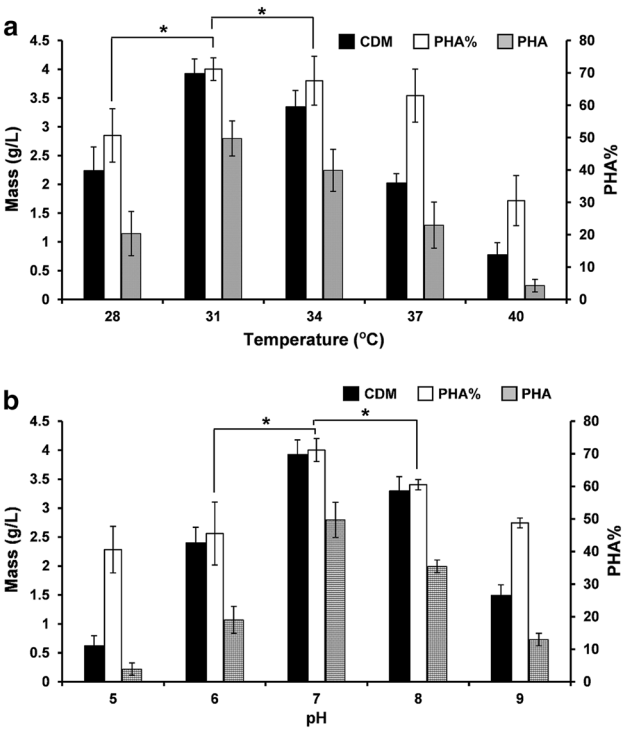
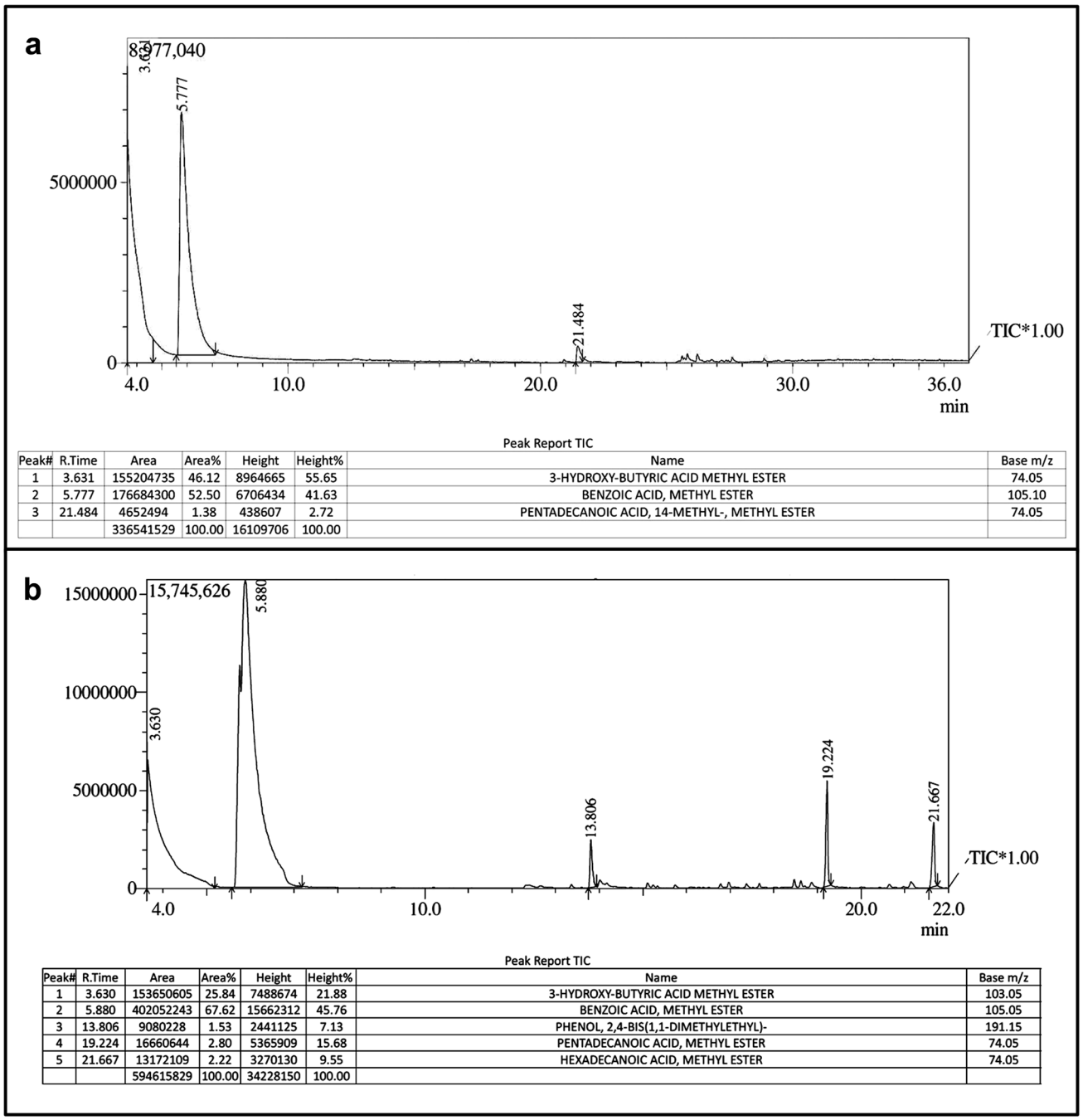


Fig. 1 Quantity of biomass (cell dry mass—CDM), PHBV and the PHBV% produced by *B. aryabhattai* PHB10 **a** at different temperatures and **b** at different pH (* represents $p < 0.05$)

database, they were identified as 3-hydroxybutyric acid methyl ester, pentadecanoic acid methyl ester and hexadecanoic acid methyl ester respectively. The 3-hydroxybutyric acid methyl ester and pentadecanoic acid methyl ester were also detected in the chromatogram of PHBV standard. The ^{1}H NMR spectrum (Fig. 3a) showed the resonances at 1.26 ppm, 2.45–2.65 ppm and 5.22–5.28 ppm representing a methyl group (–CH_3), a methylene group (–CH_2–) and a methine group (–CH–) respectively from the 3-HB monomer. The spectrum also showed resonances at 0.87 ppm, 1.62 ppm and 5.13–5.18 ppm representing a methyl group (–CH_3), a methylene group (–CH_2–) and a methine group (–CH–) respectively from the 3-HV monomer. The ^{13}C NMR spectrum (Fig. 3b) showed peaks at 19.75 ppm, 40.77–40.80 ppm and 67.61–67.69 ppm representing a methyl (–CH_3) group, a methylene (–CH_2–) group and an ester (–O–CH–) group respectively from the 3-HB monomer. The peaks at 26.85 ppm and 38.80 ppm correspond to the two methylene (–CH_2–) groups and the peak at 71.90 ppm corresponds to an ester (–O–CH–) group from the 3-HV monomer. The resonance at 169.14 represents carbonyl carbon (–C–) atom from both the 3-HB and 3-HV monomers.

The melting point of the material was determined by DSC analysis and was observed as 90 °C. Figure 4 shows the TGA thermogram of the PHA film. A 5% reduction in mass was observed around 90 °C and after that, the



Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	3.631	155204735	46.12	8964665	55.65	3-HYDROXY-BUTYRIC ACID METHYL ESTER	74.05
2	5.777	176684300	52.50	6706434	41.63	BENZOIC ACID, METHYL ESTER	105.10
3	21.484	4652494	1.38	438607	2.72	PENTADECANOIC ACID, 14-METHYL-, METHYL ESTER	74.05
		336541529	100.00	16109706	100.00		

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	3.630	153650605	25.84	7488674	21.88	3-HYDROXY-BUTYRIC ACID METHYL ESTER	103.05
2	5.880	402052243	67.62	15662312	45.76	BENZOIC ACID, METHYL ESTER	105.05
3	13.806	9080228	1.53	2441125	7.13	PHENOL, 2,4-BIS(1,1-DIMETHYLETHYL)-	191.15
4	19.224	16660644	2.80	5365909	15.68	PENTADECANOIC ACID, METHYL ESTER	74.05
5	21.667	13172109	2.22	3270130	9.55	HEXADECANOIC ACID, METHYL ESTER	74.05
		594615829	100.00	34228150	100.00		

Fig. 2 Gas chromatography analysis of PHBV. **a** PHBV standard and **b** PHBV obtained from *B. aryabhattai* PHB10. The *x*-axis shows retention time (min) and *y*-axis shows the intensity (arbitrary unit) of the signal

polymer was stable up to 220 °C. Onset of fast reduction in original mass was observed at 220 °C and reached the maximum degradation at around 255 °C. GPC analysis confirmed that the polymer had a number average molecular weight (Mn) of 128.508 kDa and a weight average molecular weight (Mw) of 362.021 kDa with a polydispersity index (PDI) 2.82. The PHBV recorded tensile strength of 10.3 MPa and elongation at break of 13.3%. A comparison of thermal and mechanical properties of the polymer is given at Table 1.

Evaluation of polymer induced cytotoxicity

Polymer discs prepared from different polymer samples with varying combinations of PEG were evaluated against HaCaT cells for cytotoxicity. Cells treated only with the

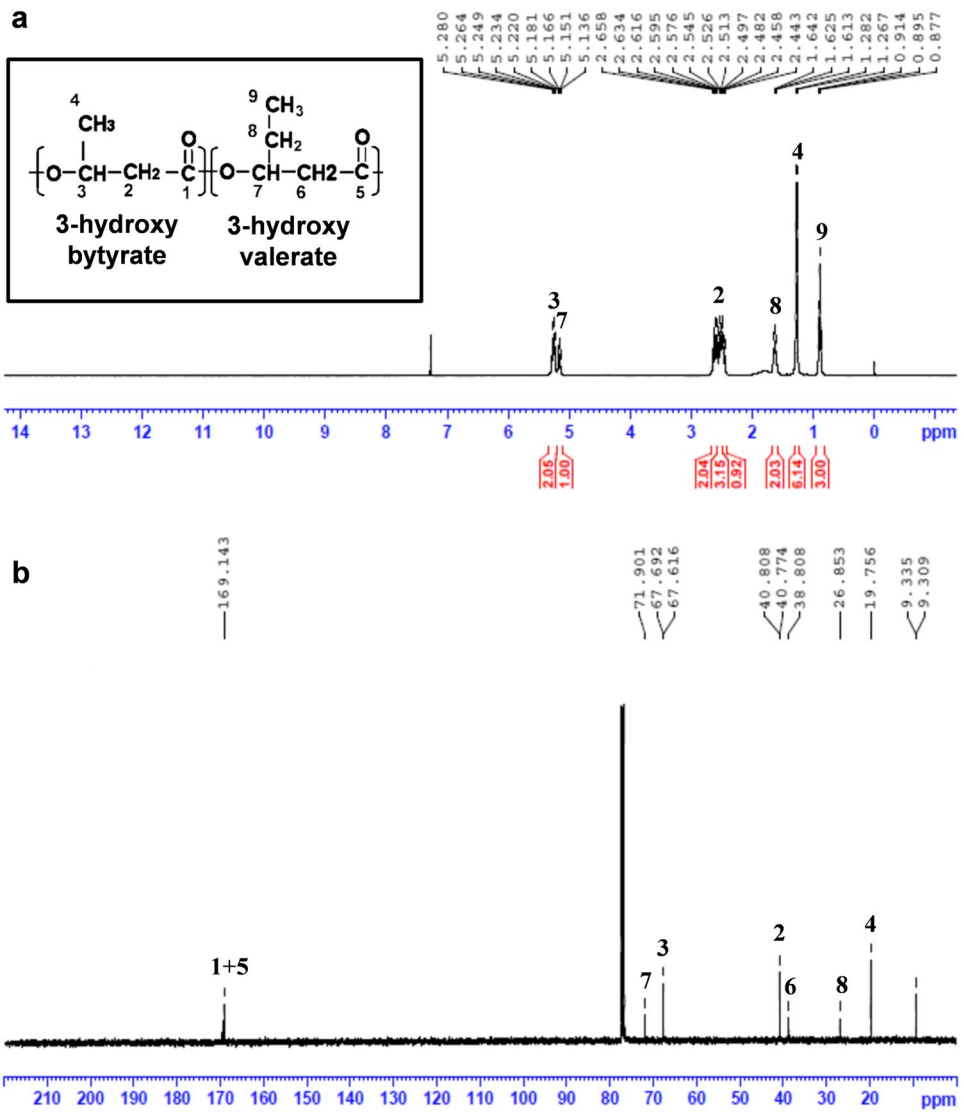


Fig. 3 NMR spectra of PHBV suspended in $CDCl_3$ **a** ^{1}H NMR **b** ^{13}C NMR

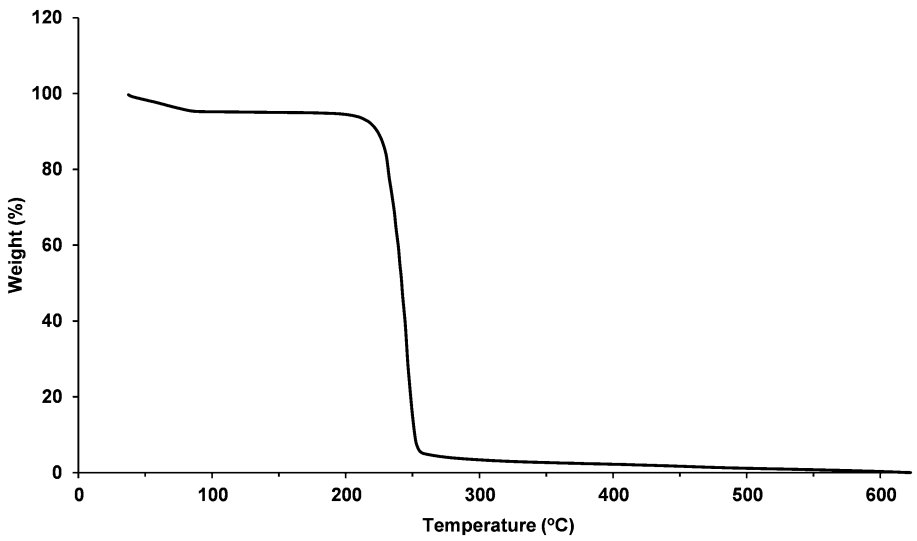


Fig. 4 TGA thermogram of PHBV

Table 1 Comparison of physical properties of PHBV with other related reports

Polymer	Mechanical properties		Thermal properties			References
	Tensile strength (MPa)	Elongation at break (%)	Melting point (°C)	Initial thermal decomposition temperature (°C)	Final Thermal Decomposition Temperature (°C)	
PHB	24.4	2.42	170	247	287	Pillai et al. (2017a)
PHB	~40	5–8	NA	NA	NA	Jiang and Zhang (2013)
PHBV	8.9	5.9	161.64	254.9	277.5	Kuciel et al. (2019)
PHBV	NA	NA	101.93	284.4	303.6	Wang et al. (2013)
PHBV	22	50.5	147	NA	NA	Myung et al. (2017)
PHBV	10.3	13.3	90	220	255	This study

NA not available

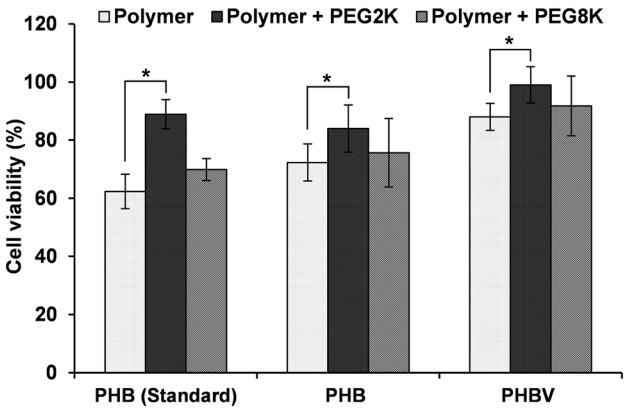


Fig. 5 Evaluation of cytotoxicity of the polymers obtained from *B. aryabhattai* PHB10 and the polymer/PEG blends. The cell viability is expressed as the percentage viability of cells with respect to the control experiment (PEG2K = PEG with Mw 2000 Da; PEG8K = PEG with Mw 8000 Da) (* represents $p < 0.05$)

fresh medium were kept as the control experiment. Per cent viability of the cells in the test samples was compared to the control experiment and is presented in Fig. 5. Normal cell growth was observed in the presence of all combinations of the polymer samples. Each set of experiment contains a polymer without PEG, polymer + PEG-2kDa and polymer + PEG8kDa. When the polymer samples without PEG were compared, PHBV showed the least cytotoxicity with 88 (± 4.69) % cell viability, whereas the PHB standard showed higher cytotoxicity with only 62 (± 5.92) % cell viability. When the samples with PEG of 2 kDa were tested, all the polymer samples showed a significant reduction in cytotoxicity. The cells incubated with the PHBV-PEG2kDa blends were 99% viable. However, the samples blended with PEG8kDa induced higher toxicity than that with PEG2kDa.

Discussion

The genus *Bacillus* is well known for its property to accumulate short chain length (SCL) PHA such as PHB, as its genome harbours gene for SCL-specific PHA synthase (Pillai et al. 2017a, b). Reports are also available on the accumulation of copolymers of PHB in several *Bacillus* spp. when culture medium was supplemented with propionic acid (Güngörmedi et al. 2014; Moorkoth and Nampoothiri 2016). *B. aryabhattai* PHB10 is an environmental bacterial strain accumulating high levels of PHB (Pillai et al. 2017a). We tested the strain PHB10 for accumulation of the copolymer PHBV, in a culture medium supplemented with propionate and the obtained yield (2.8 g/L, 71% of CDM) was better than the previously reported PHBV content of 1.9 g/L (54% of CDM) and 0.9 g/L (26% of CDM) from other *Bacillus* spp. as reported by Kumar et al. (2006) and Moorkoth and Nampoothiri (2016) respectively. This study is the first report on PHBV accumulation in a *B. aryabhattai* strain. The optimum temperature for growth and PHBV accumulation was 31 °C, which was the same as observed during the PHB production studies with this bacterium (Pillai et al. 2017a). Besides, Masood et al. (2012) and Güngörmedi et al. (2014) have also observed that optimum temperature for growth as well as PHA accumulation was in a temperature range of 30–35 °C. The optimum pH for the copolymer production was 7 and the obtained yield showed a significant decreasing trend towards either side of the pH value, which is in agreement with the previous reports on PHBV accumulation in *Bacillus* spp. (Moorkoth and Nampoothiri 2016; Masood et al. 2012). The PHB accumulation study in this strain (Pillai et al. 2017a) and the reports on PHBV accumulation in other *Bacillus* spp. suggested the optimum incubation period of 48 h for maximum polymer yield (Güngörmedi et al. 2014; Moorkoth and Nampoothiri 2016) and hence the incubation period was set as 48 h in all the shake flask experiments.

The GC–MS analysis revealed the monomer composition of the obtained polymer. The main peaks were corresponding to 3-hydroxybutyric acid methyl ester, pentadecanoic acid methyl ester and hexadecanoic acid methyl ester. The 3-hydroxybutyric acid methyl ester is the monomer methyl ester of 3-HB, whereas the pentadecanoic acid methyl ester and the hexadecanoic acid methyl ester are the trimer and tetramer methyl esters of 3-HV and 3-HB respectively (Bhuwal et al. 2014). These oligomers might have been formed as a result of incomplete digestion of the polymer sample during the methanolysis. The chromatogram of PHBV standard also showed the methyl esters of 3-hydroxybutyric acid and pentadecanoic acid. These observations confirmed the obtained polymer as PHBV. The ^{1}H NMR analysis demonstrated the resonances for the methyl group (–CH_3), methylene group (–CH_2–) and methine group (–CH–) from both the 3-HB and 3-HV monomer units. The ^{13}C NMR spectrum provided the signals for the methyl (–CH_3) groups, methylene (–CH_2–) groups, ester (–O–CH–) groups and carbonyl carbon (–C–) atoms from the 3-HB and 3-HV monomers. The chemical shift signals were in agreement with the previous findings of Abd-El-Haleem (2009) and Aramvash et al. (2016) which again confirmed that the polymer obtained from the *B. aryabhattai* was PHBV.

DSC analysis proved that the melting point of the material was 90 °C which was very low when compared to 170 °C of the homopolymer PHB, produced by the strain (Pillai et al. 2017a). Generally, PHBV has a melting point around 100–150 °C and is decreased with an increase in the amount of HV in PHBV (Wang et al. 2013; Liu et al. 2014). The TGA revealed that the obtained polymer began to lose its mass significantly with an increase in temperature from 220 °C and degrades completely at around 255 °C. This thermal behaviour was comparable to the thermal properties of PHBV, as reported by Wang et al. (2013). The observations on the thermal behaviour of the copolymer can be attributed to the higher hydroxyvalerate content in the sample, which may improve its ductility and flexibility (Wang et al. 2013).

The molecular weight (Mw) and PDI of the obtained PHBV was higher than the PHB produced by this strain (Pillai et al. 2017a) and the PHBV obtained from the *Bacillus* sp. as reported by Moorkoth and Nampoothiri (2016). Generally, higher values of PDI were observed for compounds with higher molecular weight, especially in polymers recovered by sodium hypochlorite lysis method (Berger et al. 1989). The polymer showed an elongation at break of 13.30% and tensile strength of 10.3 MPa. Previously, PHBV with very low mol% of 3-HV was reported to be with tensile strength 36.2 MPa and elongation at break 1% (Jost and Miesbauer 2018). The PHBV obtained from the PHB10 was having better mechanical properties than PHBV reported by Kuciel et al. (2019). The values are in a range suitable for tissue engineering applications (Little et al. 2011). Elongation at break represents the capability of a material to resist changes of shape without crack formation. The better elongation at break value recorded here indicates the better elastomeric character of the obtained PHBV. The mechanical properties of PHBV obtained in this study suggest that the polymer contained high 3-HV content.

PHAs are ideal candidates for biomedical engineering because of its high immunotolerance, low toxicity, and biodegradability (Lomas et al. 2013). They were reported to be more angiogenic than other similar polymers and having greater macrophage polarization properties. Hence PHA based polymer scaffolds could be an attractive candidate for skin reconstruction procedures (Castellano et al. 2017). Reports are also available on the enhancement in the biocompatibility of PHAs when blended with PEG (Cheng et al. 2003; Chan et al. 2011; Li et al. 2016). To get a more reliable information on cytotoxicity of polymer samples intended for skin graft applications, human keratinocytes which are the predominant cell type in the epidermis could be the ideal target. HaCaT cells are the immortalized human keratinocytes which have been widely applied in the studies related to epidermal homeostasis and its pathophysiology (Seo et al. 2012). Also, there are reports on the improvement in the physical properties and biodegradability of the polymer on blending with PEG (Xiang et al. 2013; Hu et al. 2017).

The cytotoxicity values obtained from the study proved that the blending PEG of 2 kDa significantly reduced the polymer induced cytotoxicity of the PHB and PHBV. In an earlier study, the PHBV surfaces, when modified with PEG decreased the nonspecific adsorption of proteins from plasma and thereby improved the blood compatibility of implanted materials (Wang et al. 2011). Similar observations of improvement in the biocompatibility of PHB were also reported in Chinese Hamster Lung (CHL) fibroblast when blended with PEG (Cheng et al. 2003). Studies on neural-associated olfactory ensheathing cells (OECs) in the presence of PHB blended with PEG2kDa also reported improved cell viability, biocompatibility and proliferation (Chan et al. 2011). It is evident from these observations that the PHBV when blended with PEG2kDa, did not affect the normal cell growth and proliferation and hence can be effectively used for skin graft applications.

Conclusions

The *B. aryabhattai* PHB10 accumulated copolymer PHBV up to 2.8 g/L (71.80% of CDM) in the presence of glucose and propionic acid. This study is the first report on PHBV accumulation in this bacterium. The polymer yield can be improved by optimization of the fermentation conditions. The blending of PHBV with PEG considerably reduced the polymer induced cytotoxicity on HaCaT cells, which

is a promising result in terms of skin graft applications. Therefore, it will be worthwhile to study further the potential of PHB/PEG blends in the purview of its biomedical applications.

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Authors contributions ABP and HK conceived and designed the experiments; ABP and AJK did the experiments; ABP, AJK and HK analyzed the data and wrote this manuscript. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals This article does not contain any studies with human participants or animals performed by any of the authors.

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