J Ind Microbiol Biotechnol (2009) 36:347–354
DOI 10.1007/s10295-008-0503-x

ORIGINAL PAPER

Exploitation of inexpensive substrates for production of a novel SCL–LCL-PHA co-polymer by *Pseudomonas aeruginosa* MTCC 7925

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Received: 30 July 2008 / Accepted: 10 November 2008 / Published online: 4 December 2008

Abstract Studies conducted with various inexpensive carbon sources such as whey, vegetable oils (palm, mustard, soybean and coconut), a low-cost source of glucose-D, rice and wheat bran, and mustard and palm oil cakes demonstrated palm oil as the best substrate for accumulation of a novel short-chain-length–long-chain-length polyhydroxyalkanoate (SCL–LCL-PHA) co-polymer containing SCL 3HAs [3-hydroxybutyric acid (3HB) and 3-hydroxyvaleric acid (3HV)] and LCL 3HAs of 3-hydroxyhexadecanoic acid (3HHD) and 3-hydroxyoctadecanoic acid (3HOD) units as constituents by a sludge-isolated *Pseudomonas aeruginosa* MTCC 7925. The co-polymer content reached up to 60% of dry cell weight (dcw) at 48 h of incubation in 0.5% (v/v) palm oil and the extract of 0.5% (v/v) palm oil cake supplemented vessels. The PHAs pool was further enhanced up to 69 and 75% (dcw), when the above culture was subjected to P- and N-limitation, respectively. The mol fraction of 3HB:3HV:3HHD:3HOD units were, respectively, 83.1:7.7:3.8:5.4 and 87.3:5.1:3.6:4.0 in P- and N-limited cultures. Consequently, a co-polymer yield of 5 g l^{-1} (approx.) was achieved, which was about 80-fold higher as compared to 69 mg l^{-1} of the control culture. On substrate basis, the accumulation reached up to 0.62 g PHAs per g substrate, which was significantly higher as compared to the yield obtained from starch by *Haloferax mediterranei* and *Azotobacter chroococum,* from molasses by *A. vinelandii* UWD, and from lactose and xylose by *Pseudomonas cepacia.* This novel P(3HB-*co*-3HV-*co*-3HHD-*co*-3HOD) co-polymer exhibited better thermal and mechanical properties as revealed from the differential scanning calorimetry and mechanical property studies, thus opens up new possibilities for various industrial applications.

Keywords Inexpensive carbon sources · N-limitation · PHAs · P-limitation · *Pseudomonas aeruginosa* MTCC 7925 · SCL–LCL-PHA co-polymer · Vegetable oils

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Introduction

Polyhydroxyalkanoates (PHAs) have attracted considerable interest in recent years. PHAs can be divided into three classes depending on the number of carbon atoms in their monomer units; short-chain-length (SCL), medium-chain-length (MCL) and long-chain-length polyhydroxyalkanoates (LCL-PHAs), composed of hydroxyacids with 3–5, 6–14 or more than 14 carbon atoms, respectively [2, 28]. About more than 100 different monomer units reported so far, none of them contain more than 14 carbon atoms as constituents of PHAs [23].

Despite the basic attractiveness as a substitute for the petroleum-derived polymers, the major hurdle facing commercial production and application of PHAs as consumer products is the high cost of bacterial fermentation, making PHAs about 15 times more expensive than the petroleum-derived polymers such as polypropylene [23]. In PHA production, about 30–50% of the total production cost is due to the raw materials [3, 19]. Therefore, research efforts are abound to exploit inexpensive raw materials as substrates in order to reduce the high cost of PHAs production.

Thus, a good candidate for PHAs production would be an organism that can store high level of PHAs while

growing on an inexpensive substrate. Several inexpensive carbon substrates such as molasses, lauric acid, whey, cellulose, plant oils and hydrolysates of starch (corn and tapioca) can be excellent substrates for bacteria to produce PHAs, which could lead to significant economical advantages. *Pseudomonas cepacia* was found to produce PHAs from whey or from its major component, lactose [32]. *Pseudomonas resinovorans* accumulated PHAs from tallow [5]. PHAs were produced by *Pseudomonas oleovorans* and *Ralstonia eutropha* when incubated in the oil remaining from rhamnose-producing process [8]. *Pseudomonas corrugata* was evaluated for PHAs production from soy molasses [27]. Biosynthesis of PHAs in *Brevundimonas vesicularis* LMG P-23615 and *Sphingopyxis macrogoltabida* LMG 17324 using acid-hydrolyzed sawdust as carbon source and conversion of agro-industrial wastewaters into biodegradable plastics were also explored [13, 25].

To the best of our knowledge, the sludge-isolated *Pseudomonas aeruginosa* MTCC 7925 is the first strain to accumulate SCL–LCL-PHA co-polymer consisting of SCL 3HAs units of 3-hydroxybutyric acid (3HB), 3-hydroxyvaleric acid (3HV) and LCL 3HAs of C_{16} [3-hydroxyhexadecanoic acid (3HHD)] and C_{18} [3-hydroxyoctadecanoic acid (3HOD)], i.e. P(3HB-*co*-3HV-*co*-3HHD-*co*-3HOD) co-polymer with more than 14 carbon atoms under the similar growth conditions [26], where other *Pseudomonads* synthesize MCL-PHAs only. In this study, attempt has been made to assess PHAs production in the test bacterium, *P. aeruginosa* MTCC 7925 by taking into account of various inexpensive carbon sources.

Materials and methods

Organism and growth measurement

Axenic cultures of the sludge-isolated *Pseudomonas aeruginosa* MTCC 7925 were grown in mineral salt medium and growth was measured in terms of dry cell weight (dcw) as detailed in Singh and Mallick [26].

Extraction and assay of polyhydroxyalkanoates (PHAs)

Extraction of PHAs from the dried biomass was carried out in hot chloroform [31] and PHAs concentration was determined following the propanolysis method [24] with the help of a Gas Chromatograph (Clarus 500, Perkin-Elmer, Shelton, CT, USA) under split mode (1:50, v/v) following Singh and Mallick [26]. The standards, poly 3-hydroxybutyric acid (PHB) and poly 3-hydroxybutyric acid-*co*-3-hydroxyvaleric acid P(3HB-*co*-3HV) co-polymer were procured from Aldrich (USA), and 3-hydroxyhexadecanoic acid (3HHD) and 3-hydroxyoctadecanoic acid (3HOD) were from Larodan Fine Chemicals (Sweden).

Impact of inexpensive carbon sources

Various inexpensive substrates such as palm, mustard, soybean and coconut oils, a low-cost source of glucose-D (Era Dextropack Company, West Bengal, India), rice and wheat bran, and mustard and palm oil cakes were obtained from the local market of Kharagpur, West Bengal, India. Whey was procured from a local dairy of Midnapur, West Bengal, India. All carbon sources were supplemented to the growth medium after autoclaving except mustard and palm oil cakes. Mustard and palm oil cakes were suspended in distilled water (50 g l^{-1}), boiled for 30 min and filtered. The filtrate was used as the stock solutions for mustard and palm oil cakes.

Studies on nitrogen and phosphorus limitations

In our earlier study, nitrogen (NH_4NO_3) and phosphate (KH_2PO_4) concentrations of 1.9 and 3.0 g l^{-1}, respectively, were found optimum for PHAs accumulation rather than complete N- or P-deficiency in the test bacterium. The above concentrations of NH_4NO_3 and KH_2PO_4 were taken to study the interactive effects of carbons and N-/P-limitation on PHAs yield. Cells pre-grown in carbon-supplemented medium were subjected to N- or P-limitation in presence of appropriate carbon doses. These doses were selected based on their performance on growth and PHAs yield.

Studies on physical properties of PHAs

For analysis of the thermal properties of the polymer, differential scanning calorimetry (DSC) was conducted with the help of a Pyris Diamond Differential Scanning Calorimeter (Perkin-Elmer, Shelton, CT, USA) following Kato et al. [12]. The stress–strain curves of the solution-cast films (0.1 mm thickness) of the samples were analyzed following Doi et al. [7].

All experiments were performed in triplicate to check the reproducibility. The results were analyzed statistically by Duncan's new multiple range test.

Results

Impact of inexpensive carbon sources on PHAs accumulation

Under batch mode study, an accumulation of 24% (dcw) was observed in control culture (grown in 0.1% glucose as

carbon source) at 48 h of incubation, where the polymer was dominated with 3HV units, i.e. 70.7% on mol fraction basis (Table 1). Interestingly, the plant oils (palm, coconut, mustard and soybean oils) depicted stimulatory effects on growth as well as PHAs accumulation (Table 1). Cakes of mustard and palm oils also exhibited positive effect on growth. PHAs accumulation, however, not registered any rise in these vessels. Maximum PHA pool of 30–33% (dcw), dominating with 3HB units was recorded in soybean and palm oil-supplemented vessels. Palm oil was selected for further study owing not only to the maximum polymer accumulation potential but also for its relatively low-cost as compared to the soybean oil.

Time-course of growth and PHAs accumulation under palm oil supplementation

The time-course of growth and accumulation of PHAs in 0.3% palm oil-supplemented condition was compared with the control cultures (Fig. 1). A profound rise in biomass yield was evident in the palm oil-supplemented vessels. Accumulation of PHAs also reached up to 33.3% (dcw) as compared to 24% in the control culture.

Impact of palm oil and its cakes on PHAs accumulation

Impact of various concentrations (0.3–2%) of palm oil on PHAs accumulation is presented in Fig. 2. PHA yield was boosted up to 62.2% (dcw) under 0.7% palm oil supplementation at 48 h of incubation. No further rise in PHA pool was observed at increasing concentrations of palm oil or further increasing the incubation period. Table 2 summarizes the maxima of PHAs yield for all the concentrations of palm oil studied along with the polymer composition. Interestingly, under palm oil supplementation a profound rise in 3HB units vis-à-vis a reduction in 3HV fraction was evident.

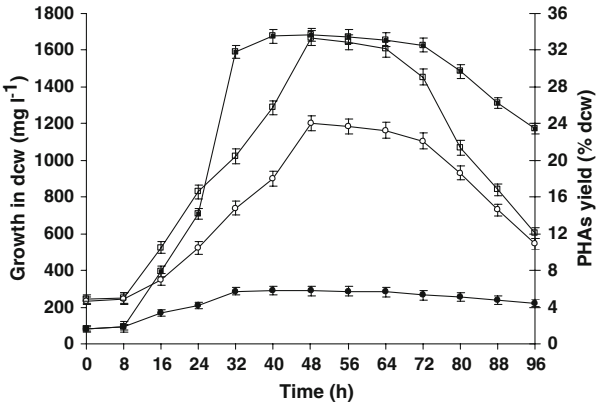

Fig. 1 Accumulation of PHAs in *P. aeruginosa* MTCC 7925 with reference to growth. Growth in 0.3% palm oil (*filled square*) and PHAs yield (*open square*). Control growth (*filled circle*) and control PHAs yield (*open circle*). Values are mean ± SE, $n = 3$. Control: 0.1% glucose

Table 3 shows the maxima of PHAs yield with polymer composition for various concentrations of palm oil (0.3–0.7%) when supplemented with the extract of palm oil cakes (0.3–0.7%). PHAs content reached up to 60% (dcw) under supplementation of 0.5% palm oil + 0.5% palm oil cake, which could be comparable with the maxima of the PHAs yield, i.e. 62.2% (dcw) in 0.7% palm oil-supplemented cultures (Fig. 2).

Table 1 Accumulation of PHAs in *P. aeruginosa* MTCC 7925 under supplementation of various inexpensive carbon sources for 48 h

Carbon source	Concentration (%)	Biomass yield (dcw, mg l^{-1})	PHA (% dcw)	Polymer composition (mol%)			
				3HB	3HV	3HHD	3HOD
Control (glucose)	0.1 (w/v)	288.2[a]	24.0[a, e]	24.7	70.7	1.5	3.1
Whey	0.3 (v/v)	220.4[b]	13.2[b]	79.2	10.5	1.9	8.4
Palm oil	0.3 (v/v)	1684.0[c]	33.3[c]	63.6	9.7	12.2	14.5
Mustard oil	0.3 (v/v)	1575.6[d]	27.3[a, d]	57.2	13.1	14.1	15.6
Soybean oil	0.3 (v/v)	1678.4[c]	30.1[c, d]	60.2	12.7	14.2	12.9
Coconut oil	0.3 (v/v)	1547.1[e]	25.3[a, e]	54.1	14.8	13.0	18.1
Glucose-D	0.3 (w/v)	376.7[f]	25.5[a, e]	89.1	3.1	3.2	4.6
Rice bran	0.3 (w/v)	161.3[g]	11.6[b]	68.4	9.1	12.4	10.1
Wheat bran	0.3 (w/v)	168.1[g]	12.5[b]	71.1	12.1	10.7	6.1
Mustard oil cake	0.3 (w/v)	533.1[h]	22.6[e]	55.4	10.8	12.1	21.7
Palm oil cake	0.3 (w/v)	760.6[i]	23.5[a, e]	61.2	11.0	14.3	13.5

Values are mean ± SE, $n = 3$

SE values range from 0.6 to 8.9

Values in the column superscripted by different alphabets are significantly different from each other ($P < 0.05$, Duncan's new multiple range test)

Separate analysis was done for each column

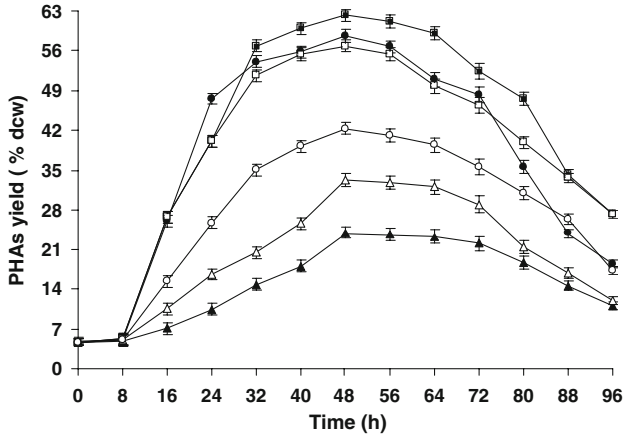

Fig. 2 Impact of various concentrations of palm oil on PHAs accumulation potential of *P. aeruginosa* MTCC 7925. Control (*filled triangle*), 0.3% (*open triangle*), 0.5% (*filled circle*), 0.7% (*filled square*), 1% (*open square*) and 2% (*open circle*) palm oil. Values are mean ± SE, $n = 3$. Control: 0.1% glucose

Impact of N-/P-limitation on PHAs accumulation

Limitations of nitrogen and phosphorus were also found stimulatory for the co-polymer accumulation (Table 4). A rise in total PHAs pool up to 44.8% (dcw) was noticed when the stationary phase *P. aeruginosa* MTCC 7925 was grown in N-limited medium under 0.3% palm oil supplementation for 48 h. Under P-limitation, the co-polymer pool also reached up to 39.4% of dcw.

Interaction of N-/P-limitation with palm oil and palm oil cakes on PHAs accumulation

PHAs accumulation was also studied under the interactive conditions of N-/P- limitation in presence of 0.5% palm oil in combination with the extract of 0.5% palm oil cakes. *Pseudomonas* cells pre-grown in 0.5% palm oil + the extract of 0.5% palm oil cake-supplemented mineral salt medium when transferred to N-limited medium in presence of 0.5% palm oil + the extract of 0.5% palm oil cakes for 48 h, a rise in PHAs pool up to 74.7% (dcw) was noticed with a mol fraction of 87.3:5.1:3.6:4.0 of 3HB:3HV:3HHD:3HOD units, respectively (Table 5). Under P-limitation, the PHAs pool also reached up to 69.4% (dcw) with a mol fraction of 83.1:7.7:3.8:5.4 in the above order (Table 5).

Discussion

Selection of suitable carbon substrates is a critical factor that determines the overall performance of the fermentation process as well as significantly influencing the cost of the final product. Plant oils are desirable feedstock for PHAs production because they are relatively cheap as compared to most sugars. Theoretically, the yield of PHAs from glucose ranges between 0.3 and 0.4 g of PHA per g of glucose [10]. On the other hand, plant oils are predicted to produce higher PHA yield because it contains higher carbon content on weight basis than simple sugars [1]. The theoretical yield of PHA from plant oils and fatty acids ranges from 0.65 to 0.98 g of PHA per g substrate [11]. Therefore, the prospect of industrial-scale production of PHAs using plant oils as the fermentation substrate is very encouraging.

As presented in Table 1, vegetable oils (palm, soybean, mustard and coconut) depicted good performance on growth as well as PHAs yield. Among them, palm and soybean oils recorded comparatively higher yields both for PHAs and growth. The stimulatory effect of plant oils on PHAs yield could be explained in the light of the findings of most *Pseudomonads* from the rRNA homology group I,

Table 2 Maximum accumulation values of SCL–LCL-PHA co-polymer in *P. aeruginosa* MTCC 7925 grown in various concentrations of palm oil

Carbon source	% Concentration (v/v)	Biomass yield (dcw, mg l^{-1})	PHA yield		Polymer composition (mol%)			
			(mg l^{-1})	(% dcw)	3HB	3HV	3HHD	3HOD
Palm oil	Control	288.2[a]	69.2[a]	24.0[a]	24.7	70.7	1.5	3.1
	0.3	1684.0[b]	561.4[b]	33.3[b]	63.6	9.7	12.2	14.5
	0.5	3545.3[c]	2078.6[c]	58.6[c]	69.1	9.4	9.7	11.8
	0.7	4258.5[d]	2649.1[d]	62.2[c]	86.6	5.8	3.2	4.4
	1.0	4192.9[e]	2387.7[e]	56.9[c]	85.3	6.6	3.0	5.1
	2.0	3168.4[f]	1342.6[f]	42.4[d]	78.4	6.3	7.6	7.7

Control: 0.1% glucose

Values are mean ± SE, $n = 3$

SE values range from 0.9 to 12.5

Values in the column superscripted by different alphabets are significantly different from each other ($P < 0.05$, Duncan's new multiple range test)

Separate analysis was done for each column

Table 3 Maximum accumulation values of SCL–LCL-PHA co-polymer in *P. aeruginosa* MTCC 7925 grown in various concentrations of palm oil with palm oil cake

Carbon source	Biomass yield (dcw, mg l^{-1})	PHA yield		Polymer composition (mol%)			
Palm oil + Palm oil cake [% concentration (v/v)]		(mg l^{-1})	(% dcw)	3HB	3HV	3HHD	3HOD
Control	288.2[a]	69.2[a]	24.0[a]	24.7	70.7	1.5	3.1
0.3 + 0.3	2139.1[b]	947.6[b]	44.3[b]	66.3	11.6	9.9	12.2
0.5 + 0.5	4111.4[c]	2467.8[c]	60.0[c]	86.2	6.0	3.3	4.5
0.7 + 0.7	4157.0[d]	2474.3[c]	59.5[c]	84.9	6.9	2.8	5.4

Control: 0.1% glucose

Values are mean ± SE, $n = 3$

SE values range from 1.4 to 8.4

Values in the column superscripted by different alphabets are significantly different from each other ($P < 0.05$, Duncan's new multiple range test)

Separate analysis was done for each column

Table 4 PHAs accumulation in *P. aeruginosa* MTCC 7925 in 0.3% palm oil supplemented medium when subjected to N- and P-limitations

Nutrient limitation	PHAs (% dcw) Time (h)			
	24	48	72	96
Untreated control	10.5 ± 0.84[a]	24.0 ± 0.94[a]	22.2 ± 0.62[a]	10.9 ± 0.59[a]
$N^+ + P^+$- medium	16.6 ± 1.26[b]	33.3 ± 1.80[b]	29.1 ± 2.06[b]	12.0 ± 0.70[a]
P-limitation	23.7 ± 2.16[c]	39.4 ± 1.89[c]	35.7 ± 2.56[c]	16.1 ± 0.67[b]
N-limitation	28.5 ± 2.48[d]	44.8 ± 2.80[d]	39.8 ± 3.20[c]	19.0 ± 1.16[c]

Values are mean ± SE, $n = 3$

Values in the column superscripted by different alphabets are significantly different from each other ($P < 0.05$, Duncan's new multiple range test)

Separate analysis was done for each column

Table 5 Maximum yield and composition of the polymer in *P. aeruginosa* MTCC 7925 pre-grown in 0.5% palm oil + the extract of 0.5% (v/v) palm oil cakes supplemented-medium, when subjected to nitrogen and phosphorus limitations in the presence of 0.5% palm oil + the extract of palm oil cakes for 48 h

Condition	Biomass yield (dcw, mg l^{-1})	PHA yield		Polymer composition (mol%)			
		(mg l^{-1})	(% dcw)	3HB	3HV	3HHD	3HOD
Untreated control	288.2 ± 1.9[a]	69.2 ± 2.3[a]	24.0 ± 0.8[a]	24.7	70.7	1.5	3.1
$N^+ + P^+$- medium	8460.1 ± 6.2[b]	5270.6 ± 7.1[b]	62.3 ± 3.6[b]	86.5	5.7	3.4	4.4
P-limitation	7887.8 ± 6.7[c]	5472.4 ± 7.6[c]	69.4 ± 6.5[cb]	83.1	7.7	3.8	5.4
N-limitation	7566.3 ± 7.3[d]	5650.8 ± 8.5[d]	74.7 ± 8.7[c]	87.3	5.1	3.6	4.0

Values are mean ± SE, $n = 3$

Values in the column superscripted by different alphabets are significantly different from each other ($P < 0.05$, Duncan's new multiple range test)

Separate analysis was done for each column

where β-oxidation pathway is the main route that supply (*R*)-3-hydroxyacyl-CoA intermediates as metabolic precursors for PHAs biosynthesis [6]. *P. aeruginosa* MTCC 7925 might degrade long chain fatty acids of plant oils by β-oxidation pathway, which is used as sole source of carbon/energy and also for PHAs accumulation. Under physiological conditions permissive for synthesis and accumulation of PHAs, the intermediates of the β-oxidation pathway are converted to (*R*)-3-hydroxyacyl-CoA by enoyl-CoA hydratases [16]. *pha* J encoded (*R*)-specific enoly-CoA hydratase has been identified from *P. aeruginosa* and *Aeromonas caviae*, which derives 3-hydroxyacyl-CoA from *trans*-2-enoyl-CoA [29]. As each round of β-oxidation releases one molecule of acetyl-CoA, its metabolism through tricarboxylic acid cycle may have provided energy and metabolic intermediates needed for growth and PHAs accumulation. Acetyl-CoA can also be utilized as a metabolic precursor of 3HB. Amongst various concentrations of palm oil studied

(Table 2), PHAs yield was boosted maximum up to 62.2% (dcw) under 0.7% palm oil supplementation at 48 h of incubation. A PHAs pool of 60% (dcw) was also recorded under supplementation of 0.5% palm oil + 0.5% of the extract of palm oil cakes. Thus, supplementation of the extract of 0.5% palm oil cakes in combination with 0.5% palm oil resulted not only in comparable PHAs yield, the composition of the polymer and biomass yield were also at par with the yield of 0.7% palm oil supplemented-cultures. Moreover, reduction of palm oil concentration from 0.7 to 0.5% resulted into further reduction in the cost of PHAs production as palm oil cake is a cheap and plentily available waste material.

Under nitrogen limitation, a rise in the co-polymer pool up to 44.8% (dcw) was observed (Table 4). Co-polymer accumulation was found to increase up to 39.4% (dcw) in P-limited condition (Table 4). The cultures grown under normal growth conditions, the flux into the TCA cycle was almost constant throughout the cultivation and therefore, the NADPH produced during the whole cultivation period did not change [17]. However, under nitrogen limitation, NADPH consumption was decreased due to unavailability of nitrogen pool, which blocks the amino acid synthesis pathways, especially the reaction from α-ketoglutarate to glutamate, thus resulting into accumulation of excess NADPH in the cells. This residual NADPH might be responsible for the enhanced PHAs synthesis in nitrogen-limited cells. The enhanced PHAs accumulation under P-limitation agrees well with the earlier report of Chen et al. [4], where the co-polymer, 3-hydroxybutyrate-*co*-3-hydroxyhexanoate, i.e. P(3HB-*co*-3HH$_X$) yield was enhanced when growth was restricted due to unavailability of phosphorus. The possible explanation for this could be the accumulation of excess reducing power and decreased ATP synthesis with the onset of phosphate limitation [15]. Enhanced polymer accumulation at the interactive conditions of N and P limitations under palm oil and palm oil cake supplementations (Table 5) could be ascribed to the availability of plenty of precursors along with reducing powers required for PHAs biosynthesis.

PHB homopolymer with SCL monomer units as the constituent is a stiff and brittle polymer, while co-polymers with high mol% of SCL monomers have properties similar to polypropylene and polyethylene [20]. In this study, P(3HB-*co*-3HV-*co*-3HHD-*co*-3HOD) co-polymer with mol fraction ranging from 83.1:7.7:3.8:5.4 to 87.3:5.1:3.6:4.0 (Table 6) did not exhibit significant variation in physical properties. Table 6 also compares the material properties of P(3HB-*co*-3HV-*co*-3HHD-*co*-3HOD) co-polymer of *P. aeruginosa* MTCC 7925 with PHB, P(3HB-*co*-3HV), P(3HB-*co*-3HA), polypropylene (PP) and low-density polyethylene (LDPE), which advocates its potential application because of comparable properties with PP and LDPE.

The relatively low growth rate of available PHA-producing bacteria seems to be a major problem when plant oils or fatty acids are used as carbon sources. Even if a bacterium that possesses a high cell growth rate on plant oils or fatty acids, the PHA content is found to be relatively low [30]. However, in this study, the novel PHAs co-polymer [P(3HB-*co*-3HV-*co*-3HHD-*co*-3HOD)] was produced from palm oil using *P. aeruginosa* MTCC 7925 with a PHA content of 74.7% (dcw) in batch-scale experiments. Fukui and Doi [9] and Kahar et al. [11] reported PHA pool of 81 and 76%, respectively in *Ralstonia eutropha* PHB^{-}4/pJRDEE32d13 and *R. eutropha* H16, when grown in palm and soybean oils. Interestingly, in this study, when compared with % dry cell weight basis, the polymer yield was found to be boosted up by 3-fold, whereas on the basis of per liter culture a tremendous increase in yield, i.e. about 80-fold as compared to the control culture (Table 5); thus resulting into a yield of 5 g l^{-1} (approx.). On substrate basis the PHAs yield reached up to 0.62 g PHAs per g palm oil + palm oil cakes, which is significantly higher than the yield obtained from starch, molasses, lactose and xylose (Table 7), except for the recombinant and wild type strains

Table 6 Comparative account on the properties of the co-polymer of *P. aeruginosa* MTCC 7925 with PHB, P(3HB-*co*-3HV), P(3HB-*co*-3HA) and other common plastics

Property	(3HB-*co*-3HV-*co*-3HHD-*co*-3HOD) (mol fraction 83.1:7.7:3.8:5.4–87.3:5.1:3.6:4.0)[a]	PHB	P(3HB-*co*-3HV) (mol fraction 80:20)	P(3HB-*co*-3HA) (mol fraction 94:06)	PP	LDPE
T_m (°C)	115 to 120	180	145	133	176	130
T_g (°C)	−13 to −14	4	−1	−8	−10	−36
Tensile strength (Mpa)	18	40	20	17	38	10
Elongation to break (%)	701 to 723	5	50	680	400	620
Young's modulus (GPa)	0.2	3.5	0.8	0.2	1.7	0.2

T_m, melting temperature; T_g, glass-transition temperature; 3HA, 3-hydroxydecanoate (3 mol%), 3-hydroxydodecanoate (3 mol%), 3-hydroxyoctanoate (<1 mol%), 3-hydroxy-*cis*-dodecenoate (<1 mol%)

[a] Polymer from this study

Table 7 A comparative account on PHA yield from inexpensive substrates

Microorganism	Substrate	PHA (% dcw)	PHA composition	PHA yield (g PHA/g substrate)	Reference
Haloferax mediterranei	Starch	60.0	PHB	0.32	Lillo and Rodriguez-Valera [18]
Azotobacter vinelandii UWD	Molasses	66.0	PHB	0.29	Page and Cornish [21]
Pseudomonas cepacia	Lactose	56.0	PHB	0.15	Young et al. [32]
Pseudomonas cepacia	Xylose	60.0	PHB	0.11	Ramsay et al. [22]
Azotobacter chroococcum	Starch	73.9	PHB	0.17	Kim [14]
Ralstonia eutropha H16	Soybean oil	76.0	PHB	0.76	Kahar et al. [11]
Recombinant Ralstonia eutropha PHB^{-}4/pJRDEE32d13	Soybean oil	74.0	P(3HB-*co*-3HHx)	0.72	Kahar et al. [11]
Pseudomonas aeruginosa MTCC 7925	Palm oil + palm oil cakes	74.7	P(3HB-*co*-3HV-*co*-3HHD-*co*-3HOD)	0.62	This study

of *R. eutropha*, where the polymer yield reached above 0.7 g per g substrate under soybean oil supplementation.

Conclusion

This study concludes that palm oil in combination with its waste cakes can be used as excellent carbon source for production of the novel SCL–LCL-PHAs co-polymer [P(3HB-*co*-3HV-*co*-3HHD-*co*-3HOD)] with better thermal and mechanical properties by a sludge-isolated *Pseudomonas aeruginosa* MTCC 7925. Therefore, further study is needed at pilot-scale level to check the economic feasibility of using palm oil and its cakes for commercial production of this novel co-polymer.

Acknowledgment One of the authors (Mr. Akhilesh Kumar Singh) is thankful to Council of Scientific and Industrial Research (CSIR), Govt. of India, New Delhi, for providing fellowship.

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