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ORIGINAL ARTICLE

Biosynthesis of poly-β-hydroxybutyrate (PHB) with a high molecular mass by a mutant strain of *Azotobacter vinelandii* (OPN)

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Abstract The aim of this study was to characterize the influence of the aeration conditions on the production of PHB and its molecular mass in a mutant strain of *Azotobacter vinelandii* (OPN), which carries a mutation on *ptsN*, the gene encoding enzyme IIA^{Ntr}, previously shown to increase the accumulation of PHB. Cultures of *A. vinelandii* wild-type strain OP and its mutant derivative strain OPN were grown in 500-mL flasks, containing 100 and 200 mL of PY sucrose medium. PHB production and its molecular mass were analyzed at the end of the culture. The molecular mass (MM) was significantly influenced by the aeration conditions and strain used. A polymer with a higher molecular weight was produced under low aeration conditions for both strains. A maximal molecular mass of 2,026 kDa (equivalent to 3,670 kDa measured by GPC) was obtained with strain OPN cultured under low-aeration conditions, reaching a value two-fold higher than that obtained from the parental strain OP (MM=1,013 kDa) grown under the same conditions. Aeration conditions and the *ptsN* mutation influence the molecular mass of the PHB produced by *A. vinelandii* affecting in turn its physico-chemical properties.

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Introduction

Poly-β-hydroxybutyrate (PHB) is an intracellular polyester of the polyhydroxyalkanoates (PHAs), a family of storage polymers produced by numerous bacteria, including *Azotobacter vinelandii*. This polymer is an aliphatic polyester composed of 3-hydroxybutyrate monomers in which the carboxyl group of one monomer forms an ester bond with the hydroxyl group of the neighboring monomer (Madison and Huisman 1999). PHB has some mechanical properties similar to conventional plastics like polypropylene or polyethylene, although it exhibits a high rate of crystallization and high degree of crystallinity, leading to brittleness and low elongation to break (Khanna and Srivastava 2005; Dominguez-Diaz and Romo-Uribe 2012).

PHB and other PHAs have been drawing attention because they are biodegradable and biocompatible thermoplastics that can be melt or solution processed to create a wide variety of consumer products, including films, nonwoven membranes and fibers (Aldor and Keasling 2003; Dominguez-Diaz et al. 2011). Recently, based on their properties of biocompatibility and biodegradability, new attractive applications for PHB have been proposed in the medicine field, where chemical composition and product purity are critical (Williams and Martin 2005).

The molecular mass (MM) of PHB determines the elastic behavior of the material and its mechanical resistance as spun fibers (Iwata 2005). PHB fibers with a molecular mass about of 300 kDa have a tensile strength of 190 MPa and an elongation at break of 5 %. In contrast, when the molecular mass is of 5,300 kDa, the fibers increase their tensile

strength 7 times (1,320 MPa) and the elongation at break is of 57 % (Iwata 2005). Therefore, for PHB commercial production it is desirable to obtain polymers with a suitable molecular mass for the final application, especially in the medical field.

The molecular mass of PHB produced by species of *Azotobacter* could be altered by the medium composition and the aeration conditions (Chen and Page 1994; Quagliano and Miyazaki 1997; Myshkina et al. 2008). Previous studies in *Azotobacter chroococcum* 6B have shown that by manipulating the aeration conditions either in shake flasks or in fermenter, it is possible to obtain PHB with a high molecular mass. Myshkina et al. (2008) found that PHB having a maximal MM of 2,215 kDa was obtained in cultures developed at low aeration (125 rpm) at pH7.0.

Mutations that increase PHB accumulation in *A. vinelandii* have been reported (Segura et al. 2003; Segura and Espín 2004; Noguez et al. 2008; Hernandez-Eligio et al. 2012). However, strains carrying these mutations have not been evaluated regarding the characteristics of the polymer produced. One of the mutations that increase PHB accumulation is the inactivation of *ptsN*, the gene coding for the IIA^{Ntr} protein of the nitrogen-related phosphoenolpyruvate-dependent phosphotransferase system (PTS-Ntr), a signal transduction system composed of the EI^{Ntr}, Npr and IIA^{Ntr} proteins that participate in a phosphotransfer chain where IIA^{Ntr} appears to be the terminal phosphoryl acceptor. This protein has been shown to negatively regulate expression of the PHB biosynthetic genes *phbBAC,* and of *phbR* the gene that codes for their transcriptional activator. Thus, a *ptsN* gene inactivation increases PHB accumulation (Noguez et al. 2008).

In the present work we report a study about the influence of aeration conditions on the molecular mass of the PHB produced by a mutant strain of *Azotobacter vinelandii* (OPN), which carries a mutation inactivating the *ptsN* gene which increases the accumulation of PHB (Noguez et al. 2008).

Materials and methods

Microbial strains

Azotobacter vinelandii strains used in this investigation were OP (Segura et al. 2009) and its *ptsN*::Km^r derivative named OPN. The strains were cryopreserved at −70 °C in a 40 % glycerol solution and maintained by monthly subculture on Burk's agar slopes (Peña et al. 1997) stored at 4 °C. To construct strain OPN, competent cells of strain OP were transformed with total DNA from the *A. vinelandii* mutant RN4, which contains a kanamycin resistance cassette inserted in a nonpolar orientation within the *Cla*I site of the *ptsN* gene (Noguez et al. 2008). A transformant resistant to kanamycin was isolated and named OPN. To confirm the correct replacement of the wild-type *ptsN* gene, a PCR analysis was carried out with primers flanking the insertion site (data not shown). This analysis confirmed the replacement of the chromosomal wild-type *ptsN* gene with the insertionally inactivated allele in strain OPN by a double crossover event.

Culture medium and growth conditions

Both strains were cultured in PY sucrose medium with the following composition (in g L^{-1}): sucrose 20; yeast extract (Difco) 3; peptone (Difco) 5. The pH was adjusted to 7.2 with a concentrated 2 N NaOH solution. The cultures were grown in a rotary shaker (New Brunswick Scientific Co., Model G 25) at 200 rpm and 29 °C, up to an absorbance (measured at 540 nm) of 0.15 (dilution 1:50). Ten milliliters of this inoculum were transferred to 500-mL flasks containing 90 mL of medium, and were cultivated under the same conditions for 60 h. Under these conditions, the cells were growing under oxygen limitation (Peña et al. 2007). In order to evaluate the influence of the aeration conditions, 100 and 200 mL of filling volume in shake flasks of 500 mL were employed. Cultures were performed in a number of parallel flasks, two of which were regularly withdrawn and submitted to analyses (10 mL). These flasks were not placed back on the shaker. All experiments were conducted in triplicate and the results presented are the average of the independent runs.

Analytical determinations

Biomass and sucrose concentration Cell dry weight was determined gravimetrically. 10 mL of culture broth were mixed with 1 mL of Na_4EDTA (0.1 M) and 1 mL of NaCl (1.0 M) and then centrifuged at 12,000*g* for 20 min. The pellet was isolated and resuspended in water and filtered through previously weighted Millipore filters (0.45-μm pore size) and then dried at 80 °C to a constant weight. Sucrose was assayed for reducing power with DNS reagent. Samples were previously hydrolyzed by using β-fructofuranosidase (Gist Brocades) to generate glucose and fructose and then assayed for reducing power with DNS reagent (Miller 1959).

Protein concentration Protein concentration was determined by the Lowry method (Lowry et al. 1951) using bovine serum albumin as a standard. The specific growth rate (μ) was calculated based on protein using the logistic model reported previously (Klimek and Ollis 1980).

Poly-β-hydroxybutyrate (PHB) concentration PHB was extracted from the cell debris obtained from plates and

liquid cultures as described previously (Peña et al. 1997). The quantification of PHB (as crotonic acid, which is a product of PHB hydrolysis) was assayed using a HPLC system with a UV detector and an Aminex HPX-87H ion-exclusion organic acid column. Elution was performed with H_2SO_4 (0.014 N) at a flow rate of 0.7 mL min^{-1} and 50 °C, as described by Karr et al. (1983).

PHB isolation and purification

To begin, 40 mL of broth culture was centrifuged at 11,000 rpm during 15 min. The pellet was separated and washed with distilled water. After this, the pellet was suspended in acetone for 20 min. This mixture was centrifuged at 11,000 rpm for 15 min. Then, the pellet was mixed with 2 mL of sodium hypochlorite and 5 mL of chloroform and incubated at 25 °C for 15 min. The suspension was centrifuged, obtaining three phases. The lower phase (where PHB is located) was isolated with the help of a Pasteur pipette. Finally, the PHB solution was spread in a glass plate for drying at room temperature for 24 h (Hahn et al. 1994).

Characterization of PHB

Molecular mass calculated from intrinsic viscosity values

The PHB molecular mass was determined by dilute solution viscosity measurements utilizing an Ubbelohde capillary viscosimeter (Cannon). Viscosity was measured at 30 °C utilizing chloroform as the solvent. For the measurement, 17 mL of the solution were poured into the viscometer, which was then placed into a thermostat strictly vertical and incubated there for 15–20 min. The flow time of PHB solutions was determined as the mean of 10 measurements. For determining intrinsic viscosity, measurements were made for four different PHB concentrations. Various PHB concentrations were obtained by diluting the starting solution of the highest concentration by adding chloroform.

Sample concentrations varied within 60 to 240 mg PHB per 100 mL chloroform. Flow times varied in the experiments within 3–15 s. The specific viscosity was calculated as:

$$\eta_{esp} = \frac{t - t_0}{t_0}$$

Where t_0 is the flow time of the solvent, s, and t is the flow time of the polymer solution, s.

Molecular mass, M_m, was calculated from intrinsic viscosity values, $[\eta_{int}]$, using the Mark–Houwink–Coon equation with the following coefficients (Marchessault et al. 1970).

$$[\eta_{int}] = 7.7 \times 10^{-5} M_m^{0.82}$$

To determine the intrinsic viscosity $[\eta_{int}]$ the values of reduced viscosity (η_{esp}/c) were plotted against the polymer solution concentration, c. The intrinsic viscosity value $[\eta_{int}]$ was obtained by extrapolating the plot to zero concentration. The accuracy of the determination was about 4 %. The accuracy of M_m calculation from the Mark–Houwink–Coon equation was within 5 %.

Mean molecular mass by GPC

The mean molecular mass of PHB was estimated by gel permeation chromatography (GPC) with a Styragel HMW column (HR5E, Waters), using a HPLC system (Waters, Alliance 2695) with a diferential refractometer detector (Waters, 2414). The eluant was chloroform at 30 °C at a flow rate of 0.7 mL min. The detector signal was processed with PC-compatible software (Empower). The column was calibrated by a standard calibration method using polystyrene as standards of molecular mass of 1.2×10^3–2.78×10^6. PHB samples were filtered through a 0.22-μm Millipore membrane to remove cellular debris. A volume of 50 μl was injected into the HPLC via a sample loop (Hahn et al. 1994).

Mechanical properties

The mechanical properties were studied under uniaxial tension. Tests were carried out on as-cast films, at room temperature, using the TST-350 tensile tester (Linkam Ltd., England) with a crosshead speed of 5 mm min. The PHB films were cast from chloroform solution, dried overnight in a hood and then further dried under vacuum at 90 °C for 4 h. The mechanical Young's modulus was determined from the elastic region of the stress–strain curves (Ward 1971).

Results and discussion

Construction of *A. vinelandii* strain OPN

The IIA^{Ntr} protein, encoded by *ptsN*, has been shown to negatively regulate expression of the PHB biosynthetic genes *phbBAC* and its activator *phbR*. Thus, a *ptsN* gene inactivation increases PHB accumulation (Noguez et al. 2008). In order to have a PHB overproducing strain derivative of *A. vinelandii* strain OP and to study the effect of the *ptsN* gene mutation on the characteristics of the polymer produced, we constructed, as described in Materials and Methods, a mutant strain (OPN) carrying the *ptsN* gene inactivated by the insertion of a Kanamycin resistance cassette in a non-polar orientation. As expected, mutant OPN showed a PHB overproducing phenotype when cultured on PY sucrose agar plates, reaching a specific production of 0.52 g $_{PHB}$ g $_{protein}^{-1}$; whereas the strain OP produced 0.30 g $_{PHB}$ g $_{protein}^{-1}$;

It is important to point out that the conditions on solid medium can be considerable different from those occurring in a submerged culture (Galindo et al. 2007) and for that reason it is not expected that a clear correlation between the PHB production levels observed on agar plates and in submerged conditions will be found. Because the fermentation system that will be used for the PHB production at a commercial level is the submerged culture, cultures in liquid medium should be used to characterize the actual potential of the mutant strains of *A. vinelandii.*

Characterization of PHB and biomass production in cultures of the OP and OPN strains

Figure 1a shows the growth kinetics by the OPN mutant and the wild type OP cultured in PY sucrose medium. A higher maximum biomass concentration (measured as protein content) and specific growth rate (μ) was achieved with the cultures of *A. vinelandii* strain OP (Fig. 1a). The μ for this strain was of 0.085 h^{-1}, with a maximum protein concentration of 2.3 g L^{-1}, compared to 0.073 h^{-1} and 1.5 g L^{-1} respectively, for cultures of the OPN mutant strain.

Although the OPN mutant strain reached a lower biomass, it accumulated a higher amount of PHB, reaching a concentration of 4.1 g L^{-1} at 60 h of cultivation (Fig. 1c); whereas in the case of the OP cultures the PHB content was of 3.5 g L^{-1} at the same cultivation time. As shown Table 1, the specific production of PHB (g PHB g prtotein^{-1}) was higher in the OPN cultures. The mutant reached a maximum of 2.69 g PHB g protein^{-1}, which was almost two times higher than that observed for the OP wild type strain (1.52). The sucrose uptake rate for both strains tested was very similar, 0.25 and 0.23 g $L^{-1}h^{-1}$ for OP and OPN mutant, respectively (Table 1). At the end of cultivation, the OP wild type strain consumed 75 % of initial sucrose, whereas the OPN mutant strain consumed only 65 % of sucrose (Fig. 1c). The yield of product based on the consumed substrate (Y p / s) was 0.30 g $_{PHB}$ g $_{Suc}^{-1}$ for OPN mutant, and 0.22 mutant strain g $_{PHB}$ g $_{Suc}^{-1}$ for the OP strain (Table 1).

The high specific production and yield of PHB observed for the culture of *A. vinelandii* mutant OPN is clearly related to the genotype of this strain, because the inactivation of *ptsN* gene eliminates IIA^{Ntr}, which is a negative regulator of *phbBAC* and *phbR* expression. Thus, this mutation has been shown to increase the production of PHB (Noguez et al. 2008). However, growth of this mutant is negatively affected. This result is in general agreement with previous reports showing that when mutants of *A. vinelandii* overproducing alginate and/or PHB are cultured in shake flasks or in fermenter, the growth rate is negatively affected with respect to that obtained with the parental strain (Segura et al. 2003; Galindo et al. 2007). These studies have reported that mutations which have a stimulatory effect on alginate yield (per cell basis), such as the one blocking PHB synthesis (Segura et al. 2003) also have a negative effect on the growth capacity of *A. vinelandii*. The negative effect of the *ptsN* mutation on growth could be a consequence of a higher PHB production. If the synthesis of this polymer competes with other essential metabolic pathways for the carbon source or the reducing power available, the consequence of an increased expression of the PHB biosynthetic genes would be a lower growth; however, a non-PHB related explanation for this phenotype cannot be discarded, because the II^{Ntr} protein has been shown to have several other regulatory roles in *A. vinelandii* and other bacteria (Segura and Espín 1998; Pflüger-Grau and Görke 2010). Therefore, it is likely that most mutations increasing alginate or PHB production will have a negative effect upon cell growth.

Influence of the aeration conditions on growth and PHB production (OP and OPN)

In order to investigate the influence of aeration conditions on the production of PHB, cultures at 100 and 200 mL of filling volume were undertaken. It is known that increasing the filling volume, decreases the oxygen transfer rate in shake flasks and therefore, the availability of oxygen in the liquid (Peña et al. 2007). Figure 2 shows the growth kinetics, sucrose consumption and PHB production by the OPN mutant and the wild type OP strains cultured under low and high aeration in PY sucrose medium. As it was expected, increasing the filling volume (from 100 mL to 200 mL) negatively influenced the growth rate of both strains, reaching a maximal protein concentration of 1.2 g L^{-1}, with a specific growth rate of 0.066 h^{-1} during the culture of OP and OPN strains respectively (Fig. 2a and Table 2). As a consequence of a low oxygen transfer rate in the shake flasks with 200 mL of filling volume, the sucrose uptake rate decreased dramatically, obtaining values of 32 and 56 % of those reached at high aeration conditions (Fig. 2b).

It is important to note that the aeration conditions also influenced the volumetric production of PHB from the cultures of both strains evaluated. In both cases, a decrease in the PHB production was observed, reaching 1.5 and 2.3 g L^{-1} of PHB at 60 h of cultivation for the OP and OPN strains, respectively (Fig. 2c). With respect to the specific production of PHB (SPHB), this was practically the same (1.5 g PHB g protein^{-1}) in the cultures with the wild type OP, under low-aeration conditions, with respect to that obtained at high aeration. In contrast, using the OPN mutant the SPHB decreased from 2.69 g to 1.94 g PHB g protein^{-1} in the cultures conducted under low aeration conditions (Table 2).

Our results are in agreement with those reported by Ryu et al. (2008). These authors evaluated the effect exerted by the aeration conditions (using 50 and 100 mL of filling volume in shake flasks of 250 mL) on the production of

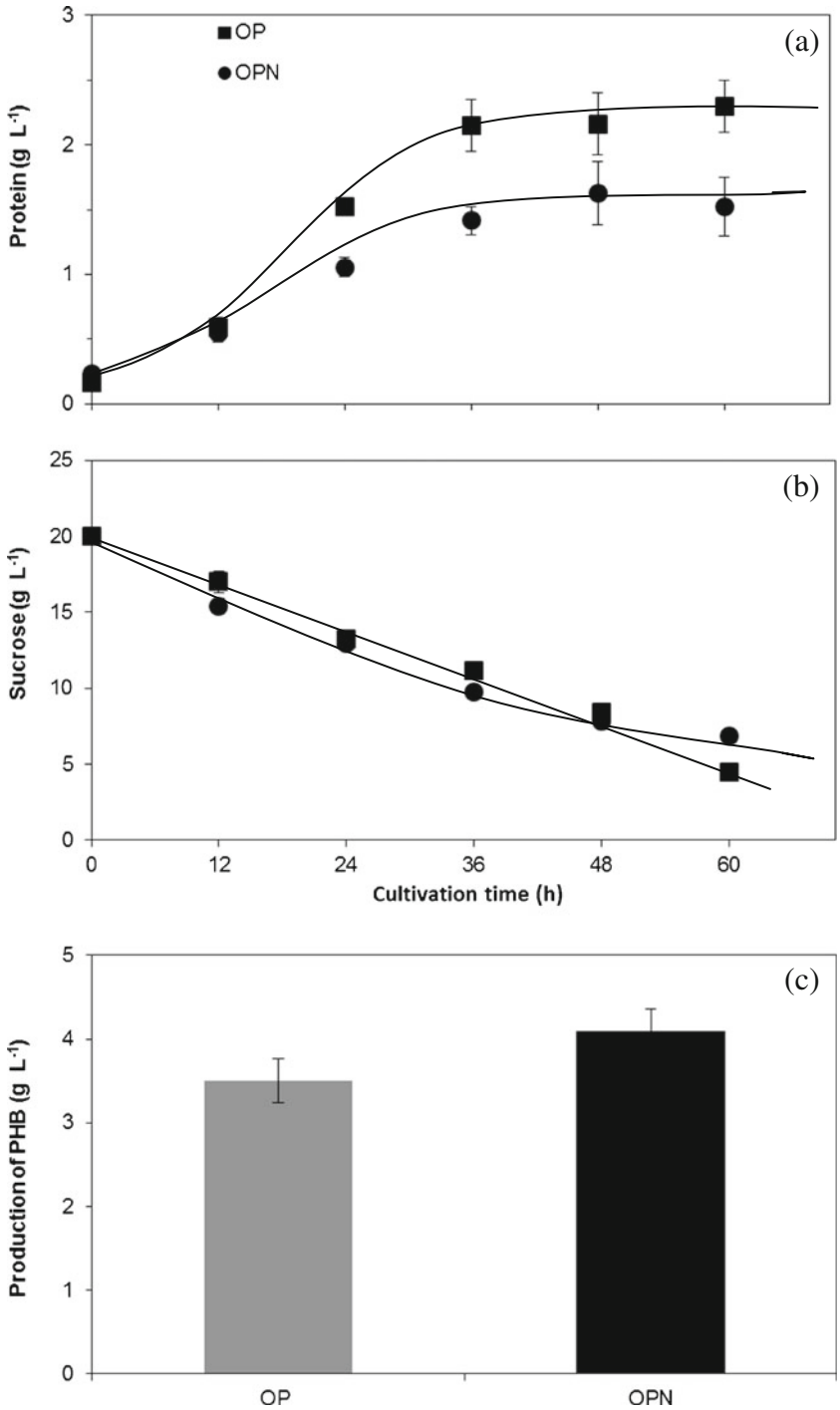

Fig. 1 Kinetics of growth as protein (**a**), sucrose consumption (**b**), and PHB production at 60 h of culture (**c**), of strains OP and OPN cultured in shake flasks in PY sucrose medium

Table 1 PHB specific production (g $_{PHB}$ g $_{Prot}^{-1}$), sucrose uptake rate and PHB yield of *A. vinelandii* OP and OPN strains in PY sucrose medium

Strain	PHB specific production (g $_{PHB}$ g $_{Prot}^{-1}$)	Sucrose uptake rate (g L $^{-1}$h^{-1})	Y $_{PHB\ /\ Sucrose}$ (g $_{PHB}$ g $_{sucrose}^{-1}$)
OP	1.52	0.25	0.22
OPN	2.69	0.23	0.30

PHAs in *Azotobacter vinelandii* UWD. They found that, under high aeration (50 mL), a higher cell growth (12.84 g L^{-1}) and a higher volumetric production of PHB (7.39 g L^{-1}) were obtained. In contrast, the cultures at low aeration (100 mL) showed a cellular growth of 9.40 g L^{-1} and a volumetric production of PHB 5.48 g L^{-1}.

It has been reported for some *A. vinelandii* strains, that oxygen limitation leads to increased PHB production, due to an accumulation of reducing power that causes inhibition of the tricarboxilic acids cycle and the activation of the

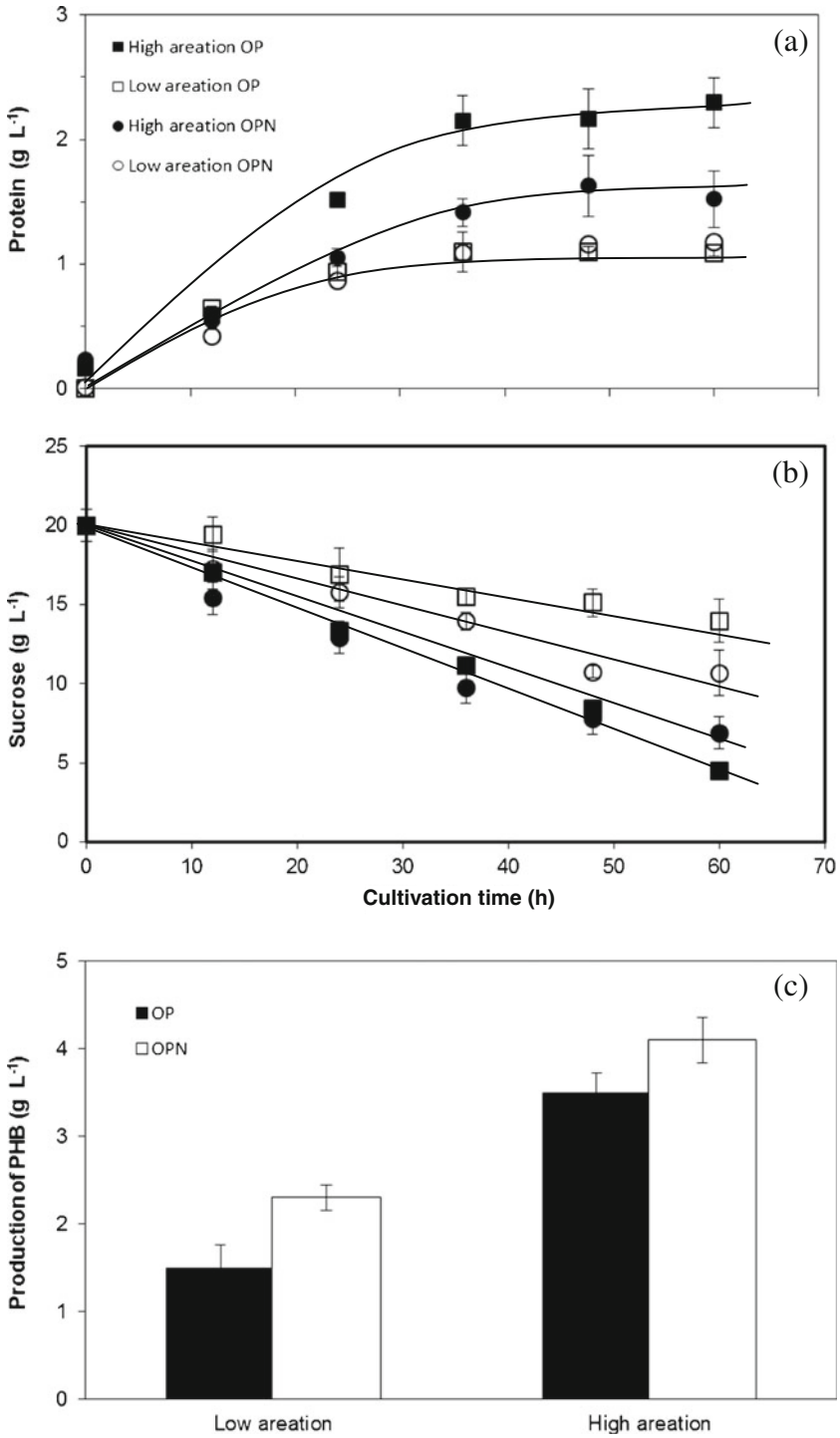

Fig. 2 Influence of the aeration conditions on growth (as cell protein) (**a**), sucrose consumption (**b**), and PHB production (**c**), of cultures of OP and OPN strains conducted in shake flasks in PY sucrose medium

Table 2 Specific growth rate (μ), PHB specific production ($g_{PHB}\ g_{Prot}^{-1}$) and PHB yield of *A. vinelandii* OP and OPN strains cultured at low- and high-aeration

Strain	Condition	Specific growth rate (h^{-1})	PHB specific production ($g_{PHB}\ g_{Prot}^{-1}$)	$Y_{PHB\ /\ Sucrose}$ ($g_{PHB}\ g_{sucrose}^{-1}$)
OP	Low-areation	0.066±0.003	1.38	0.25
	High-areation	0.085±0.004	1.52	0.22
OPN	Low-areation	0.066±0.003	1.94	0.24
	High-areation	0.076±0.002	2.69	0.30

β-ketothiolase enzyme, and therefore, the synthesis of PHB (Galindo et al. 2007). As it is shown in the Table 2, this behavior was not observed in our experiments with both strains, because under low aeration conditions and therefore oxygen limitation, the specific production of PHB was similar for strain OP or even higher in the case of the of the OPN mutant. Although no conclusive explanation can be given for this result, it is possible that this behavior could be due to differences between the strains used in previous reports and the strains tested in the present study. The regulatory mechanisms present could have different range of oxygen sensitivity, or the respiratory activity could differ in different strains of *A. vinelandii*. On the other hand, the behavior observed in the OPN mutant could be related with the absence of IIA^{Ntr} that leads to a deregulated PHB synthesis; however, further studies would be necessary to elucidate the reason for these differences.

Influence of the aeration conditions on the molecular mass of PHB

The molecular mass of PHB determines its mechanical properties, and in turn, the applications of this polymer. For that reason, this parameter was analyzed for the OP and OPN mutant. Figure 3 shows that the molecular mass is strongly influenced by both the aeration condition and the strain tested. A maximal molecular mass of 2,026 kDa was observed for the PHB isolated from the cultures of OPN mutant under low aeration conditions at 60 h of cultivation. A similar behavior was observed in the polymer produced by the OP strain, obtaining a PHB with a M*m* of 1,657 kDa at the same time (Fig. 3a). In contrast, in the cultures at high aeration the molecular mass of PHB decreased to 1,013 kDa and 551 kDa, for the OPN and parental strain (OP) respectively (Fig. 3b).

In addition, the molecular mass was analyzed by GPC, as it was described in Materials and methods. In general, the values obtained using this method were coincident with the data calculated from intrinsic viscosity, determining a maximal weight-averaged molecular mass for the polymer isolated from the cultures with the OPN strain under low aeration condition (3,670±270 kDa) and of 3,500± 300 kDa for the parental strain (Table 3). As it was expected, in the cultures with the OPN strain at high aeration, the molecular mass of PHB decreased to 2,600±170 kDa

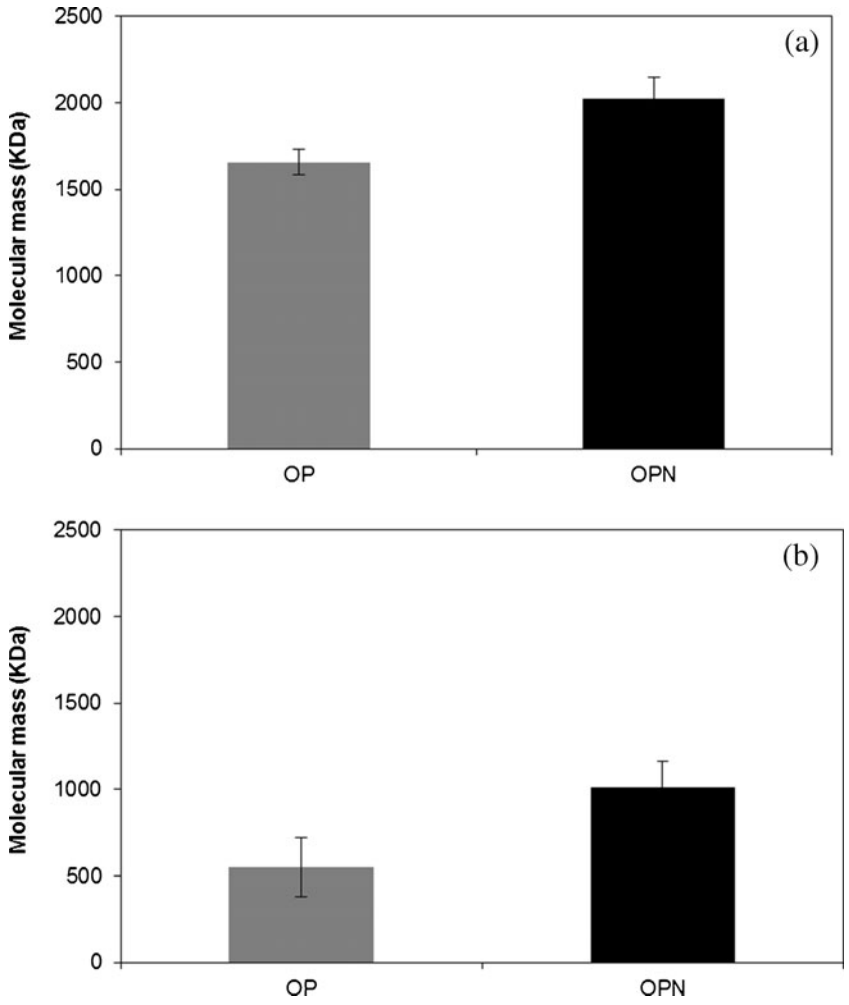

Fig. 3 Molecular mass of the PHB obtained from the cultures of *A. vinelandii* strains OP and OPN under low (**a**) and high (**b**) aeration conditions

Table 3 Molecular mass of the PHB, determined by gel permeation chromatography (GPC) and mechanical Young's modulus obtained from the cultures of *A. vinelandii* strains OP and OPN under low and high aeration conditions

Strain	Condition	Maximal molecular mass (kDa)	Young's modulus (MPa)
OP	Low aeration	3500±300	320
	High aeration	ND	12
OPN	Low aeration	3670±270	560
	High aeration	2600±170	180

(Table 3). Although the trends in the molecular weights were similar, the values obtained by GPC technique were higher than those estimated by intrinsic viscosity. These differences may be due to the polydispersity of the molecular mass of the samples, where it is known that weight-averaged molecular mass >viscosity-averaged molecular mass >number-averaged molecular mass (Rubinstein and Colby 2003).

The uniaxial tensile tests on as-cast films showed that the Young's modulus increased as the molecular weight increased, ranging from 12 MPa to 560 MPa (Table 3). The tensile tests also showed that the strain at fracture increased as the molecular mass increased, in agreement with other reports (Iwata 2005). It is noted that the tensile modulus values reported here are smaller than those reported for as-spun fibers. These differences may be due to the high molecular alignment attained in a fiber spinning process, as opposed to a solution casting process, where there is no polymer chain alignment (Rubinstein and Colby 2003).

Our results contrast with the previously reported by other authors (Chen and Page 1994), who did not find differences in the M_m of PHB changing the aeration conditions in terms of the dissolved oxygen (DO) in fermenters in the range of 5–20 %. These differences could be due to the much lower ranges of DO and oxygen transfer rate (OTR) obtained in the shake flasks (vessels used in the present study) in comparison to that in the fermenter (Peña et al. 2011) and therefore, the changes in the M_m of PHB would occur under microaerobic conditions. The studies reported by Myshkina et al. (2008) for the production of PHB with *A. chroococcum* 6B support that hypothesis. They found that by decreasing the agitation rate (and therefore the oxygen transfer rate) in shake flasks, it was possible to obtain PHB with a higher molecular mass (up to 2,215 kDa), with respect to the polymer produced at higher aeration. On the other hand, it has been reported by Zhang et al. (2008) that changes in the MM of PHB produced by *Methylosinus trichosporium* could be related to the expression of key enzymes related to the PHB metabolism, such as PHB synthase, which is responsible for the polymerization of PHB, or PHB depolymerase, which degrades the polymer. In our case, the production of PHB with a higher molecular mass in the OPN mutant could be due to differences in expression of some of these enzymes, as the *ptsN* mutation present in this strain has been found to affect expression of the biosynthetic genes, and it may also affect the expression of other genes involved in the metabolism of PHB such as the depolymerizing enzymes.

Conclusions

In summary, our results show that the aeration conditions, as well as the *ptsN* mutation affect the molecular mass of PHB produced by *A. vinelandii* allowing the synthesis of a polymer with a maximal M_m of 2,026 kDa (3,670 kDa determined by GPC) with the culture of the OPN mutant under low aeration conditions. Furthermore, the mechanical properties were influenced by the molecular mass. PHB with a high molecular mass, such as that obtained from the OPN strain, had a higher tensile modulus and elongation at break, consistent with other reports (Iwata 2005). From a technological point of view, it should be highlighted that the manipulation of the molecular weight of PHB by means of changes in the aeration conditions of the culture, is a convenient method that could considerably improve the properties of PHB, expanding the potential application of this polymer, especially in the medical field.

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