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RESEARCH ARTICLE

Comparative evaluation of physico-chemical characteristics of biopolyesters P(3HB) and P(3HB-co-3HV) produced by endophytic *Bacillus cereus* RCL 02

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BACKGROUND: Bacteria endogenously residing within the plant tissues have attracted significant attention for production of biopolyester, polyhydroxyalkanoates (PHAs). *Bacillus cereus* RCL 02 (MCC 3436), a leaf endophyte of oleaginous plant *Ricinus communis* L. accumulates 81% poly(3-hydroxybutyrate) [P(3HB)] of its cell dry biomass when grown in mineral salts (MS) medium.
METHODS: The copolymer production efficiency of *B. cereus* RCL 02 was evaluated in valeric acid supplemented MS medium under biphasic cultivation condition. The copolymer so produced has been compared with the P(3HB) isolated from RCL 02 in terms of thermal, mechanical and chemical properties.
RESULTS: Valeric acid supplementation as co-substrate in the medium has led to the production of copolymer of 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV) [P(3HB-co-3HV)] with 14.6 mol% 3HV. The identity of the polymers has been confirmed by X-ray diffraction (XRD) analysis, Fourier transform infrared (FTIR) and nuclear magnetic resonance (NMR) spectroscopic studies. Thermogravimetric analysis (TGA) revealed that P(3HB) and P(3HB-co-3HV) films degraded at 278.66°C and 273.49°C, respectively. The P(3HB-co-3HV) showed lower melting temperature (165.03°C) compared to P (3HB) (170.74°C) according to differential scanning calorimetry (DSC). Incorporation of 3HV monomers decreased the tensile strength (21.52 MPa), tensile modulus (0.93 GPa), storage modulus (E′) (0.99 GPa) and increased % elongation at break (12.2%) of the copolyester. However, P(3HB) showed better barrier properties with lower water vapor transmission rate (WVTR) of 0.55 g-mil/100 in^2/24 h.
CONCLUSION: These findings emphasized exploration of endophytic bacterial strain (RCL 02) to produce biodegradable polyesters which might have significant potential for industrial application.

Keywords poly(3-hydroxybutyrate), poly(3-hydroxybutyrate-co-3-hydroxyvalerate), biodegradable polyester, *Bacillus cereus*, endophytic bacteria

Introduction

Endophytic bacteria, the treasured biological resources, have been explored for production of diverse biopolymers including the biodegradable biopolyester, polyhydroxyalkanoates (PHAs) (Ryan et al., 2008). The intracellularly accumulated biopolyesters might contribute to the survival of these endosphere associated bacteria in a highly competitive microenviroment inside the plants. Moreover, metabolism of these intracellular PHAs might be one of the strategies by which these endogenous bacteria can improve their establishment and survival within the internal tissues of the plant (Castro-Sowinski et al., 2010). However, literature pertaining to the production and characterization of the PHAs by endophytic bacteria appears to be scanty (Catalan et al., 2007).

Biosynthesis and accumulation of PHA by a wide variety of microorganisms derived from diverse ecological conditions has been well explored (Saharan et al., 2014) and the

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biopolyesters so produced have gained significant interest as a promising alternative to conventional petrochemical based thermoplastics due to their material properties, biodegradability and biocompatibility (Urtuvia et al., 2014). Poly(3-hydroxybutyrate) [P(3HB)], the most common member of the PHA family has been studied extensively but its widespread usage has been restricted due to high crystallinity and brittleness (Tanase et al., 2015). In contrast, copolymers of 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV) [P(3HB-co-3HV)] are characterized by improved material properties and higher biodegradability (Anjum et al., 2016). It is also evident that the variation of material properties of PHAs can be tailored to specific applications by varying their chemical composition, which could be achieved by variation of producer organisms, carbon sources, co-substrates and cultural conditions (You et al., 2003; Winnacker and Rieger, 2017).

Members of the genus *Bacillus* including (Kumar et al., 2013; Tajima et al., 2003) *Bacillus cereus* are capable of utilizing wide variety of carbon sources for biosynthesis of homopolyesters (Valappil et al., 2007; Sharma and Bajaj, 2015a). However, biosynthesis of copolyesters by *B. cereus* is confined to a very few reports and needs extensive investigation. *B. cereus* DSM 31 was previously reported to accumulate P(3HB-co-3HV) when odd-chain length n-hydroxyalkanoic acids such as propionic acid, valeric acid and heptanoic acid were used as co-substrates (Chen, 1991). Later, Valappil et al. (2008) and Masood et al. (2012a) documented the accumulation of P(3HB-co-3HV) by *B. cereus* SPV and *B. cereus* FA11, respectively. Moreover, *B. cereus* was reported to produce copolymers of P(3HB-co-3HHx) and P(3HB-co-3HO) when grown in caproate and octanoate, respectively (Caballero et al., 1995). In addition, biosynthesis of tercopolymer [P(3HB-co-3HV-co-6HHx)] by *B. cereus* UW85 utilizing ϵ-caprolactone as the sole source of carbon was documented by Labuzek and Radecka (2001).

An endophytic bacterium, *Bacillus cereus* RCL 02 (MCC 3436; GenBank accession no. KX458035) isolated from internal leaf tissues of oleaginous plant *Ricinus communis* L. has been reported to produce P(3HB) accounting about 81% of cell dry weight (CDW) (Das et al., 2017). The present investigation represents the synthesis and intracellular accumulation of copolymer of 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV) [P(3HB-co-3HV)] by the endophytic *Bacillus cereus* strain RCL 02. Attempts have also been made to characterize and compare the thermal, mechanical as well as chemical properties of the homo- and copolyesters produced by *B. cereus* RCL 02 for potential applications.

Materials and methods

Bacterial strain and maintenance

Bacillus cereus RCL 02 (MCC 3436; GenBank accession no. KX458035), a leaf endophyte of oil-yielding plant *R. communis* L. was used throughout this study. The culture was grown on slopes of tryptic soy agar at 32°C for 24 h and maintained by regular sub-culturing on the same medium.

Growth and polymer production

For the production of P(3HB), mineral salts medium (Ramsay et al., 1990) containing 2.5% (w/v) glucose and 0.4% (w/v) yeast extract (50 mL per 250 mL Erlenmeyer flask) was inoculated with freshly prepared inoculum of *B. cereus* RCL 02 and incubated at 32°C under continuous shaking at 120 rpm for 56 h.

Production of the copolymers was evaluated following biphasic cultivation method (Choi et al., 1999). Cells from 24 h old culture grown in mineral salts medium were harvested aseptically by centrifugation (10000 r/min × 10 min), washed with normal saline and transferred to the same medium supplemented with valeric acid and incubated under continuous shaking at 32°C.

Estimation of growth

Growth was determined by measuring the dry weight of the biomass. Cells were harvested by centrifugation (10000 r/min × 10 min) after definite period of incubation, washed thoroughly with deionized water and acetone. Finally, the biomass was determined after drying to a constant weight at 80°C.

Extraction and purification of the polymer

The polymer accumulated in the dried cell mass was extracted thrice in warm chloroform (50°C), filtered through glass wool, concentrated and precipitated with double volumes of pre-chilled diethyl ether (Ramsay et al., 1990). The precipitated polymer was recovered by centrifugation (12000 r/min × 12 min) and dried to obtain the powdered polymer.

Crotonate assay of the polymer by UV spectroscopy

The extracted polymer was converted to crotonic acid following treatment with concentrated H_2SO_4 in a boiling water bath for 10 min (Law and Slepecky, 1961), cooled to room temperature and the absorbance was recorded at 235 nm in a UV-VIS spectrophotometer (Jenway, Model 6505) using authentic P(3HB) from Sigma, USA as standard. While, the amount of P(3HB) was quantified from the calibration curve, total PHA content of the dried cell mass was quantified gravimetrically.

Preparation of the polymer films

Films of PHA were prepared by casting 2% (w/v) chloroform solution of the polymer onto a glass Petriplate following slow evaporation of the chloroform under regular humidity at 50°C. The remaining solvent was evaporated by vacuum drying for two days.

Characterization of the polymer

Thermal property analysis

Thermogravimetric analysis
Thermogravimetric analysis was made in a TA STDQ 600 thermogravimetric analyzer operating with nitrogen flow of 100 mL/min. The samples (10 mg) were heated from room temperature to 500°C in alumina crucible with a heating rate of 10°C/min under the dinitrogen atmosphere.

Differential scanning calorimetry

Melting temperature (T_m) and the heat of fusion (ΔH) of the PHA samples were determined by differential scanning calorimetry (DSC) using Perkin-Elmer Diamond DSC thermal analyzer at a heating rate of 10°C/min in the temperature range of −50 to 200°C and N_2 flow rate of 150 mL/min. Only the second heating curve was provided in the result.

Mechanical property analysis

Mechanical properties of the P(3HB) and P(3HB-co-3HV) films were determined using Zwick Roell (ZO10) at room temperature in regular humidity. The specimens of ~ 0.1 mm thickness were cut into rectangular shape with 5 mm width. Initial separation between two grips was set as 22 mm and cross head speed of 2 mm/min according to the ASTM method D882-95a. Tensile strength, tensile modulus as well as % elongation at break were calculated from the stress-strain plot.

Dynamic mechanical analysis

The dynamic mechanical analysis (DMA) of the homo- as well as copolyester films were carried out in a Perkin Elmer DMA 8000 instrument in tension rectangle mode in the temperature range of 40 to 180°C with a heating rate of 5°C/min at a frequency of 1 Hz and sinusoidal deformation at 5 µm amplitude. The films were cut into ~ 13.02 × 3 × 0.1 mm^3 dimension for the analysis.

Water vapor transmission rate

The water vapor transmission rate (WVTR) of P(3HB) as well as P(3HB-co-3HV) films was performed using the modified ASTM E96-00 (ASTM, 2000) method. Thin films were sealed on the top of a 60 mm circular opening glass container containing calcium chloride, maintaining ~ 0% relative humidity inside the container. The glass container was then placed in a 75% constant relative humidity chamber. The weight of the container was measured at an interval of 24 h until the constant weight was reached. The WVTR of the films were calculated using the following equation,

$$Q = \frac{W \times L}{100 \times S}$$

where, W is the increased weight of the permeation cell every 24 h interval, L and S are the thickness (mil) and the exposed area (in^2) of the specimen, respectively. Q is the water vapor transmission rate (g-mil/100 in^2/ 24 h).

X-Ray Diffraction analysis

The wide angle X-ray diffractograms of the purified polymer sheets were recorded in X'pert PRO PANalytical X-ray diffractometer. The nickel filtered CuKα radiation (λ = 0.154 mm, 40 kV, 30 mA) was used at room temperature. The scan speed was 2θ = 5°/min.

Chemical property analysis

Elemental analysis
Elemental composition of purified PHAs was determined by analyzing the carbon, hydrogen, nitrogen, oxygen and sulfur content in Perkin Elmer 2400 CHNS/O.

Fourier transform infrared spectroscopic analysis
For Fourier transform infrared (FTIR) spectroscopy, the pellets were prepared by using purified polyesters and KBr and scanned in Perkin Elmer RX-1 FTIR spectrophotometer in the range of 4000 to 400 cm^{-1}.

Nuclear magnetic resonance spectroscopic analysis
Monomer composition of the purified polyester samples were elucidated from proton (^1H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectroscopic analysis.

^1H NMR
The polyester samples dissolved in deuterated chloroform ($CDCl_3$) were analyzed in a Bruker AV300 Supercon NMR (at 300 MHz) spectrophotometer. A multinucleate probe head at 30-degree flip angle was used. The chemical shift-scale was in parts per million and tetramethylsilane (Me_4Si) was used as the internal standard.

^{13}C NMR
The ^{13}C NMR spectral analysis was performed at 75 MHz in Bruker AV300 Supercon NMR spectrophotometer. The chemical shift-scale was in parts per million (ppm) and tetramethylsilane (Me_4Si) was used as the internal standard.

Results

Biosynthesis of copolymer by valeric acid supplementation

Biosynthetic production of copolyester by the endophytic isolate *B. cereus* RCL 02 was evaluated following biphasic cultivation method. Freshly grown cells of RCL 02 were harvested during logarithmic phase of growth in glucose containing mineral salts medium and the washed cells were transferred to the same fresh medium supplemented with valeric acid (0.2%) as co-substrate. Growth kinetics indicated that after 60 h of incubation under continuous shaking, during the second phase, the isolate RCL 02 produced maximum biomass (8.05 g/L) and PHA accounting 72.34% of CDW. The accumulated polyester showed incorporation of 14.6 mol % 3HV in PHA chain leading to the production of copolyesters of 3HB and 3HV [P(3HB-co-3HV)] (Fig. 1). Further studies with variation in concentration of valeric acid (0.05-0.5%) revealed enhanced biomass (10.85 g/L) and PHA production (78.9%, CDW) at lower concentration (0.05%) but with reduced 3HV incorporation (8.4 mol%). On the contrary, valeric acid concentration beyond 0.2% showed a pronounced negative impact on growth, PHA accumulation and 3HV content (Table 1).

The endophytic isolate *B. cereus* RCL 02 also produced 81% P(3HB) of its CDW during early stationary growth phase in glucose containing mineral salts medium (Das et al., 2017). Both the homo- and the copolyester so produced have been extracted, purified and characterized for their thermal, mechanical as well as chemical properties.

Characterization of the homo-and copolymer

Thermal property analysis

Thermogravimetric analysis

Thermo-gravimetric analysis (TGA) (Fig. 2A) and first order derivative (DTG) of weight loss (Fig. 2B) of P(3HB) and P(3HB-co-3HV) clearly indicated that in both the cases, weight

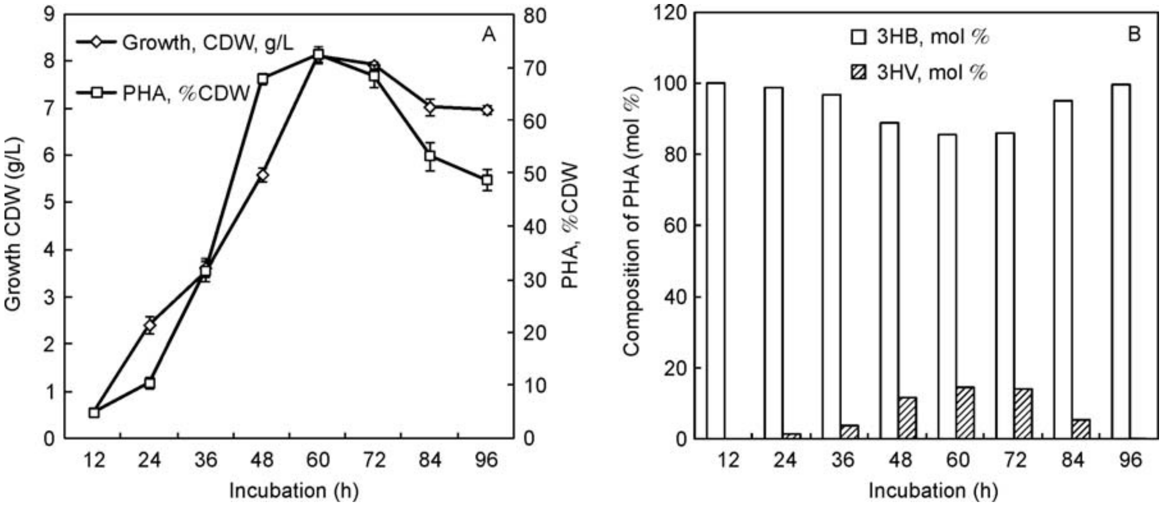

Figure 1 Time course of growth, PHA content (A), and composition (B) of the copolymer accumulated by the endophytic bacterial isolate *B. cereus* RCL 02 cells grown in glucose containing mineral salts medium supplemented with valeric acid during biphasic cultivation.

Table 1 Influence of valeric acid on growth, PHA content and monomer composition of polyester accumulated by the endophytic *B. cereus* RCL 02 during biphasic cultivation condition

Valeric acid, % (v/v) [a]	Growth CDW (g/L) [b]	PHA (% CDW) [c]	PHA (g/L)	PHA composition (mol %) [d]	
				3HB	3HV
0.05	10.15±0.08	78.89±2.21	8.00±0.07	91.6	8.4
0.1	8.54±0.11	72.98±1.15	6.23±0.08	87.5	12.5
0.2	8.05±0.14	72.34±1.42	5.82±0.11	85.4	14.6
0.3	7.65±0.07	70.93±1.18	5.43±0.05	85.6	14.4
0.4	1.28±0.03	29.04±2.16	0.37±0.04	97.3	2.7
0.5	0.62±0.05	15.53±1.14	0.10±0.05	98.5	1.5

[a] Valeric acid of different concentration was added individually to the glucose (2.5%) containing MS medium under biphasic cultivation condition
[b] Growth was determined by measuring the cell dry weight (CDW)
[c] P(3HB) was determined by chemical estimation method and the PHA content was quantified gravimetrically
[d] The mol% of monomers were calculated following ^{1}H NMR analysis of the purified samples

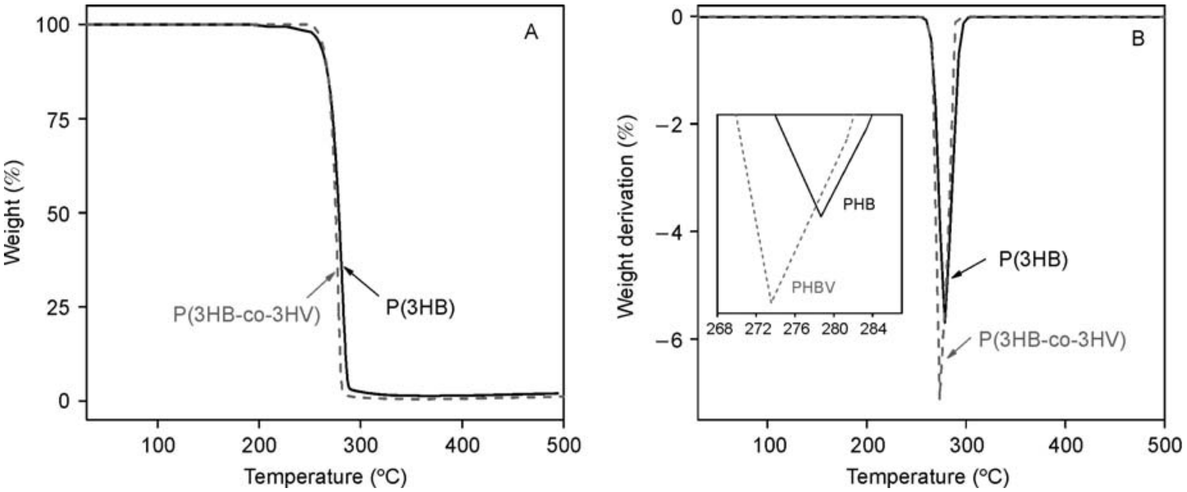

Figure 2 TGA (A) and DTA (B) curves of P(3HB) and P(3HB-co-3HV) isolated from *B. cereus* RCL 02 as a function of temperature.

loss occurred between temperature 230 to 300°C as single exotherm due to the decomposition of polymer chains. The TGA curves of both the homo as well as copolymer showed smooth weight loss from beginning until completion. The DTG curves, on the other hand revealed that incorporation of 3HV monomer (14.6 mol%) into the polymer chain, decreased the degradation temperature (in terms of 50% weight loss) to 273.49°C as compared to 278.66°C of P(3HB).

Differential scanning calorimetry

Melting temperature (T_m) and heat of fusion (ΔH_m) of the polyester samples were determined following differential scanning calorimetry (DSC). It was clearly evident that incorporation of 3HV monomer reduced the T_m as well as ΔH_m value of P(3HB-co-3HV) as compared to P(3HB) (Fig. 3). While, the purified P(3HB) showed a T_m of 170.74°C and ΔH_m of 92.52 J/g, P(3HB-co-3HV) showed comparatively lower T_m (165.03°C) and ΔH_m (84.61 J/g).

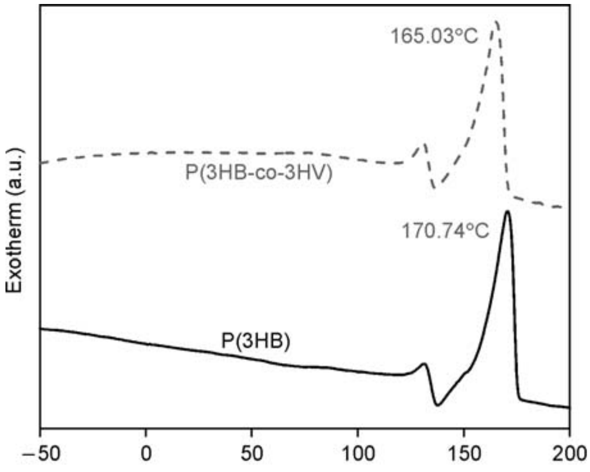

Figure 3 DSC curves of P(3HB) and P(3HB-co-3HV) isolated from *B. cereus* RCL 02 showing melting temperature (T_m).

Mechanical property analysis

The stress *vs.* strain curves of solution cast P(3HB) as well as P(3HB-co-3HV) films are represented in Fig. 4A. The tensile strength of P(3HB) and P(3HB-co-3HV) films were recorded 25.22 and 21.52 MPa, respectively (Fig. 4B), while the tensile modulus at 0.01% strain decreased from 1.86 GPa of P(3HB) to 0.93 GPa of P(3HB-co-3HV) (Fig. 4C).

The % elongation at break of solution cast P(3HB) and P(3HB-co-3HV) films were recorded as 2.1% and 12.2%, respectively (Fig. 4D). Incorporation of 14.6 mol% 3HV into the 3HB polymer chain significantly increased the elongation at break and thus the flexibility of the copolymer increased approximately 480%.

Dynamic mechanical analysis

The storage modulus (E′) of P(3HB) as well as P(3HB-co-3HV) as a function of temperature at a fixed frequency (1Hz) is represented in Fig. 5. The storage modulus indicates the stiffness of the polymer as a function of temperature. It was observed that P(3HB) retained its initial storage modulus (1.06 GPa) up to 80°C and beyond that a significant drop occurred. Whereas, P(3HB-co-3HV) showed lesser storage modulus (0.99 GPa) at 40°C as compared to the P(3HB). Moreover, it gradually decreased with increase in temperature. The incorporation of 3HV monomers reduced the melt viscosity of the copolymer and thereby reduced the stiffness of P(3HB-co-3HV).

Water vapor transmission rate

The WVTR of purified P(3HB) and P(3HB-co-3HV) isolated from endophytic isolate RCL 02 was found 0.55 and 2.01 g-mil/100 in^2/ 24 h, respectively (Fig. 6). The homopolymer with lower WVTR exhibited better barrier

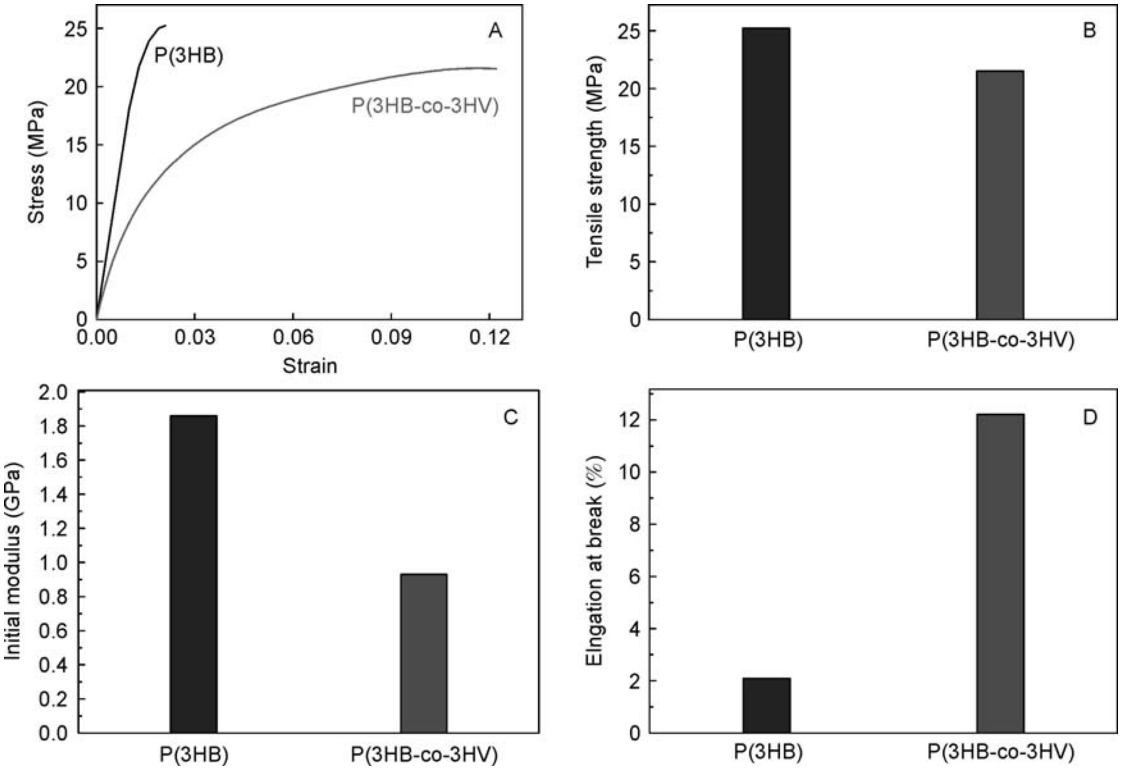

Figure 4 Mechanical properties of P(3HB) and P(3HB-co-3HV) films; stress *vs* strain curve (A), tensile strength (B), tensile modulus (0.01% strain) (C) and elongation at break (D).

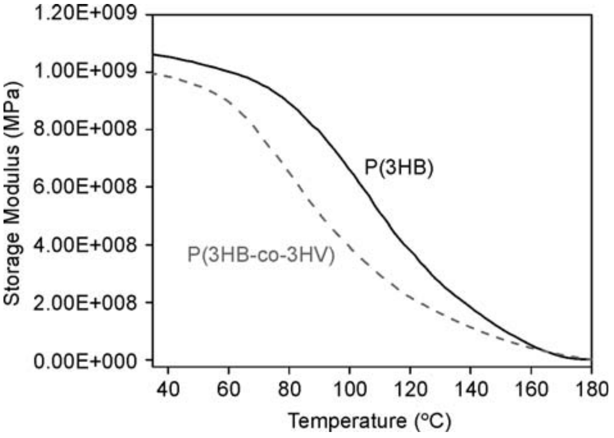

Figure 5 Storage modulus of P(3HB) and P(3HB-co-3HV) isolated from *B. cereus* RCL 02 as a function of temperature.

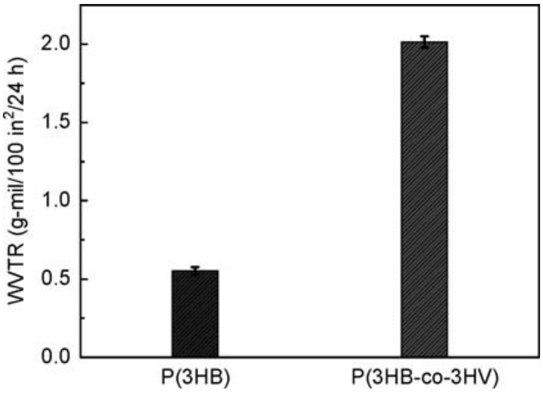

Figure 6 Water vapor transmission rate (WVTR) of P(3HB) and P(3HB-co-3HV) films prepared from the polyesters isolated from *B. cereus* RCL 02.

properties as compared to the copolymer.

X-Ray Diffraction analysis

X-ray diffractograms showed characteristic diffraction peaks of P(3HB) as well as P(3HB-co-3HV) (Fig. 7). While, the sharp and slender peaks up to 31° were attributed to high crystallinity of P(3HB), the copolymer was comparatively soft and less crystalline.

Chemical property analysis

Elemental analysis

The elemental composition of the purified polyesters from *B. cereus* RCL 02 showed that the homopolyester [P(3HB)] was composed of 56.4% carbon, 7.35% hydrogen and 36.25% oxygen as against 56.54% carbon, 7.39% hydrogen and 36.07% oxygen in the co-polyester [P(3HB-co-3HV)]. Both

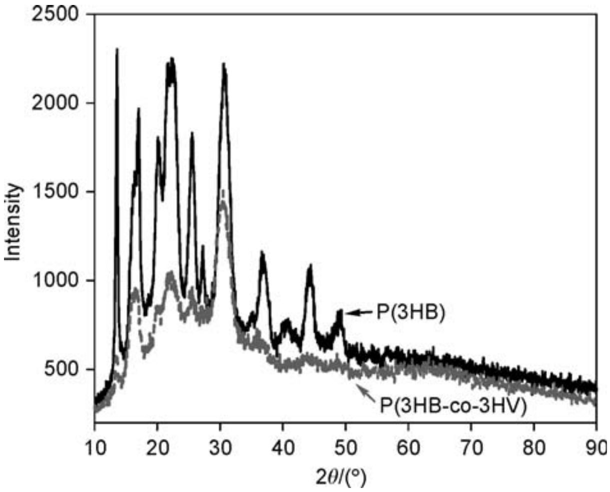

Figure 7 XRD analysis of P(3HB) and P(3HB-co-3HV) isolated from *B. cereus* RCL 02.

the homo-as well as copolyester was free from nitrogen and sulfur.

Fourier transform infrared spectroscopic analysis

The FTIR spectra of the purified homopolymer (Fig. 8A) showed characteristic absorption spectra for esters; –OH bending at 3437 cm^{-1}, C-H stretching at 2920-2980 cm^{-1}, strong absorption band of aliphatic carbonyl C = O at 1723 cm^{-1} and –CH group of aliphatic compound at 1230-1380 cm^{-1}.

The FTIR spectra of copolymer revealed the presence of absorption bands at 1723 cm^{-1} and 1277 cm^{-1} corresponding to C = O and C-O, respectively. The other characteristic bands appeared at 3436 cm^{-1}, 2933-2976 cm^{-1} and 1230-1380 cm^{-1} representing the –OH bending, C-H stretching and –CH group, respectively (Fig. 8B).

Nuclear magnetic resonance spectroscopic analysis

^{1}H NMR

The ^{1}H NMR spectrum (at 300 MHz) (Fig. 9A) of the purified homopolyester showed chemical shifts (δ) at 1.26, 2.4- 2.6 and 5.3 ppm which were assignable to the methyl group (CH_3) coupled to one proton, methylene group (CH_2) adjacent to an asymmetric carbon atom bearing a single proton and methyne group (CH), respectively.

The ^{1}H NMR spectrum (at 300 MHz) of the copolyester displayed characteristic peaks at 1.26 and 0.89 ppm indicating the presence of methyl (CH_3) group of 3HB (85.4 mol%) and 3HV (14.6 mol%) monomer unit, respectively (Fig. 9B). The presence of valerate monomer in copolymer was confirmed by the existence of triplet at 0.89 ppm.

^{13}C NMR

The ^{13}C NMR (at 75 MHz) spectrum of the homopolyester showed chemical shifts (δ) at 19.75, 40.78, 67.61 and 169.14 ppm, which were assigned to the presence of (CH_3), (CH_2), (CH) and (C = O) groups, respectively in P(3HB) (Fig. 10A).

The peaks identified by ^{13}C NMR spectrum (at 75 MHz) of P(3HB-co-3HV) at 19.77, 40.78, 67.61 and 169.16 correspond to (CH_3), (CH_2), (CH) and (C = O) groups, respectively (Fig. 10B).

Discussion

Bacteria endogenously residing within the internal tissues of plants have attracted considerable attention in the recent past for the production of diverse biopolymers including eco-benign biopolyester, polyhydroxyalkanoates (PHAs) (Ryan et al., 2008). While, the production of homopolyester, P(3HB) by endosphere associated bacteria under different cultural conditions have been explored extensively (Catalan et al., 2007; Das et al., 2016; Das et al., 2017), studies on accumulation of copolyester by the endophytes are scanty. In the present study, we report the utilization of valeric acid as a co-substrate for production of coployester, P(3HB-co-3HV) with 14.6 mol% 3HV by the endophytic bacterium *B. cereus* RCL 02. This observation corroborated well with the production of P(3HB-co-3HV) by the non-endophytic strains of *B. cereus*. Earlier, Valappil et al. (2008) have documented accumulation of P(3HB-co-3HV) with 10 mol% 3HV by *B. cereus* SPV under potassium limiting condition. Moreover, Mizuno et al. (2010) also reported P(3HB-co-3HV) accumulation by *B. cereus* YB-4 with a 3HV fraction up to 2 mol% with glucose as the carbon source.

It was evident that endophytic isolate *B. cereus* RCL 02 when grown in glucose containing MS medium synthesized and accumulated only P(3HB) (Das et al., 2017). Supplementation of valeric acid as co-substrate during biphasic cultivation condition (Choi et al., 1999) induced the incorporation of valerate monomers and synthesis of P (3HB-co-3HV) (Fig. 1).

Introduction of valerate monomers (14.6 mol%) in the PHA chain resulted significant variation in themal as well as mechanical properties. Thermogravimetric analysis revealed that the thermal degradation of the copolymer occurred at comparatively lower temperature (273.49°C) as compared to the homopolymer (278.66°C) (Fig. 2A and 2B). Such decrease in thermal stability could be explained by the incorporation of 3HV monomers which might have reduced the interaction between polymer chains. Moreover, 3HV monomers decreased the melting temperature (165.03°C) as well as the enthalpy of fusion (84.61 J/g) of the copolymer matrix and thus reduced its crystallinity (Fig. 3). These results are in well conformity with the documentation of Reddy et al. (2009) where the P(3HB-co-3HV) copolymers produced by *B. megaterium* showed T_m values ranging from 174.34 to 178.12°C. Decrease in melting temperature widens the processing range of P(3HB-co-3HV) as compared to P(3HB).

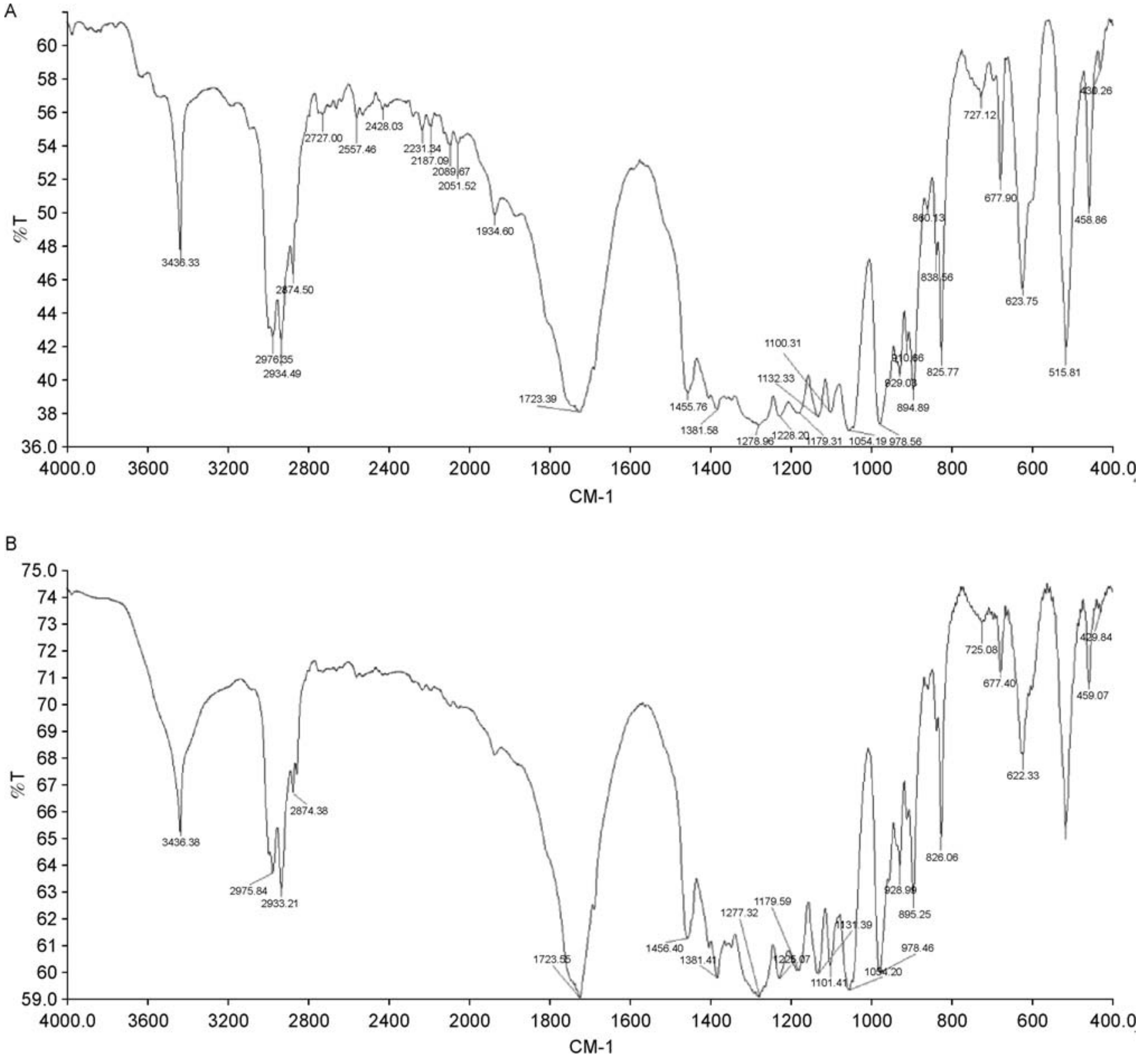

Figure 8 FTIR spectral analysis of purified P(3HB) (A) and P(3HB-co-3HV) (B) isolated from *B. cereus* RCL 02.

While, the tensile strength and modulus of P(3HB) were 25.22 MPa and 1.86 GPa, respectively, P(3HB-co-3HV) exhibited tensile strength of 21.52 MPa and tensile modulus of 0.93 GPa (Fig. 4B and 4C). Such decrease in tensile strength and tensile modulus of copolymer may be attributed to reduction of intermolecular force of attraction resulting from incorporation of 3HV. The % elongation at break of solution cast P(3HB) and P(3HB-co-3HV) films revealed that the flexibility of the copolyester increased to approximately 480% making it a better alternative for widespread commercial application.

Dynamic mechanical analysis (DMA) is used for the determination of temperature dependent storage modulus of polymers in dynamic condition. DMA of P(3HB) and P(3HB-co-3HV) showed the storage modulus (E′) of 1.06 GPa and 0.99 GPa, respectively (Fig. 5). It was apparent that with the reduction in E′, the copolymer appeared to be a better quality polymer for packaging industry.

Moisture permeability plays significant role in determining the competence of a material for its commercial application especially in food packaging industry as the water vapor may transfer from the internal or external environment through the polymer package wall, resulting in a continuous change in product quality and shelf-life. The quality of packaging material is inversely proportional to the value of water vapor permeability. In general, as compared to the conventional synthetic polymers, biodegradable polymers are characterized by lower barrier properties. While, the water vapor transmis-

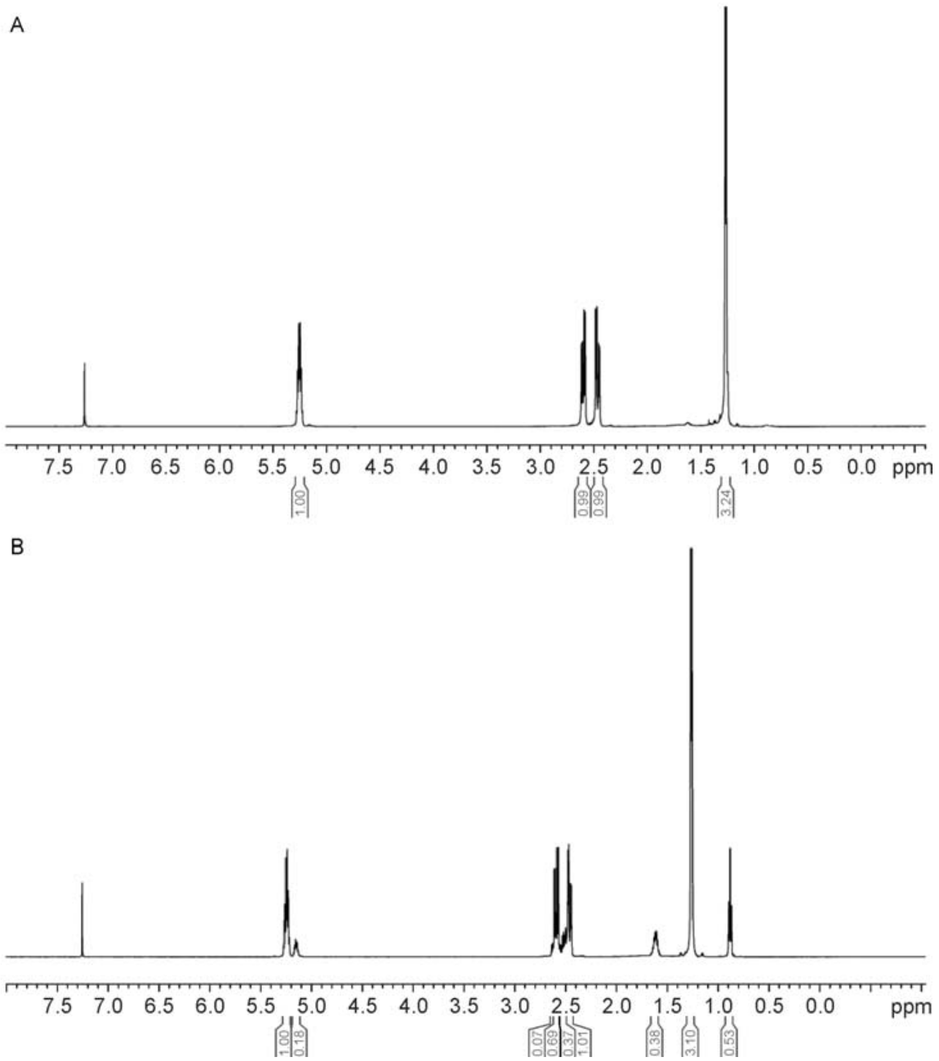

Figure 9 ^{1}H NMR spectra of purified P(3HB) (A) and P(3HB-co-3HV) (B) isolated from *B. cereus* RCL 02.

sion rate (WVTR) of synthetic polymers viz, polyvinyl chloride (PVC), low density polyethylene (LDPE), high density polyethylene (HDPE), polypropylene (PP), polystyrene (PS) and polycarbonate (PC) are 0.2, 1, 0.3, 0.2, 5 and 3 g-mil/100 in^2/24 h, respectively, the WVTR of commercially available biodegradable polymer like polylactic acid (PLA) is 6.04 g-mil/100 in^2/24 h (Grewal et al., 2012). The barrier properties of biodegradable polyesters produced by the endophytic isolate RCL 02 appeared to be quite satisfactory and compared well with other conventional non-biodegradable thermoplastics (Fig. 6). The 3HV monomers reduced the interaction between the polymer chains and increases the void spaces which in turn increased the WVTR of the P(3HB-co-3HV) compared to the P(3HB). So the polymers, especially the homoplymer of endophytic origin with lower WVTR (0.55 g-mil/100 in^2/24 h) may find better application in food packaging.

The powder diffraction pattern of the homo- and copolyester represented characteristic peaks of semi-crystalline polymer (Fig. 7). While, the wide-angle X-ray diffractograms of P(3HB) corroborated well with the polymer isolated from *B. cereus* PS 10 (Sharma and Bajaj, 2015b), the XRD pattern of the copolymer was consistent when compared with previous crystallographic studies of P(3HB-co-3HV) (Sato et al., 2011).

The majority of the bands in the infrared spectra of P(3HB) and P(3HB-co-3HV) were found to be associated with both crystalline and amorphous region (Bayari and Severcan, 2005). However, using KBr pelletized PHA, it is possible to distinguish short and medium chain length PHA, but within short chain length PHA, HV could not be conclusively distinguished from HB (Shamala et al., 2009). While, the FTIR spectra of purified P(3HB) (Fig. 8A) isolated from *B. cereus* RCL 02 was consistent with the documentation of Sindhu et al. (2013), the absorption peaks of P(3HB-co-3HV) (Fig. 8B) showed close proximity with P(3HB-co-5 mol% 3HV) isolated from *B. cereus* S10 (Masood et al., 2012b) and provided evidence for the accumulation of homo- as well as

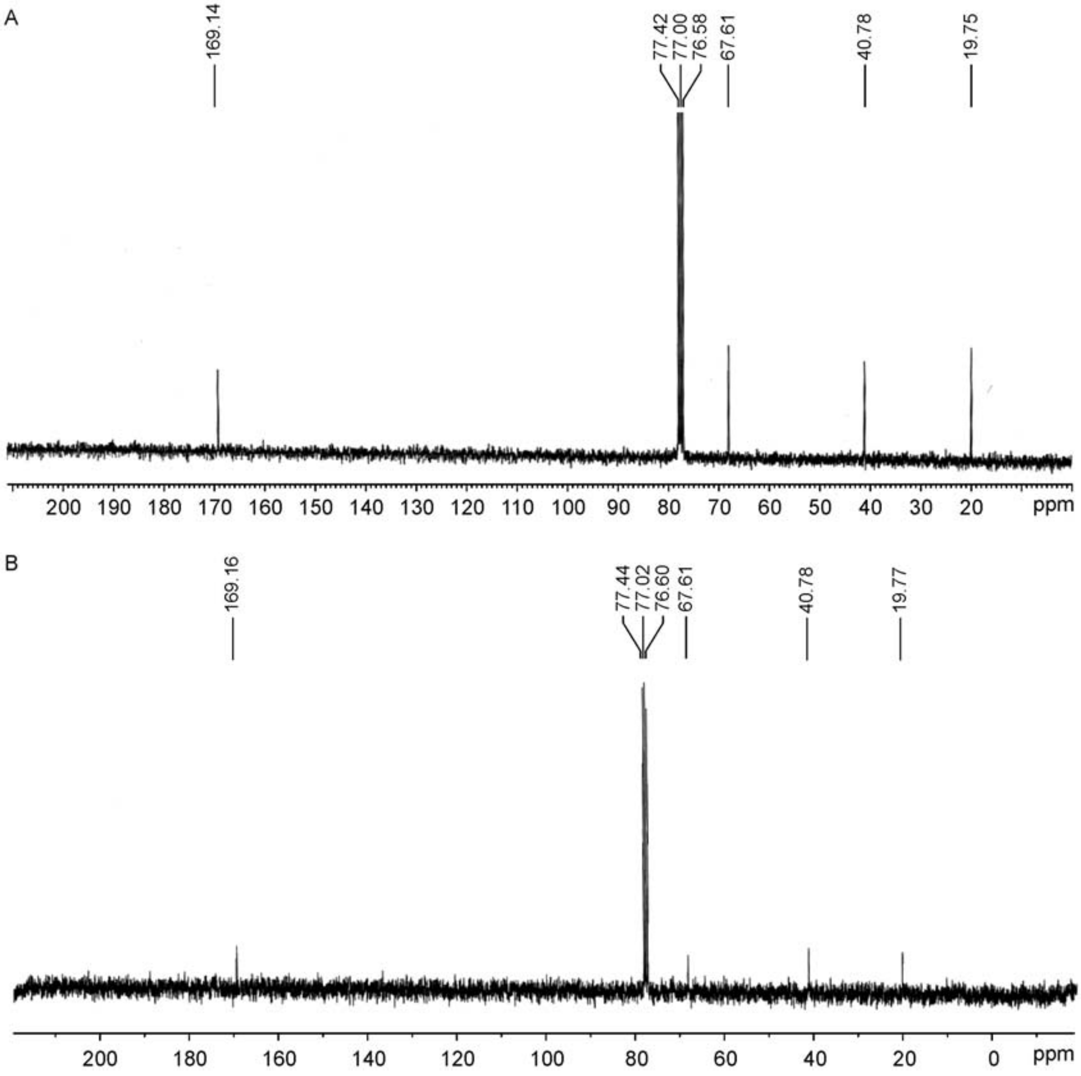

Figure 10 ^{13}C NMR spectra of purified P(3HB) (A) and P(3HB-co-3HV) (B) isolated from *B. cereus* RCL 02.

copolyester accumulation by the endophytic bacterium *B. cereus* RCL 02.

The solution state NMR (^{1}H and ^{13}C) spectra of the homopolyester showed characteristic signals of 3-hydroxybutyrate and was consistent with the previous findings of Doi et al. (1989). Furthermore, the incorporation of 3HV (14.6 mol%) in the PHA was determined through the integration of well resolved signals of ^{1}H and ^{13}C NMR spectra (Mitomo et al., 1993) (Figs. 9 and 10).

Conclusion

The present study has clearly revealed that the internal tissues of oleaginous plants, hither to an underexplored area represent a niche for novel bacterial strains, which could serve as potential bioresource for production of biodegradable plastics of commercial importance. The leaf endophyte *B. cereus* RCL 02 isolated from oleaginous plant *R. communis* L. accumulated P(3HB) as well as copolymers of 3HB and 3HV and represents an ideal resource for PHA production. The endophytic isolate accumulated P(3HB-co-3HV) with 14.6 mol% 3HV during growth under biphasic cultivation condition. Incorporation of 3HV monomers has improved the material as well as thermal properties of the copolymer making it less crystalline and more ductile and thereby indicating its possibility for wide scale industrial application. Moreover, the homopolymer also showed good barrier properties and may find potential packaging applications.

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Compliance with ethics guidelines

The authors, Rituparna Das, Nayan Ranjan Saha, Arundhati Pal, Dipankar Chattopadhyay and Amal Kanti Paul declare that they have no conflict of interests. This article does not contain any studies with human and animal subjects performed by any of the authors.

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