Camelina Oil as a Promising Substrate for mcl-PHA Production in *Pseudomonas* sp. Cultures

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Abstract

Background and objective: Polyhydroxyalkanoates are biodegradable polyesters synthesized by some prokaryotic organisms from renewable sources. Medium-chain-length Polyhydroxyalkanoates show interesting properties as elastic and adhesive specialty polymers. Medium-chain-length Polyhydroxyalkanoates producers such as *Pseudomonas* sp. have demonstrated high yields on fats and oils. *Camelina sativa* is non-food chain competing crop, whose seed contain about 43% (w w^{-1}) oil in dry matter with about 90% (w w^{-1}) of unsaturated fatty acids. Camelina oil was for the first time tested for the production of medium-chain-length Polyhydroxyalkanoates by different Pseudomonas strains.

Material and methods: The production of Polyhydroxyalkanoate was evaluated in a nitrogen-limited minimal medium supplemented with crude Camelina oil or saponified oil to compare the production capability of *Pseudomonas* sp. strains. A phosphates-limited medium was used to optimize polyhydroxyalkanoate production in fed-batch assays. Experiments were carried out by duplicates.

Results and conclusion: *Pseudomonas resinovorans* was used for direct fermentation of Camelina oil without prior hydrolysis. A first approach to process development in bioreactor has provided up to 40% (w w^{-1}) polymer content, matching highest medium-chain-length polyhydroxyalkanoates titer reported from plant oils (13.2 g l^{-1}). Camelina oil was shown to be a suitable substrate for production of medium-chain-length polyhydroxyalkanoates. This non-food vegetable oil gave good results for *Pseudomonas resinovorans* DSM 21078 without any pre-treatment.

Conflict of interest: The authors declare no conflict of interest.

1. Introduction

Food surplus and byproducts frequently produced by agriculture and other industries can be exploited as low-cost substrates in biotechnological processes as a driver for bio-based and circular economy strategies. The production of bacterial polyesters by fermentation from such substrates, specifically the production of polyhydroxy-alkanoates (PHAs), could be one of the possible applications of this concept. The synthesis and use of biodegradable plastics is a promising alternative to replace the use of petroleum derivatives and research in the generation of bioplastics such as PHAs is an effective solution towards sustainable development [1,2].

PHAs are biodegradable and biocompatible polyesters that are accumulated as intracellular carbon and energy reserves in many prokaryotic organisms. Different types of polymers can be produced depending on the selected bacterial species and on the carbon source used during fermentation process: short-chain length PHA (*scl*-PHA), with monomers 4 to 5 carbon atoms are often stiff and brittle and medium-chain length PHA (*mcl*-PHA), with monomers from 6 to 16 carbon atoms are elastomeric in nature. PHAs are thermoplastic polymers with similar behavior to polyolefins, but with the advantage of being from renewable origin, biodegradable and biocompatible, therefore they can be used in a wide range of application from medicine to food packaging, however their price is still higher than their conventional counterparts [3-8].

PHA-storing microorganisms commonly produce PHA from simple carbon sources, such as sugars, but fatty acids have traditionally been the preferred substrates for studies investigating the production of *mcl*-PHA for their compa-rably higher transformation yields due to the metabolic pathway of fatty acids degradation ("β-oxidation"), that provides the structurally related precursors for PHA

production. Other organisms than Pseudomonades produce *scl*-PHA when supplied with oils [9,10]. However, the high price of those feedstocks accounts for up to 50% of the overall PHA production costs. In addition, most of the PHA-accumulating bacterial strains are not capable to convert triglycerides into fatty acids, then prior hydrolysis to yield free fatty acids or saponification is required, which increase the overall production costs [11,12]. Transesterification to (methyl) esters is a third possibility to convert triacyl-glycerides to low molecular mass units to be converted by microbes, but the strategies for reducing the costs of PHA production are focused on reducing raw material pretreatment stages as well as optimizing PHA recovery and purification; or even using some C1 carbon sources, e.g., methane, methanol, and CO_2 are cheap substrates and have received attention due to their serious role in greenhouse problem [10,13-15].

Plant oil as raw material for PHA production is interesting due to its relative low cost and the minimal processing required compared to fatty acids. As the demand for vegetable oils for food has increased dramatically in recent years, it is not realistic to justify the use of these oils in other purposes such as fuel or bioplastics production [16]. Non-edible vegetable oils can be considered as promising substitutions for traditional edible food crops and in this context *Camelina (C.) sativa* can be a sustainable plant for biofuel and biomaterial sustainable production [17,18].

C. sativa is a member of the *Brassicaceae* family and is adaptable to many different environmental conditions. The use as food is partly limited due to Camelina's smaller oil yields per hectare, as well as its strong taste and smell caused by components that are difficult to remove from the oil [19,20]. Camelina is an ideal crop for use on less productive lands due to the relatively low inputs required for its cultivation. Therefore, Camelina crops could be a good candidate to produce biofuels or biopolymers without displacing crops used for food production [21]. Poly (3-hydroxybutyrate) (PHB) production in plastids of transformed *C. sativa* seeds was investigated before, but products of this plant have never been tested for PHA production by microorganisms, particularly *mcl*-PHA with *Pseudomonas* strains [22].

Mcl-PHA is accumulated by some Pseudomonas species from different oily substrates but some of them are opportunistic pathogens unsuitable for PHA production at an industrial level [23,24]. Some of these studies compared the growth on oils to the growth on individual pure fatty acids, mainly oleic acid. Genetic engineering has also been used to express lipase genes in well-known PHA-producing strains, such as *Pseudomonas (P.) putida*, *P. oleovorans* and *P. corrugata* to allow them to grow directly on oils [25,26]. Recent studies show the ability of some strains to grow to high cell density when they are cultivated on plant oils and compare their performance using plant oils with crude fatty acid mixtures generated from the hydrolysis of oils [11].

The objective of this work is to validate the use of Camelina oil for the production of *mcl*-PHA by Pseudomonas strains, exploiting its potential as a renewable, non-food competing, lipid rich substrate.

2. Materials and methods

2.1 Bacterial strains, media and growth conditions

The bacterial strains employed in this study are *P. putida* KT2442 (a rifampycin sensitive mutant from KT2440 strain), *P. oleovorans* GPo1 and *P. resinovorans* DSM 21078. Cells were initially cultured in an undefined rich medium (Luria-Bertani, LB) and stored in 20% glycerol for long-term preservation. Solid media were supplemented with 1.5% (w v^{-1}) agar. Seed cultures were prepared in flasks containing LB medium by incubating in a rotary shaker overnight at 30°C and 180 rpm.

The production of PHA was evaluated in flaks cultures with 0.1 N M63, which is a nitrogen-limited minimal medium [27], supplemented with crude Camelina oil at 15 g l^{-1} or saponified oil to compare the production capability of the strains. Mineral Modified-R medium described by Lee and colleagues [28] with the initial KH_2PO_4 concentration set at 4 g l^{-1} was used to optimize PHA production in flasks cultures. The pH value was set to 7 in all cases and Camelina oil was added at different concentrations ranging from 2.5 to 10 g l^{-1} to the cultures after 20 hours. Bacteria were incubated in flasks with stirring, at 30°C and 180 rpm during 48 hours. Growth was measured by absorbance at 600 nm in a Biochrome libra S60 spectrophotometer.

2.2 Bioreactor assays

Fermentation was performed in the 1.5-L Applikon® reactor with a working volume of 0.5 liter at 30°C. pH was controlled at 7.0 by the addition of 12.5% (v v^{-1}) ammonia also provided as a nitrogen source. The oxygen saturation was controlled at 20% by controlling the agitation speed between 300 and 1000 rpm with up to 1 vvm of air flow rate. Foaming was controlled by adding Antifoam 204 (Sigma-Aldrich) when necessary. Fermentation were carried out with *P. resinovorans* DSM 21078 in Modified-R medium with the initial KH_2PO_4 concentration set at 4 g l^{-1} and $(NH_4)HPO_4$ at 1 g l^{-1} [28,29]. Initial oil concentration was 10 g l^{-1}, and several pulses were added up to total oil concentration of 60 g l^{-1} to prevent starvation of the bacteria and therefore polymer degradation; a first addition of 10 g l^{-1} of oil was added at 10 hours after the beginning of the assay; another pulse of 10 g l^{-1} was added at 20 hours, and a final pulse of 30 g l^{-1} was added at 32 hours. The addition of the pulses were performed at the moment the oxygen saturation started to increase above 20%.

2.3 Analytical procedures

PHA was determined by acidic methanolysis and gas chromatography of lyophilized culture samples. For this purpose, after 48 hours incubation, biomass was collected from 50 ml broth samples by centrifugation (3000 $\times g$, 20 min., Eppendorf 5810R Centrifuge) and lyophilized. Methanolysis reaction was performed weighing 5-7 mg of dry cells (assuming that at least 10% (w w^{-1}) of PHA accumulation is achieved), using 2 ml of H_2SO_4 in 15% (v v^{-1}) in methanol and 2 ml chloroform, and incubating for 4 hours at 100°C to transform the PHA into hydroxymethyl esters. Methyl benzoate was used as an internal standard. The resulting methyl esters were collected in the chloroform phase and analyzed by gas chromatography [30]. PHA produced by *P. putida* KT2442 from different fatty acids or glucose were used as bioplastic standards. The equipment used was an Agilent 6890N gas chromatograph with flame ionization detector and HP5-MS column.

Fatty acid profiles of Camelina oil were obtained by gas chromatography of the fatty acid methyl ester derivatives with the method mentioned above but individual peaks were identified by referring to a fatty acids methyl esters standard solution, analyzed under the same operation conditions.

2.4 Camelina oil treatments

In order to test the potential of the Camelina oil as a substrate for the production of PHA a fraction of oil was saponified. A sample of 10 g of Camelina oil was added to the ethanolic potassium hydroxide solution prepared by dissolving 3 g potassium hydroxide in 100 ml ethanol. The mixture was refluxed gently for 60 min. The ethanol was then removed by rotary evaporation under reduced pressure, leaving behind solid potassium salts of fatty acids [31]. On the other hand, two lipases were added to cultures of *P. putida* KT2442 to compare the accumulation of PHA versus the accumulation of *P. resinovorans* DSM 21078 from crude Camelina oil: Lipase A "Amano" 12 (Amano Enzyme U.S.A.) and Lipase CALB-L (Novozymes) at 1.0 g l^{-1} and 3 ml per liter respectively.

2.5 Polymer recovery and characterization

Cells were harvested from bioreactor cultures by centrifugation (3000 $\times g$, 20 min., Eppendorf 5810R Centrifuge) and then lyophilized. The lyophilized cells were mixed with chloroform (25 g l^{-1}) and kept at 30°C, during 24 h, with constant stirring. The mixture was allowed to decant, and the supernatant was filtered using a 0.2 μm PTFE filter and concentrated by rotary evaporation under vacuum. The polymer was precipitated using 10 vol. of cold methanol and the supernatant was decanted. PHA was resuspended in a minimum amount of acetone and allowed to dry before further analysis.

The average molecular mass (M_w), the molecular number (M_n), and the polydispersity index of the polymer were measured by Gel permeation chromatography using a pre-column PSS GRAM 10 μm, 30 Å; ID 8.0 mm × 50 mm and a column PSS GRAM 10 μm; 30, 100, 3000 Å; ID 8.0 mm × 50 mm with a PSS SECcurity 1260 Differencial Refractometer RID. DMF with 0.01 M LiBr was used as the eluent at flow rate of 1.0 ml min^{-1} at 70°C. Monodisperse polystyrene was used as standard material. Sample concentration of 0.5% (w v^{-1}) and injection volumes of 50 μl were used.

3. Results and discussion

3.1 Characterization of Camelina oil and its use for PHA production by *Pseudomonas* sp.

Fatty acid composition of Camelina oil was determined (Table 1). The determination of the composition of the oil was carried out by comparison with standards of main fatty acids according to literature [32]. The principal fatty acids found in Camelina oil were eicosenoic, linoleic, linolenic, and oleic. This crop presents variability in its composition attributable to factors such as climate and particular conditions at growing location [20].

Table 1. Fatty acid composition of Camelina oil used in this work (triplicate results).

Fatty acid	Carbon number	(% w w^{-1})
Stearic	(C18:0)	2.5 ± 0.3
Eicosenoic	(C20:1)	16.7 ± 0.5
Linoleic	(C18:2)	18.4 ± 0.7
Linolenic	(C18:3)	36.3 ± 2.1
Oleic	(C18:1)	15.1 ± 1.5
Palmitic	(C16:0)	5.6 ± 0.2
Erucic	(C22:1)	2.8 ± 0.1
Others	-	5.1

A nitrogen-limited culture medium (0.1 N M63) was chosen to compare the accumulation of PHA with the selected strains: *P. putida* KT2442, *P. oleovorans* GPo1 (nowadays called *P. putida* GPo1 [33]) and *P. resinovorans* DSM 21078. These strains can accumulate considerable amounts of *mcl*-PHA with excess carbon source and nitrogen source limitation, therefore the 0.1 N M63 culture medium is the most suitable for this purpose. On the other hand, few strains of the Pseudomonas genus have the ability to assimilate the triglycerides of the oil directly. Therefore, a sample of oil has been saponified to compare the accumulation of PHA with crude oil and with saponified oil (Figure 1). The strains *P. putida* KT2442 and *P. oleovorans* GPo1 showed accumulation of PHA only when saponified oil was added to the culture medium unlike the strain *P. resinovorans* DSM 21078 which produced PHA with both substrates. This strain has lipases that can hydrolyse the triglycerides of the oil and assimilate the free fatty acids to accumulate PHA [34,35]. These results showed that Camelina oil is a very suitable candidate as a substrate for the production of *mcl*-PHA with *P. resinovorans* without any pre-treatment.

In addition, phosphorous limitation conditions were also evaluated. This feeding strategy has been previously reported to deliver high PHA concentrations with *P. putida* KT2440 using oleic acid as a carbon source in high-cell density cultures [28]. These culture conditions were tested with strain *P. resinovorans* DSM 21078 using R-modified medium and adding 15 g l^{-1} of crude oil. *Pseudomonas putida* KT2442 is a model bacteria used commonly for *mcl*-PHAs production [36], therefore in parallel the same

test was carried out with this strain but adding two different lipases, Lipase A and CALB-L, directly to the culture as previously explained (see Materials and Methods section). Results are shown in Figure 2. The *P. resinovorans* strain grew better than the *P. putida* strain and produce a considerable amount of PHA from crude Camelina oil (28.8% of CDM and 3.8 g PHA per liter), therefore, this culture medium was selected to initiate trials of fed-batch cultures with *P. resinovorans* DSM 21078.
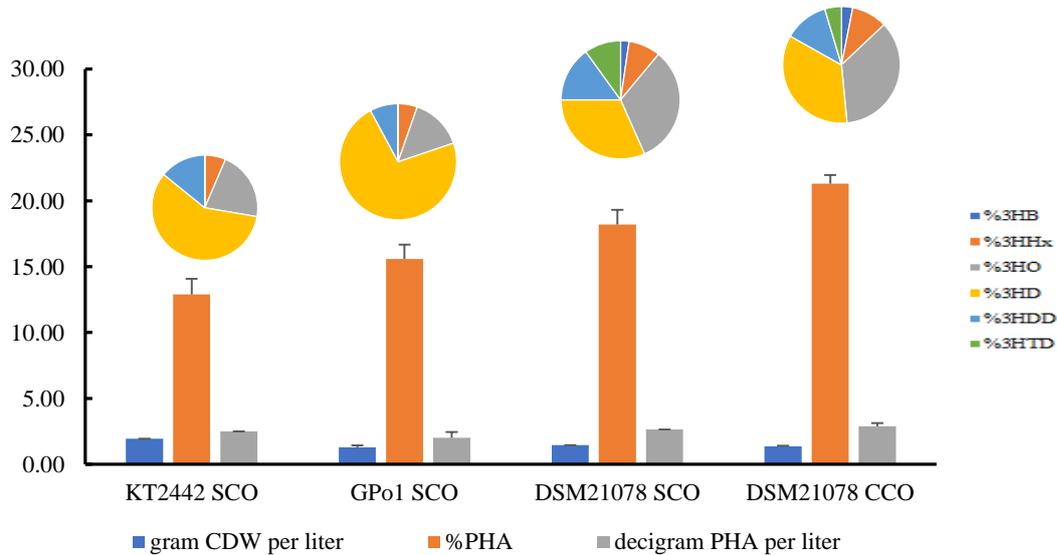

Figure 1. Production of *mcl*-PHA (medium-chain-length polyhydroxyalkanoates) by *P. putida* KT2442, *P. oleovorans* GPo1 and *P. resinovorans* DSM 21078 using 0.1 N M63 culture medium with 15 g l^{-1} of crude Camelina oil (CCO) or 15 g l^{-1} of saponified oil (SCO). 3HB: 3-hydroxybutiric acid, 3HHx: 3-hydrodxyhexanoic acid, 3HO: 3-hydroxyoctanoic acid, 3HD: 3-hydroxydecanoic acid, 3HDD: 3-hydroxydodecanoic acid, 3HTD: 3-hydroxytetradecanoic acid. *P.* = Pseudomonas
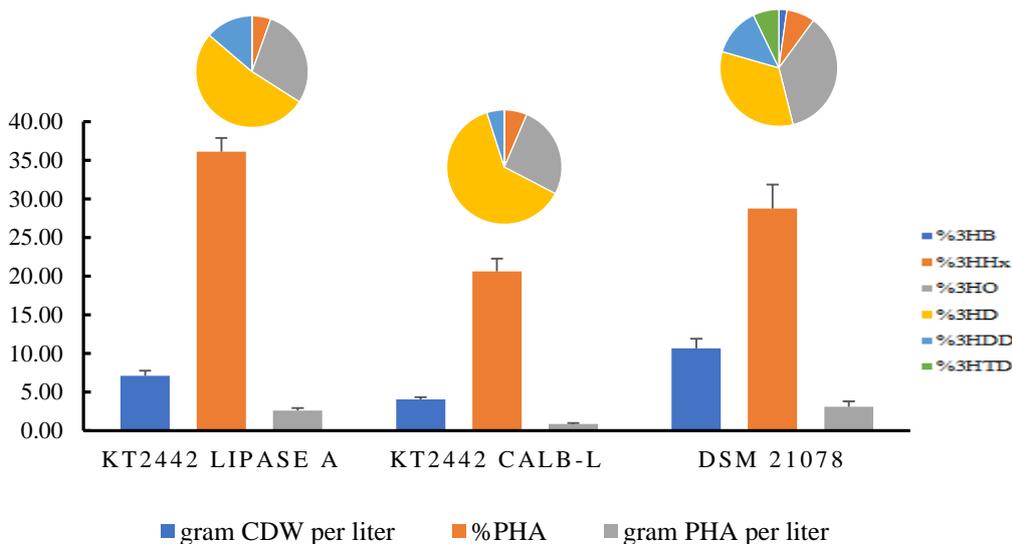

Figure 2. Production of *mcl*-PHA (medium-chain-length polyhydroxyalkanoates) ith R-Modified culture medium and 15 g l^{-1} of Camelina oil. 3HB: 3-hydroxybutyric acid, 3HHx: 3-hydrodxyhexanoic acid, 3HO: 3-hydroxyoctanoic acid, 3HD: 3-hydroxydecanoic acid, 3HDD: 3-hydroxydodecanoic acid, 3HTD: 3-hydroxytetradecanoic acid

3.2 Production of PHA in fed-batch cultures with *P. resinovorans* DSM 21078.

Fed-batch cultures have been performed to find the optimal growing conditions and regulate the production of *mcl*-PHA. Cultures of *P. resinovorans* DSM 21078 were prepared in R-modified medium with different initial quantities of crude Camelina oil: 2.5, 5 and 10 g l^{-1} and with a substrate addition at 20 hours of 10 g l^{-1} of Camelina oil (Figure 3, A-C) when the cells have consumed part of the initial substrate. These flask cultures were incubated at 30°C and 180 rpm for 48 hours and samples were taken at 20 and 48 hours of fermentation. Figure 3 shows the results obtained. While for an initial substrate concentration of 2.5 g l^{-1} and at 20 hours of culture the growth of the strain is low with an accumulation of PHA around 4%, a significant increase in the growth of the strain with an accumulation of 17.5% was observed when the substrate concentration was 5 g l^{-1} at the beginning of the fermentation. The best result in the accumulation of *mcl*-PHA was obtained with 10 g l^{-1} of substrate at the beginning of the fermentation (44% of CDM and 4.2 g PHA per liter), although it is similar to that obtained in the previous case with 5 g l^{-1} of substrate at the end of the assay (41% of DCM and 3.9 g PHA per liter). Therefore, R-modified medium is a suitable medium for the fed-batch production of *mcl*-PHA with an initial concentration of 10 g l^{-1} to avoid substrate depletion on batch stage taking into account that growing conditions are better in bioreactor cultivations. Figure 3D shows the monomeric composition of the biopolymer obtained in each case where the proportion of the monomers was similar in all cases with variations in C8, C10 and C14 monomers.
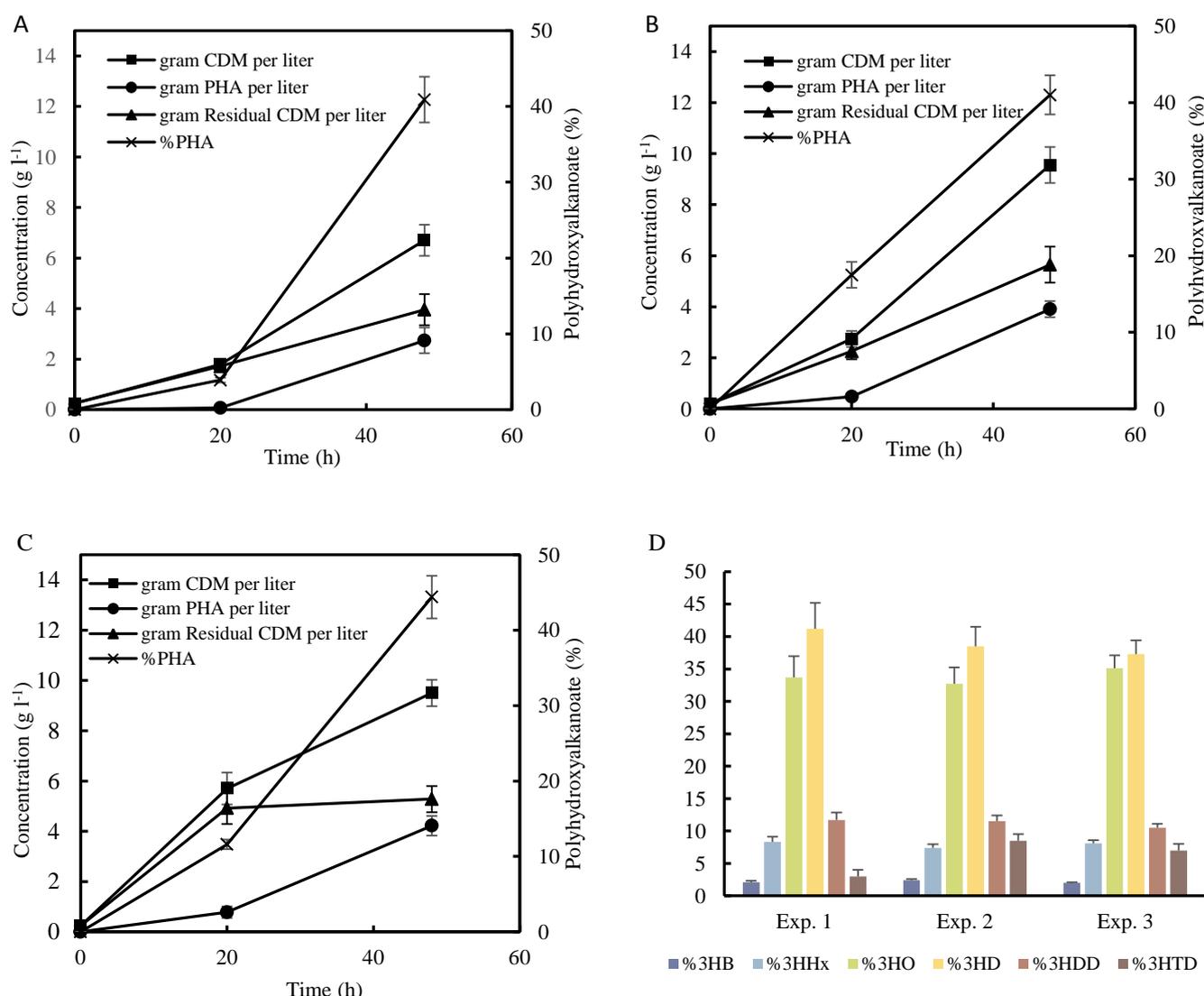

Figure 3. Shake flasks production of *mcl*-PHA by *Pseudomonas resinovorans* DSM 21078 using different initial concentration of Camelina oil. A pulse of 10 g l^{-1} of substrate at 20 hours was made. A. 2.5 g l^{-1}. B. 5 g l^{-1}. C. 10 g l^{-1}. D. Composition of polymers obtained at the end of each assay. 3HB: 3-hydroxybutyric acid, 3HHx: 3-hydroxyhexanoic acid, 3HO: 3-hydroxyoctanoic acid, 3HD: 3-hydroxydecanoic acid, 3HDD: 3-hydroxydodecanoic acid, 3HTD: 3-hydroxytetradecanoic acid

Bioreactor assays were used to evaluate the behavior of the selected strain during PHA production and to optimize the fermentation to achieve high cell density cultures. As mentioned before, PHA is an intracellular product, so it is very important to obtain high cell density cultures as a step prior to the industrial scale-up [37]. Fermentation was performed using the R-modified medium with less quantity of nitrogen source (at 1 g l^{-1} of $(NH_4)_2HPO_4$) to ensure that growth is regulated by the demand for ammonia during fermentation and to induce PHA accumulation by phosphate limitation. The ammonia solution for pH correction was not replaced by NaOH solution after the growth phase. The experiment was carried out in fed-batch because in batch mode the whole crude oil tended to emulsify the culture medium. The protocol was elaborated observing its behavior during the test and adding the oil pulses when the saturation of oxygen started to increase

above 20%, as explained in materials and methods section. The results of the experiment are shown in Figure 4.

Fermentation was finished after 45 hours; and under these conditions the strain achieved an accumulation of mcl-PHA of 35.6% of its cell dry mass, 13.5 g l^{-1} of PHA was obtained. The production of PHA obtained with this experiment is about three to four times higher than the value obtained in flask and matches the highest titer of mcl-PHA (13.2 g l^{-1}) obtained in bioreactor cultivations with *P. cloloraphis* using plant derived oils as a substrate (Table 2). *Mcl*-PHA content (around 40%) is within the range of PHA accumulation found with pure fatty acid substrates, a relevant requirement for efficient downstream recovery. It is also worth noting that *mcl*-PHA is produced directly from the substrate without previous hydrolysis or further conditioning, highlighting the potential of the combination of Camelina oil and *P. resinovorans* for efficient *mcl*-PHA production.
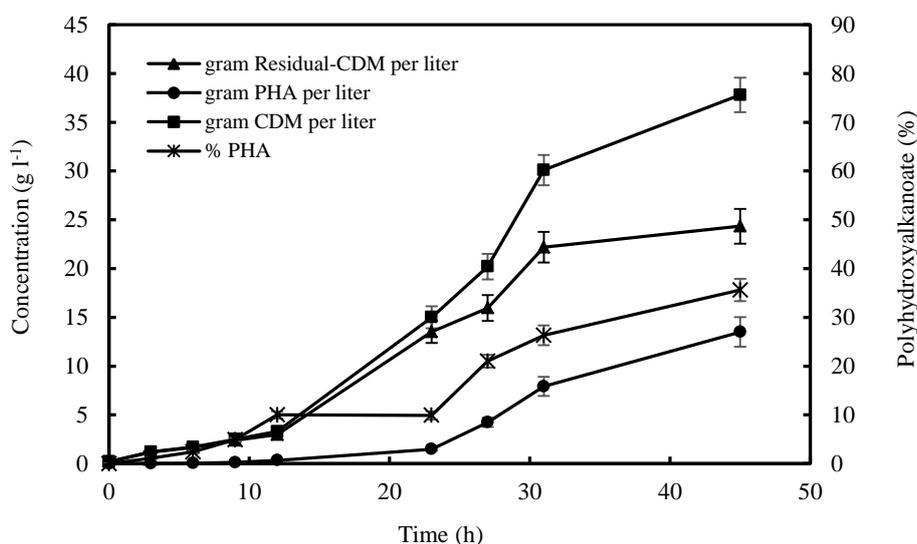

Figure 4. Growth and *mcl*-PHA production by *Pseudomonas resinovoras* DSM 21078 for the fed-batch fermentation with additions of Camelina oil

Table 2. Production of medium-chain-length polyhydroxyalkanoates from vegetable oils in shaking flask and bioreactor by *Pseudomonas* sp. strains: overview of literature data.

Microorganism	Plant oil	Device	CDM (g l^{-1})	%PHA	Reference
P. aeroginosa	Palm oil	Bioreactor	2.7	39	23
	Brassica carinata oil	Flask	1.0	5	24
P.chlororaphis	Different plant oils	Flask	0.7-0.9	27-34	11
	Rapeseed oil	Bioreactor	51	26	11
	Palm kernel oil	Bioreactor	3.3	45	38
P. putida	Different plant oils	Flask	0.3-0.5	11-25	11
P. resinovorans	Different plant oils	Flask	2.9-3.8	40-51	34
	Camelina sativa oil	Flask	10.6	28.7	This study
	Camelina sativa oil	Bioreactor	37.9	35.6	This study
P. saccharophila	Soybean, sunflower oils	Flask	-	-	39
Pseudomonas sp.	Corn oil	Flask	0.96	37.3	40

P= Pseudomonas

3.3 Recovery and characterization of PHA produced by *P. resinovorans* DSM 21078.

After fermentation the PHA produced was recovered from freeze-dried cell powder in chloroform. The recovery rate was 92%, and then the polymer was precipitated three times in cold methanol to increase its purity (see materials and methods section) and recovered as a film. The monomer composition of the PHA produced by *P. resinovorans* DSM 21078 was analyzed by GC-FID (Table 3) and compared to reference composition reported in previous studies. The monomer composition was: 2% 3-hydroxybutyrate (3HB), 7.5% 3-hydroxyhexanoate (3HHx), 38% 3-hydrxyoctanoate (3HO), 37% 3-hydroxydecanoate (3HD), 13% 3-hydroxy-dodecanoate (3HDD) and 2.5% 3-hydro-xytetradecanoate (3HTD), similar to any other mcl-PHA composition produced by other Pseudomonas strains using vegetable oils [11]. Previous studies suggest that polymer composition can be manipulated on the basis of the substrate used which is interesting to modify the characteristics and properties of the polymer if necessary [35]. Finally, the biopolymer has a M_n of 52.4 kDa and M_w of 131 kDa with a PDI of 2.5, analyzed by GPC. Molecular weights of PHA depend on the substrate and the strain PHA synthase Reported Mn and PDI values for PHA produced with *P. resinovorans* from vegetable oils rank from 65 to 100.1 KDa, and 1.65-1.81, respectively [4,41]. It is not possible to compare with other publications regarding Camelina oil, as this is the first example of the use of this vegetable oil.

Camelina oil contains high fractions of unsaturated fatty acids according to the composition of the oil presented above and Pseudomonades form unsaturated *mcl*-PHA

building blocks to a certain extend by the use of these substrates due to β-oxidation pathway. Therefore, follow-up studies will encompass the more in-depth investigation of the monomeric composition of the polymer.

4. Conclusion

In this work, we tested *Pseudomonas* sp. as bacterial strains to utilize fatty substrates and produce *mcl*-PHA. *C. sativa* has several favorable agronomic characteristics which give it potential to the obtention of bio-based products and was shown to be a suitable substrate for production of *mcl*-PHA. This non-food vegetable oil, which had previously not been tested, gave good results for *P. resinovorans* DSM 21078 without any pre-treatment. The strain produced 3 g PHA per liter in discontinuous culture and 13.5 g PHA per liter in bioreactor fed-batch culture with additions of Camelina oil pulses during fermentation. Thus, this study shows the first steps in the development of a sustainable process for the production of bioplastics using oil from *C. sativa* which is a low-input crop with high per acre yield and does not interfere with food industry. Further studies and optimization of the process could provide the productivity gains required to improve cost effectiveness.

5. Acknowledgements

6. Conflict of interest

The authors declare that there exist no conflicts of any interest.

Table 3. Molar composition of the medium-chain-length polyhydroxyalkanoates produced by *Pseudomonas resinovorans* DSM 21078 using Camelina oil (duplicate results) compared with polymers produced by *Pseudomonas resinovorans* NRRL B-2649 with different vegetable oils [35].

Strain	Monomers% (w w^{-1})						M_n (kDa)	M_w (kDa)	PDI
	3HB	3HHx	3HO	3HD	3HDD	3HTD			
Pseudomonas resinovoras DSM 21078	2±0.5	7.5±1.0	38±2	37±3	13±1	2.5±0.7	0.5± 0.2	1.3±0.5	2.5
Pseudomonas resinovoras NRRL B-2649	<1-1	8-9	29-37	30-35	5-14	2-3	0.7-1.0	1.1-1.8	1.6-1.8

3HB: 3-hydroxybutyric acid, 3HHx: 3-hydrodxyhexanoic acid, 3HO: 3-hydroxyoctanoic acid, 3HD: 3-hydroxydecanoic acid, 3HDD: 3-hydroxydodecanoic acid, 3HTD: 3-hydroxytetradecanoic acid

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روغن کاملینا، زی‌مایه‌ای مناسب برای تولید پلی‌هیدروکسی‌آلکانوآت‌های زنجیره متوسط با کشت گونه‌های سودوموناس

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چکیده

سابقه و هدف: پلی‌هیدروکسی‌آلکانوآت‌ها پلی‌استرهایی زیست‌تجزیه‌پذیری هستند که توسط برخی ارگانیسم‌های پروکاریوت از منابع تجدیدپذیر تولید می‌شوند. پلی‌هیدروکسی‌آلکانوآت‌های زنجیره متوسط خواص جالبی ماننـد بسپارهای چسبناک و الاستیک دارنـد. نشـان داده شـده اسـت کـه میکروارگانیسم‌هـای تولیدکننـده پلی‌هیدروکسی‌آلکانوآت‌های زنجیره متوسط مانند گونه‌هـای سـودوموناس حـاوی مقـادیر زیـادی روغن و چربی می‌باشند. کاملینا ساتیوا محصولی رقابتی در زنجیره غذایی است که دانه آن حاوی حدود ۴۳ درصد مـاده خشـک روغن است که ۹۰٪ آن را اسیدهای چرب غیراشباع تشکیل می‌دهد. روغن کاملینا برای اولین بار به منظـور تولیـد پلی‌هیدروکسی‌آلکانوآت‌های زنجیره متوسط از سوش‌های گوناگون سودوموناس مورد بررسی قرار گرفت.

مواد و روش‌ها: تولید پلی‌هیدروکسی‌آلکانوآت‌ها در محیط کشت حاوی حداقل میزان نیتروژن که روغن کاملینـا یا روغن صابونی شده به آن افزوده شده بود، انجام و قابلیت تولید سوش‌های سـودوموناس بـا هـم مقایسـه شـدند. محیط محدود از فسفات‌ها برای بهینه‌سازی تولید پلی‌هیدروکسی‌آلکانوآت به روش خـوراک‌دهـی ناپیوسـته مـورد استفاده قرار گرفت. آزمون‌ها در دو تکرار انجام شدند.

یافته‌ها و نتیجه‌گیری: *سودوموناس رزینوورانس* برای تخمیر مستقیم روغن کاملینا بـدون آبکافـت¹ اولیـه مـورد استفاده قرار گرفت. اولین روش برای انجام فرایند در بیوراکتور با میـزان بسـپار تـا ۴۰ درصـد وزنـی وزنـی بـود و بیشترین میزان تطابق تیتر پلی‌هیدروکسی‌آلکانوآت‌های زنجیره متوسط با روغن گیاهی (۱۳/۲ گـرم در لیتـر) بـه دست آمد. مشاهده شد که روغن کاملینا زی‌مایه‌ای مناسب برای تولید پلی‌هیدروکسی‌آلکانوآت‌های زنجیره متوسط می‌باشد. استفاده از این روغن گیاهی غیرخوراکی بدون هیچ‌گونه پیش‌تیمـاری نتـایج خـوبی بـرای سـودوموناس رزینوورانس DSM 21078 را به همراه داشت.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

Hydrolysis ¹