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Purification and characterization of polyhydroxybutyrate produced from thermophilic *Geobacillus* sp. AY 946034 strain

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Thermophilic *Geobacillus* sp. AY 946034 strain was found to accumulate poly-3-hydroxybutyrate (PHB). The highest PHB percent yield was found under phosphorous limiting conditions (68.9% of cell dry weight), using glucose as the sole carbon source. Higher values of enzyme (ketothiolase, CoA reductase and PHA synthetase) activities of thermophilic strain indicated that bacteria use the classical pathway of PHB biosynthesis when are cultured on glucose as the sole carbon source.

The characterization of biopolymer was carried out using FT-IR, DSC and capillary viscosity methods. The FT-IR analysis of the purified polymer revealed similarities in the spectra of the microbial and standard PHB at 2 983, 2 935, 1 722, 1 456, 1 278 and 1 053 cm^{-1} wave numbers. The thermal analysis showed the accumulated PHB to be semicrystalline polymer (42%), having a thermal degradation temperature of 280 °C, melting temperature of 168.8 °C and apparent melting enthalpy of 62.98 Jg^{-1}. Molecular weight of the polymer was found to be 556 kDa.

Key words: biopolymers, poly-3-hydroxybutyrate, thermophilic *Geobacillus*, thermal analysis, FT-IR spectroscopy

INTRODUCTION

Due to chemical and physical characteristics of polyhydroxyalkanoates (PHA), PHAs have a wide range of applications including conventional plastic materials, utensils and cosmetic containers, as well as in medicine, in the pharmaceutical and food industries, and as biodegradable carriers for the long-term dosage of drugs, medicines, hormones, insecticides and herbicides [1]. They can be used either in their pure form or as additives to conventional plastics such as polyethylene or polypropylene. However, PHAs are currently far more expensive than chemically based plastics and are therefore used mostly in applications that conventional plastics cannot perform, such as medical applications. In order to make the PHA production economically attractive, many goals have to be addressed simultaneously. PHA can be synthesized either by chemical means or by biological approaches. PHA can be synthesized by over 30% of soil-inhabiting bacteria [2]. Chen et al. [3] reported that many species of the genus *Bacillus* produce PHA. Recombinant microbial strains are being developed to achieve both a high substrate conversion rate and close packing of PHAs granules in the host cell [4–8]. The efficiency and economics of the manufacturing process of PHA is determined by the carbon source, bacterial strain, fermentation process and purification of the polymer [9–12].

Chen and authors [3] revealed that PHA biosynthesis is a complex process controlled by several enzymes catalyzing different metabolic pathways. The PHA biosynthesis pathway requires three enzymatic activities: b-ketothiolase, NAD(P)H-dependent acetoacetyl-CoA reductase and PHA synthetase. The most studied PHA is poly-3-hydroxybutirate. The characteristics of the biopolymer are similar to conventional plastics such as polypropylene [13–15]. Polyhydroxybutirate is a biodegradable thermoplastic which can be extracted from a wide range of bacteria. PHB and PHV [poly (3-hydroxyvalerate)] form a class of PHAs typically referred to as short-chain-length PHAs (scl-PHAs) containing 4 or 5 carbon atoms [16]. It has been suggested that PHB "homopolymer", synthesized by bacteria, always contains less than 1 mol% of 3-hydroxyvalerate monomers [14]. PHB is a highly crystalline polymer, very stiff and brittle, and degrades at the temperatures slightly above its melting point [17–19].

Thermophilic bacteria have received greater attention because of numerous advantages of operating PHA fermentation at medium and elevated temperatures [20]. The solubility and diffusion rates of chemicals in such conditions

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increased because of the acceleration of chemical reactions. Higher temperatures can reduce the cost because of less cooling and heating processes, and the risk of cross contamination by other microorganism decreases significantly because fewer microorganisms can survive at medium and elevated temperatures [20]. Therefore, it is promising to screen novel, thermophilic microorganisms for possible use in industrial production, such as production of PHB.

In this work we have investigated the production and physico-chemical properties of PHB in thermophilic *Geobacillus* sp. AY 946034 strain.

EXPERIMENTAL

Microorganism growth medium and cultivation

Thermophilic bacterial strain designated as *Geobacillus* sp. AY 946034 was isolated from the oil field of Lithuania [21]. The bacterial strain *Geobacillus* sp. AY 946034 has been studied for PHB synthesis. The strain was tested for PHB production using Sudan Black B qualitative analysis [22]. The inoculated plates were incubated at 60 °C for 24 hours. Solution of 0.2% Sudan Black B was spread over the bacterial colonies and the plates were kept undisturbed for 30 minutes. The plates were washed with ethanol (96%) to remove the excess stain from the colonies. The dark blue stained colonies were taken as a positive for PHB production.

The bacterial strain *Geobacillus* sp. AY 946034 was grown in the mineral salt medium (MSM) containing (g/L^{-1}): $Na_2HPO_4 \cdot 7H_2O$ – 6.7, KH_2PO_4 – 1.5, $(NH_4)_2SO_4$ – 1.0, $MgSO_4 \cdot 7H_2O$ – 0.2, $C_6H_7NaO_7$ – 0.03, $FeCl_3$ – 0.01, $CaCl_2 \cdot 2H_2O$, H_3BO_3 – 0.3, $ZnSO_4 \cdot 7H_2O$ – 0.1, $MnCl_2 \cdot 4H_2O$ – 0.03, $Na_2MoO_4 \cdot 2H_2O$ – 0.03, $NiCl_2 \cdot 6H_2O$ – 0.02, $CuSO_4 \cdot 5H_2O$ – 0.01 and 1% of glucose as the carbon source for the PHB synthesis. The pH of the medium was adjusted to 7.0. The flask was inoculated with 10% (v/v) of pre-culture grown overnight in the same mineral salt medium at 60 °C to an OD_{590} of 1.0. Bacterial culture was incubated at 60 °C for 48 h with shaking (180 rpm). The biomass was harvested via centrifugation 6 000 × g for 10 min for the analysis of dry cell weight and PHB accumulation.

Time course study on the PHB production was done. Samples of 10 mL were taken at regular time intervals (4 h) to determine growth (dry cell weight) and PHB content during 72 h cultivation.

Effect of different carbon sources on PHB production. Different carbon sources (1% w/v) like glucose, fructose, sucrose, maltose, lactose, sodium acetate, and glycerol were amended in 250 ml of mineral salts medium to find out a suitable carbon source for the optimum PHB production of the bacterial strain and incubated at 60 °C on a rotary shaker (180 rpm). The PHB production and dry cell weight after 48 h was determined.

PHB production undergrowth limiting conditions. Different phosphorus (P) and nitrogen (N) limited conditions for *Geobacillus* sp. AY 946034 were tested for PBH production. The P limited mineral salt medium with three times less P concentration and 1% of glucose, and the N limited media with three time less N and 1% of glucose were tested.

Experiments were performed three times under the same testing conditions.

Extraction and purification of PHB

Cells were harvested by centrifugation at 6 000 × g for 10 min and washed twice with water. The cells were disrupted by ultrasonic treatment for 6 min at 22 kHz, 65 μA by using a sonicator with 30 s sonication and 30 s for cooling. The supernatant was removed (5 000 × g, 15 min), and the pellet was washed (1 h) with acetone and alcohol (1 : 1) mixture to remove low-molecular-weight lipids and pigments. The pellet was dissolved by extraction with boiling chloroform solution and concentrated. PHB was then precipitated by dropping the polymer solution into 10 volumes of 95% ethanol. The white precipitate was formed at the interface of ethanol and then separated by centrifugation at 8 000 × g for 20 min at room temperature. The PHB precipitate was dried at room temperature for 24 h.

Enzyme assay

Culture for enzyme analysis was grown in 25 mL of MSM under phosphorus limiting conditions. The cells were harvested at the late exponential growth phase (48 h), centrifuged (6 000 × g, 10 min, 4 °C), washed twice in 10 mL of 50 mM sodium phosphate buffer (pH 7.0) and were re-suspended in the same volume of the buffer. The cells were disrupted by ultrasonic treatment for 5 min at 22 kHz by using a sonicator. The cell debris was removed by centrifugation (14 000 × g, 25 min, 4 °C). The supernatant was used as the cell free extract.

Enzyme activities were assayed by monitoring the disappearance of substrate or appearance of products with a UV–visible spectrophotometer (Perkin Elmer, Italia) with a thermojacketed cuvette at 60 °C. The following enzymes were assayed according to the reported methods given in the reference [23]. One unit of enzyme activity (U) was defined as that catalyzing either the degradation of 1 μmol of substrate or the formation of 1 μmol of degradation product per minute. Protein concentration was determined by spectrophotometric measurement at 280 nm.

Analytical methods

Characterization of PHB Fourier Transform Infrared Spectroscopy (FT-IR). The presence of different functional groups in PHB was analyzed by FT-IR. Extracted PHB (2 mg) and standard PHB from Sigma (2 mg) were dissolved in 500 μl of chloroform and layered on NaCl crystal. After evaporation of chloroform, the PHB polymer film was subjected to FT-IR. A Perkin-Elmer spectrum-400 spectrometer was used with a spectral range of 650 to 4 000 cm^{-1} (10 scans) to record the IR spectra.

Differential scanning calorimetry of polymer. Differential scanning calorimetry (DSC) thermograms were recorded by DSC 800 (Perkin Elmer Pyris 6), calibrated with indium

(m. p. 156.61 °C; H = 28.54 J/g). The data was collected by a heating and cooling method. Samples of pure powder weighed 10 mg were packed in an aluminium pan and then heated from –10 °C to 300 °C at a scanning rate of 10 °C per minute under nitrogen atmosphere. The melting temperature (T_m) and melting enthalpy (H_f) were determined from DSC endothermal peaks. The crystallinity (X_c) of PHB was calculated as per equation given below:

$$X_c = H_f \cdot 100 / H_o \cdot w,$$

where H_f is the melting enthalpy of the sample (J/g), H_o is the melting enthalpy of the 100% crystalline PHB which is assumed to be 146 J/g, w is the weight of fraction of PHB in the sample [24].

Molecular weight determination. The viscosity-average molecular weight (M_w) of the PHB sample was measured at 30 °C using a Ubbelode viscosimeter. 35 mg of the polymer was dissolved in 30 mL of chloroform and intrinsic viscosity (ŋ) was determined. Molecular weight of the polymer was calculated using the Mark-Houwink equation:

$\eta = K \cdot M_w^{\alpha}$, where K is the consistency index $1.18 \cdot 10^{-4}$, and α is the flow behavior index 0.78 [1].

RESULTS AND DISCUSSION

PHA has been industrially produced by pure cultures including *Alcaligenes latus*, *Azotobacter vinelandii*, *Pseudomonas oleovorans*, recombinant *Alcaligenes eutrophus* and recombinant *Escherichia coli* [25–27]. Recovery methods for PHAs of various purities from microorganisms have received attention. The study of PHA accumulation in thermophilic bacteria is an interesting approach aiming at investigating more and more of such microorganisms for new PHA synthesis. Several kinds of thermophilic PHA-accumulating microorganisms have been found in hot springs [28, 29] or aerobic organic waste treatment plants [20], but the amount of them is still extremely lower than that of PHA-accumulating mesophiles. In the present study, we succeeded in isolating a thermophilic PHA accumulating bacteria *Geobacillus* sp. AY946034 strain from the oil fields of Lithuania. The yield of polymer on particular carbon source is an important parameter in the bacterial production PHB. It is well known that any bacteria capable of producing PHB need an excess carbon source in addition to a limited other source such as nitrogen or phosphate [1].

Bacterial PHB production conditions

Bacterial strain *Geobacillus* sp. AY946034 was found to be positive for Sudan black staining which indicated PHA accumulation in bacterial cells (Fig. 1A). Cell mass attained its maximum in 48 h of cultivation and then declined. Accumulation of the PHB started from the beginning of growth and continued until the late exponential phase (48 h) and was the best. Different carbon sources were used to find out a suitable carbon source for the optimum PHB production. The highest PHB amount (1.03 g/L) and product yield (41.1%) was determined using glucose as the major source of maintaining energy for cell metabolisms (Table 1). This proved that glucose was a better substrate for production of PHB by *Geobacillus* strain when compared to other carbon sources.

Nutrient limitation is necessary to trigger PHB accumulation in bacteria [30, 31]. PHB production by *Geobacillus* sp. strain under different culture conditions with limitation of nitrogen and phosphate was investigated (Table 2). The highest PHB production and productivity percentage were found under phosphorous limiting conditions (1.3 g/L, 68.9%), whereas the lowest percent yield of PHB according to dry cell weight was determined in the unlimiting (40.9%) and nitrogen–phosphorus limited (40.0%) conditions, using glucose as the sole carbon source. Thus in both cases the results suggest that any of the above nutrient deficiency can trigger PHB production in this strain. Valappil et al., [32] has reported varied production of PHA by *B. cereus* SPV in different

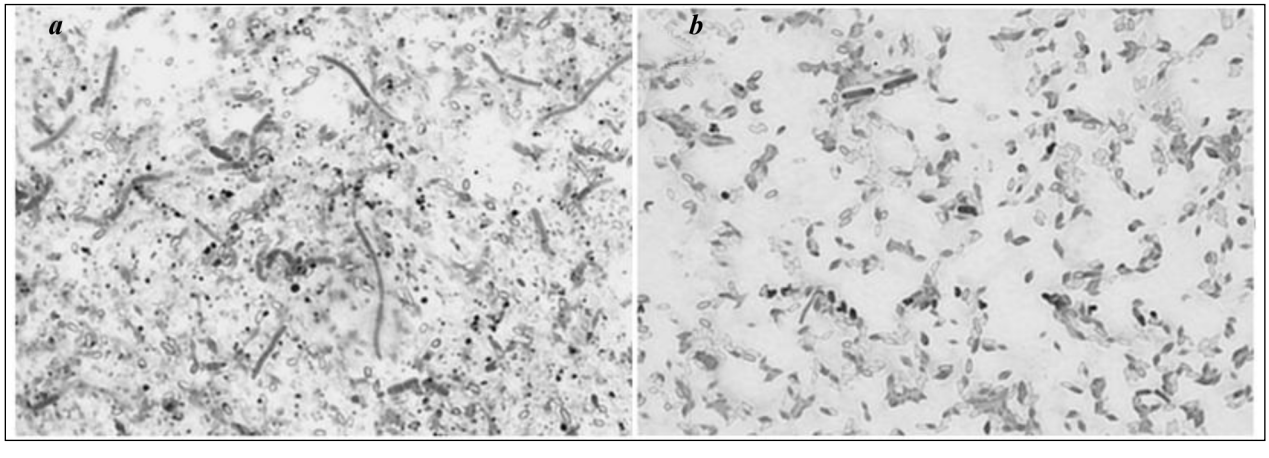

Fig. 1. Light micrograph of strain *Geobacillus* sp. AY946034 stained with Sudan Black B at × 1 000. *a* – The cells were cultivated in nutrient agar media with 10 gL^{-1} glucose; *b* – the same media with 1 gL^{-1} glucose

Table 1. **Effect of different carbon sources on growth and accumulation of PHB in cells of *Geobacillus* sp. AY946034 strain, after 48 h incubation at 60 °C in MSM without limitation N and P**

Carbon source	Dry cell weight, g/L	Weight of PHB, g/L	Yield of PHB, %
Sodium acetate	0.76 ± 0.01	0	0
Glucose	2.50 ± 0.50	1.03 ± 0.01	41.1
Maltose	1.88 ± 0.10	0.15 ± 0.03	8.1
Sucrose	1.37 ± 0.10	0.28 ± 0.01	20.0
Lactose	1.25 ± 0.90	0.13 ± 0.01	10.3
Glycerol	0.66 ± 0.01	0	0
Starch	2.10 ± 0.10	0.33 ± 0.02	15.7

Table 2. **Effect of under growth limiting conditions on PHB yield in *Geobacillus* sp. AY 946034 strain cultivated for 48 h at 60 °C in MSM with glucose as a carbon source**

Growth conditions; C/P or C/N ratio*	Dry cell weight, g/L	Weight of PHB, g/L	Yield of PHB, %
MSM	2.2 ± 0.1	0.9 ± 0.2	40.9
Phosphorus limited MSM; C/P (5/0.3)	1.9 ± 0.1	1.3 ± 0.5	68.9
Nitrogen limited MSM; C/N (7/0.3)	1.6 ± 0.2	0.8 ± 0.3	50.0
Nitrogen and phosphorus limited MSM; C/P/N (5/0.3/0.2)	1.0 ± 0.1	0.4 ± 0.1	40.0

* C/mole of P or N.

culture conditions with limitation of potassium, nitrogen, sulphur and phosphate. The phosphorous-deficient medium used in our study was by far the best medium in terms of both the cellular growth and PHA accumulation. The nitrogen source was used as the limiting one, but the *Geobacillus* strain yielded less percent of PHB (50%). According to literature various strains of *Bacillus* sp. have produced PHB in the range of 38–71% of cell dry weight using glucose as a carbon source with limitation of nitrogen [33]. The thermophilic bacteria are capable of accumulating PHB from 40 up to 76% of cell dry weight [28, 29].

Determination of enzymatic activities involved in PHB biosynthesis

Biosynthesis of PHB has been shown to proceed by a number of different pathways in mesophilic bacteria [34]. The most commonly found and studied pathways involve the presence of three enzymatic activities: β-ketothiolase, acetoacetyl-CoA reductase and PHB synthetase [1]. The enzymatic activities from cell-free extracts prepared from thermophilic *Geobacillus* sp. AY946034 strain were shown in Table 3.

Table 3. **Specific enzyme activities in the cell-free extract of *Geobacillus* sp. AY946034 strain grown on glucose and phosphorus limited MSM cultivated for 48 h at 60 °C**

Enzyme	Specific activity, μmol min^{-1} mg^{-1}
β-ketothiolase	1.89 ± 0.05
Acetoacetyl-CoA reductase	26.00 ± 1.03
PHB synthetase	11.16 ± 0.06
PHB depolymerase	5.89 ± 0.11

The activities of three enzymes involved in the classical pathway for PHB synthesis were determined in strain cultured on glucose as the sole carbon source. Specific activities of β-ketothiolase, NADH-dependent acetoacetyl-CoA reductase and PHB synthetase were comparable with those of *Azotobacter beijerinckii* [35], *Methylosinus trichosporium* [23] and *Herbaspirillum seropedicae* [36], previously described as PHB-producing strains. The significant levels of these enzymatic activities in thermophilic strains indicated that they utilized the classical pathway for PHB biosynthesis when were cultured on glucose as the sole carbon source.

Extraction and purification of PHB

From 9.4 g of dry bacterial cell mass 6.47 g PHB was obtained, i. e. 68.8% of dry cell weight basis. The extracted PHB was a white colored powder (Fig. 2). The *Geobacillus* cells grown in the nutrient deficient medium were harvested and subjected to ultrasonic treatment. PHB is an intracellular and hence acceptable method of isolation from the cells, it is essential for economic recovery of the product. A number of methods for the recovery of PHA have been developed [37]. Sodium hypochlorite is used for cell hydrolysis, which leads to the reduction in molecular weight of the polymer. Enzymatic digestion of cells has been developed as an alternative to chemicals but this consists of several steps before the purified product is obtained [38]. Most of the methods require the large amount of chemicals, which includes organic solvents for solubilization and isolation of the product. Therefore, in the present work, bacterial cells were disrupted by ultrasonic treatment for a short time to cause the lower damage of the PHB molecular weight. The disrupted cells were further subjected to extraction in

Fig. 2. Isolated and purified bacterial polymer PHB

hot chloroform and the dissolved PHB was separated by precipitation by adding ethanol.

The isolated and purified PHB was dissolved in chloroform and subjected to UV–Vis scanning over the range of 200–800 nm for preliminary identification. The obtained result has shown a sharp peak and absorbance at 240 nm (Fig. 3). But in solvent control there was no such absorbance at 240 nm. This clearly indicated the presence of PHA compounds in the isolated sample.

Characterization of PBH

The purity, yield, and molecular size are three major factors in PHA recovery.

FT-IR spectroscopy is a robust method for the identification of PHAs, with the absorption CO double bond (CAO) acting as the diagnostic signal. For PHB, one type of PHA, the CAO FT-IR peak is at about 1 728 cm^{-1}, and this has been used for PHB identification. The PHA extract from *Geobacillus* strain was analyzed along with a PHB standard (Sigma) through the FT-IR approach. The FT-IR spectra of the PHB standard (Fig. 4, upper spectrum) and extract (Fig. 4, bottom spectrum) of *Geobacillus* sp. AY946034 cultured with glucose as the carbon source show peaks at similar positions. The peak absorption band of the PHB standard, at about 1 729 cm^{-1}, corresponds to the ester carbonyl group of PHB. The peak of *Geobacillus* sp. AY946034, at about 1 722 cm^{-1}, represents the vibration of the CAO bond of ester functional groups, primarily from lipids, fatty acids, and PHB [39]. In this study the FT-IR absorption band at about 1 722 cm^{-1} is a characteristic of carbonyl group and a band at about 1 280–1 053 cm^{-1} characterizes the valence vibration of the carboxyl group. These are characteristics of polyhydroxybutyrate. The exact position of PHB absorbance depends on the degree of crystallinity of PHB [40]. The spectra of the polymer extract from *Geobacillus* strain and the PHB standard (Sigma) were almost identical. The peaks at 2 983 and 2 935 cm^{-1} belong to the COH stretching vibration of methyl and methylene [41]. At about 1 455 cm^{-1}, the asymmetric stretching peak of $-CH_3$ and $-CH_2$ group emerges. The peak at about 1 382 cm^{-1} is the symmetric deformation peak of $-CH_3$ and $-CH_2$ groups and the symmetric stretch of C–O

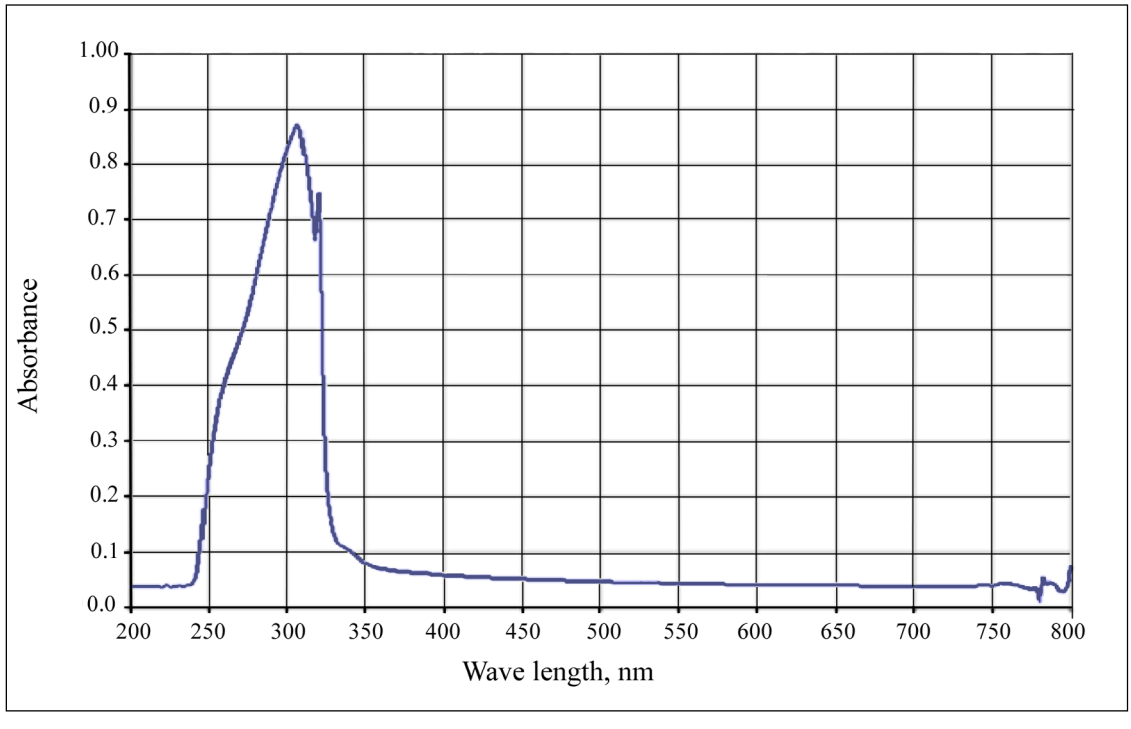

Fig. 3. UV–Vis spectrum of the PHB extracted from *Geobacillus* strain

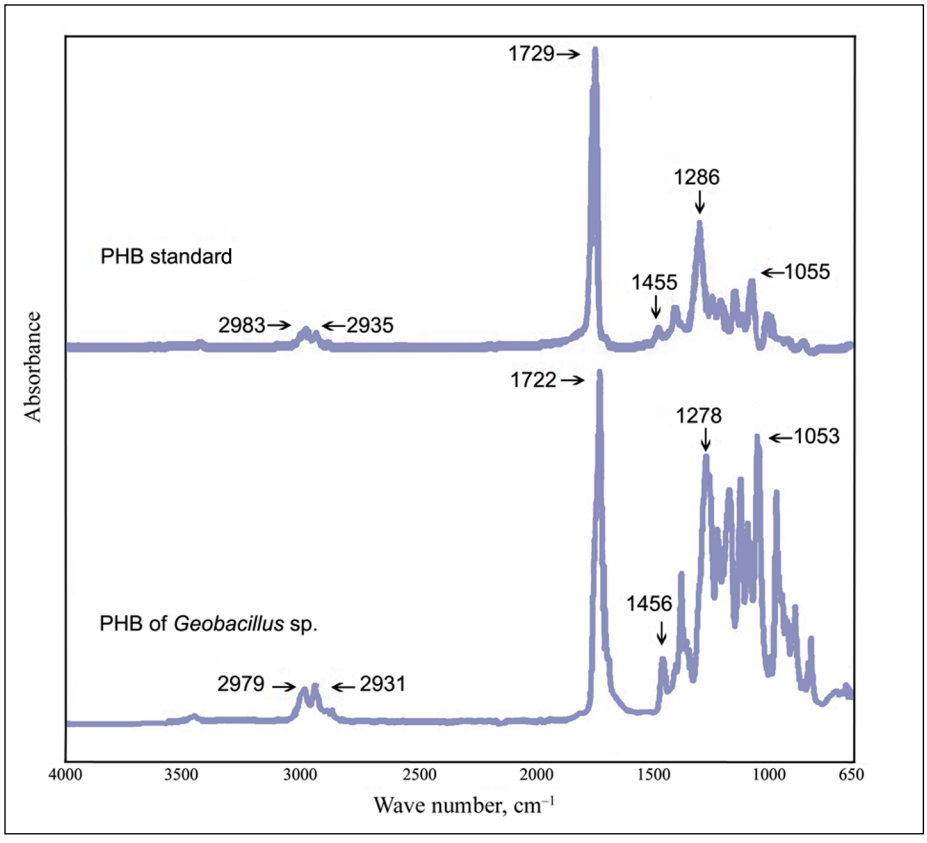

Fig. 4. FTIR spectra of PHB standard (upper) and PHB extract (bottom) from *Geobacillus* sp. AY946034 cultured with glucose as the carbon source

bonds of COO^- groups; the positions of these assignments could also vary with contributions from PHB. The peaks between 1 200 cm^{-1} and 900 cm^{-1} are due to the vibration of C–O–C bonds [39], with contributions from PHB [40].

Thermal properties of PHB. The calorimetric scan of PHB extracted from thermophilic strain was shown in Fig. 5. The melting temperature and enthalpy of melting of the polymer were 168.80 °C and 62.98 J/g, respectively. The polymer degraded rapidly over 260 °C with a peak at 280 °C. The difference between the melting temperature and the decomposition temperature (280 °C) was high enough to facilitate processing of the polymer.

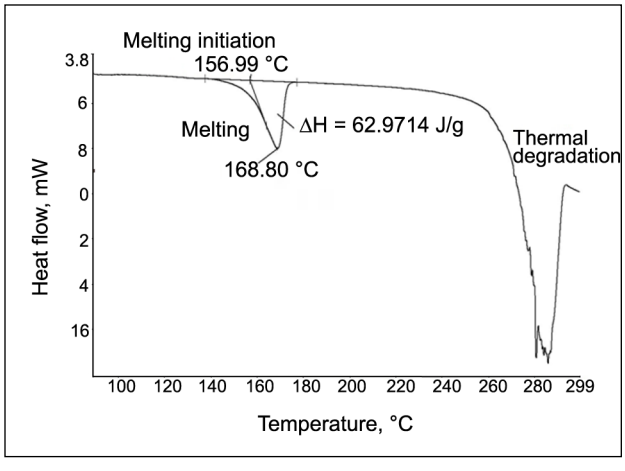

Fig. 5. The DSC thermogram of the polymer purified from *Geobacillus* sp. AY946034

The melting enthalpy led to calculate the degree of crystallinity, which is an important characteristic of a polymer in order to regulate the mechanical properties of PHB. In the present study, the crystallinity obtained for PHB was found to be in the lower range of that reported in the literature, 42% crystallinity. The results of DSC indicate that PHB from thermophilic *Geobacillus* sp. AY946034 strain has lower melting temperature, enthalpy and crystallinity (42%) than other PHBs from mesophilic *Bacillus* spp. [42] due to its much smaller molecular weight. Highly crystalline polymers are usually stiff and brittle resulting in very poor mechanical properties with low extension at break [43]. In nature, poly-3-hydroxybutirate exists as a brittle material and has high melting temperature (175 °C) which is very close to the degradation temperature of 200 °C, thus leaving only a narrow temperature window for thermoplastic processing [44].

Molecular weight determination. Polymer molecular weight determines many physical properties. There are drawbacks to the use of PHB as a plastic material because of its tendency to be brittle. This problem could be solved by the synthesis of copolymer or homopolymers with a relatively low molecular weight and melting point [45]. The average molecular weight of the PHB polymer from *Geobacillus* sp. AY946034 strain was 556 ± 21 kDa. The average molecular weight of the polymer extracted from mesophilic *Bacillus megaterium* was 519 kDa when the isolate was grown on glucose [46]. PHB with the molecular weight 20 000 kDa was obtained from mixed bacterial culture by Reis and co-workers [47]. At the other end of the

range, Lawrence et al. have reported PHB of 75 kDa molecular weight produced by *Allochromatium vinosum* [48]. Intermediate molecular weights have also been reported [49]. The molecular weight of PHA is usually larger till 1 000–2 000 kDa compared to those of synthetic polyesters 100–200 kDa. Reduction of the molecular weight to some extent does not affect the mechanical properties of the biopolyesters, however, when the molecular weight is reduced till 100–200 kDa, the biopolymer becomes brittle or less ductile what makes processing more difficult.

As far as we know, the PHB isolated in this study is the first polymer purified and characterized from thermophilic bacterium *Geobacillus* sp. The biosynthesis of PHBs in thermophilic bacteria was reported in *Thermus thermophilus* HB8 [28], *Chelatococcus daeguensis* TAD1 [50], *Caldinamonas taiwanensis* [29] and *Bacillus* spp. [51]. However, the main focus of research has been directed to the PHB yield and bacteria growth conditions, PHB has not been purified and characterized. Further studies are needed in order to optimize the biopolymer production process and to assess the potential of the material for different applications. Investigations would be dedicated to the PHB production through the molar ratio of carbon to nitrogen (C/N) and carbon to phosphorus (C/P), temperature and synthesis of various PHA copolymers.

CONCLUSIONS

On the basis of the data obtained in the present work it can be concluded that *Geobacillus* sp. AY946034 strain isolated from the oil field of Lithuania can be employed in the industrial production of PHB.

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POLIHIDROKSIBUTIRATO, IŠSKIRTO IŠ TERMOFILINĖS BAKTERIJOS *GEOBACILLUS* SP. AY 946034 KAMIENO, GRYNINIMAS IR SAVYBIŲ NUSTATYMAS

S a n t r a u k a

Poliesteriai bakterijose sintetinami ir kaupiami kaip anglies ir energijos šaltinis trūkstant tam tikrų maisto medžiagų (azoto arba fosforo), bet esant pertekliniam anglies šaltiniui. Šiame darbe nustatyta, kad didžiausia polimero išeiga termofilinėje *Geobacillus* sp. bakterijoje (68,9 % sauso ląstelių svorio) pasiekiama druskų terpėje su gliukozės pertekliumi ir sumažintu fosforo šaltinio kiekiu. Iš bakterinių ląstelių išskirtas ir išgrynintas polimeras buvo identifikuotas FT-IR spektroskopijos ir diferencinės skenuojančios kalorimetrijos metodais. FT-IR spektroskopijos metodu įvertinta, kad tiriamasis polimeras yra polihidroksibutiratas. Diferencinės skenuojančios kalorimetrijos metodu nustatyta polihidroksibutirato lydymosi temperatūra (168,8 °C), lydymosi proceso entalpija (62,9 Jg^{-1}) ir terminės destrukcijos temperatūra (280°). Polimero kristališkumo laipsnis – 42 %; polihidroksibutirato molekulinė masė – 556 kDa.