J Ind Microbiol Biotechnol (2014) 41:1375–1382
DOI 10.1007/s10295-014-1477-5

SIMB
Society for Industrial Microbiology and Biotechnology

Effects of metabolic pathway precursors and polydimethylsiloxane (PDMS) on poly-(gamma)-glutamic acid production by *Bacillus subtilis* BL53

Alessandra de Cesaro · Suse Botelho da Silva · Marco Antônio Záchia Ayub

Received: 5 May 2014 / Accepted: 16 June 2014 / Published online: 11 July 2014

Abstract The aims of this study were to evaluate the effects of the addition of metabolic precursors and polydimethylsiloxane (PDMS) as an oxygen carrier to cultures of *Bacillus subtilis* BL53 during the production of γ-PGA. Kinetics analyses of cultivations of different media showed that *B. subtilis* BL53 is an exogenous glutamic acid-dependent strain. When the metabolic pathway precursors of γ-PGA synthesis, L-glutamine and a-ketoglutaric acid, were added to the culture medium, production of the biopolymer was increased by 20 % considering the medium without these precursors. The addition of 10 % of the oxygen carrier PDMS to cultures caused a two-fold increase in the volumetric oxygen mass transfer coefficient (k_La), improving γ-PGA production and productivity. Finally, bioreactor cultures of *B. subtilis* BL53 adopting the combination of optimized medium *E*, added of glutamine, α-ketoglutaric acid, and PDMS, showed a productivity of 1 g L^{-1} h^{-1} of g-PGA after only 24 h of cultivation. Results of this study suggest that the use of metabolic pathway precursors glutamine and a-ketolgutaric acid, combined with the addition of PDMS as an oxygen carrier in bioreactors, can improve γ-PGA production and productivity by *Bacillus* strains.

A. de Cesaro · M. A. Z. Ayub (✉)
Food Science and Technology Institute, Federal University of Rio Grande do Sul State, Av. Bento Gonçalves 9500, PO Box 15090, Porto Alegre, RS 91501-970, Brazil
e-mail: mazayub@ufrgs.br

S. B. da Silva
Food Engineering, Polytechnic School, University of Vale do Rio dos Sinos, Av. Unisinos, 950, São Leopoldo, RS 93022-000, Brazil
e-mail: susebs@unisinos.br

Keywords Poly-gamma-glutamic acid · Polydimethylsiloxane (PDMS) · Oxygen carrier · *Bacillus subtilis* · L-glutamine · α-ketoglutaric acid

Introduction

Poly-γ-glutamic acid (γ-PGA) is an anionic, naturally occurring homo-polyamide, consisting of D and L-glutamic acid monomers ligated by amide linkages between α-amino and γ-carboxyl groups. First identified by Ivanóvisc and co-workers as a capsule of *Bacillus anthracis*, γ-PGA is also produced as an extracellular biopolymer by bacteria belonging to *Bacillus* sp. and other gram-positive genera [25]. *B. subtillis* and *B. licheniformis* are the main strains used for the γ-PGA production, and they have been isolated mainly from soil and traditional foods in Asian countries [1, 25, 31]. γ-PGA is soluble in water, biodegradable, edible, non-toxic, and environmentally safe [1]. These characteristics make it a product of high interest, finding applications in several industry sectors, such as in the food manufactory, used as thickener, bitterness relieving agent, and cryoprotectant [25]. It is also used as a heavy metal absorber and flocculant agent in wastewater treatment [26], and as a drug delayed-release carrier in medicine [19].

The microbial biosynthesis of γ-PGA is carried out a two steps reaction, with the formation of glutamic acid, which is then polymerized, yielding the biopolymer molecule. The synthesis occurs through the metabolic pathway of the TCA cycle [15], and culture media components might have an influence on the regulation of this enzymatic machinery. Medium "E" (ME), developed by Leonard and co-workers [18] is frequently used to grow *Bacillus* for γ-PGA production in submerged cultivations. Glycerol, citric acid, and L-glutamic acid are the main components of ME,

and these compounds are necessary for biomass and γ-PGA production. Citric and L-glutamic acids act as precursors of polymer [4, 25], whereas glycerol acts as a co-substrate [9]. Glucose has been shown to be an excellent carbon source for both cell growth and γ-PGA production by *Bacillus*. However, glycerol presents the advantage of stimulating polyglutamyl synthetase [29], the enzyme required to catalyze the polymerization of L-glutamic acid to γ-PGA. Intermediate metabolites of TCA cycle, such as L-glutamine and α-ketoglutaric acid, can also enhance enzymatic activity necessary for the biopolymer production [2].

During the γ-PGA synthesis, oxygen plays an important role because it is the last electron acceptor in the respiratory chain, allowing for the storage of energy in the form of ATP, which is essential for cell growth and metabolites production [32]. In microbial submerged cultivations, mass transport resistances must be overcome because during growth the produced γ-PGA greatly increases media viscosity, because of its high molecular mass (10^5–10^6 Da) [22]. Very often in industrial operations, increasing the stirring rate or the inlet airflow in bioreactors is not practically feasible in order to enhance oxygen supply of microbial cultures. One possible approach would be increasing the solubility of oxygen in the medium by the addition of oxygen carriers such as hydrocarbons, hemoglobin, perfluorocarbons, and silicones [21, 32].

Recently, polydimethylsiloxane (PDMS) has been tested in submerged bacteria cultures and proved to greatly enhance oxygen supply, consequently improving cell metabolism and growth [21]. PDMS is a polymer of silicon and oxygen atoms, alternately linked by siloxane bonds in the main chain, with two methyl groups attached to each silicon atom [11]. PDMS is chemically and enzymatically inert. The solubility of oxygen in PDMS is 45–50 times higher than that in water [7]. To our knowledge, there are no literature reports on the use of PDMS in γ-PGA production systems.

In this context, the aims of this study were to evaluate the effects of the metabolic precursors (glutamic acid, L-glutamine, and α-ketoglutaric acid) in the γ-PGA synthesis by *Bacillus subtilis* BL53 through detailed analysis of the cultivation kinetics. Furthermore, cultures were scaled up to bioreactors and the use of PDMS as oxygen carrier was also evaluated, analyzing the γ-PGA production and the impact on the oxygen mass transfer coefficients (k_La) of cultures.

Materials and methods

Strain

The strain used in this research was isolated from the dumped soils of the Amazonian environment and subsequently identified as *Bacillus subtilis* (strain BL53) [27]. Bacterium stocks are kept at the microbiology culture collection of our laboratory (UFRGS, Brazil). The 16S rDNA sequence was deposited in the NCBI GenBank database under the accession number JQ359757 [27]. Working stocks of cultures were maintained at −18 °C in 20 % glycerol cell suspensions, whereas for immediate use, cells were kept at 4 °C on Luria–Bertani agar plate.

Culture media

Besides the traditional Luria–Bertani broth (LB, composed of (in g L^{-1}): peptone, 10; yeast extract, 5; NaCl, 10), the following media were tested: LBGlu was Luria–Bertani broth supplemented with glutamic acid (20 g L^{-1}); medium E refers to the medium developed by Leonard et al. [18] supplemented with Zn^{2+} [27], and composed of (in g L^{-1}) glutamic acid, 20; citric acid, 12; glycerol, 80; NH_4Cl, 7; $MgSO_4.7H_2O$, 0.5; $FeCl_3.6H_2O$, 0.04; K_2HPO_4, 0.5; $CaCl_2.2H_2O$, 0.15; $MnSO_4.H_2O$, 0.04; $ZnSO_4.7H_20$, 0.35; medium E(-glu) was medium E without glutamic acid; finally, medium E(+prec) was medium E supplemented with the metabolic pathway precursors of γ-PGA L-glutamine (0.075 g L^{-1}), and α-ketoglutaric acid (1.46 g L^{-1}), according to Bajaj and Singhal [2]. The pH of the media were always adjusted to 7.0 using either NaOH or HCl 1 M.

Pre-inocula test

Three media were used (LB, E, and LBGlu) for the evaluation of the influence of the pre-inoculum medium on subsequent γ-PGA production (which was carried out in medium E). For the preparation of inoculum, a loopful of cells was inoculated into Erlenmeyer flasks containing the specific medium, and incubated at 37 °C and 180 rpm on a rotary shaker. The inocula for all cultivation essays were standardized to 1.0 ± 0.1 OD (optical density) at 600 nm and were added to culture medium at 10 % (volume fraction). The cultivation was carried out at 37 °C and 180 rpm, on a rotatory shaker for 96 h. Samples were taken along the time course of cultivations to quantify γ-PGA, viable cells, citric acid, glutamic acid, and glycerol. All the experiments were carried out in duplicates. The inocula for all cultivations were standardized as described above.

Shaker flask cultures

LBGlu and E(-glu) were used for the evaluation of the physiological influence of precursor L-glutamic acid, whereas medium E(+prec) was used for the effects of the addition of L-glutamine and α-ketoglutaric acid on γ-PGA production. Flask cultures were carried out in

125 mL Erlenmeyer flasks filled with 30 mL of the specific medium. The flasks were incubated for 96 h on a rotary shaker at 37 °C and 180 rpm.

Samples were taken along the time course of cultivation to quantify γ-PGA, viable cells, citric acid, glutamic acid, and glycerol. All the experiments were carried out in duplicates.

Effects of the addition of PDMS and metabolic pathway precursors in bioreactor cultivations

Batch cultivations were carried out in 5 L working volume stirred tank bioreactors (Biostat B model, B. Braun Biotech International, Germany) filled with 2.5 L of culture medium. The bioreactor was equipped with temperature, agitation, aeration, and pH controllers and two Rushton turbines with six flat-blades, 3 cm distant from each other. Dissolved oxygen in the culture broth was measured using a polarographic electrode (Mettler-Toledo, Germany). Four different culture media were evaluated: E, E(+prec), E supplemented with 10 % (volume fraction) of polydimethylsiloxane (PDMS; 200 Fluid, Food Grade, 350 cSt., Dow Corning, USA), and E(+prec) supplemented with 10 % (volume fraction) of PDMS. The amount of PDMS added to cultures was chosen based on results from previous works where the optimized addition of this oxygen carrier was studied Rech et al. [21]. A small amount of 0.1 % (volume fraction) of Antifoam 204 (organic non-silicone Emulsion, Sigma, USA) was added for foaming control in cultures carried out using medium E and E(+prec) without PDMS.

Cultivations were carried out at 37 °C, pH 7.0, 1,000 rpm and 2 vvm (volumes of air per volume of broth) of aeration, for 96 h. Samples were taken along the cultivations in order to quantify γ-PGA, viable cells, citric acid, glutamic acid, and glycerol. Cultivations were carried out in duplicates.

Analytical procedures

γ-PGA was quantified by the complexation spectrophotometric method using cetyltrimethylammonium bromide, which was adapted by Silva et al. [27] from Kanno and Takamatsu [14]. γ-PGA (MM: 70–100 kDa, Sigma-Aldrich) was used as spectrophotometric standard and the absorbance of the complex was measured at 400 nm. For extraction of polymer from culture medium, trichloroacetic acid solution (6.25 % as mass fraction) was added to medium until the pH was reduced to 3.0. The mixture was then centrifuged at 15,000*g*, 15 °C and 30 min. The supernatant was collected, the pH adjusted to 7.0 ± 0.1 and five volumes of ethanol were added. Centrifugation was repeated at 15,000*g*, 4 °C and 30 min; the obtained pellet was resuspended in buffer solution pH 7.0 ± 0.1 and the complexation reaction was carried out [27].

The viable cells were quantified by the Spread Plate technique. After successive decimal dilutions of the culture broth, defined dilutions were platted in Plate Count Agar (PCA) and incubated at 37 °C for 18 h.

Citric acid, glycerol, and glutamic acid concentrations were determined using a HPLC (Shimadzu Corp., Japan) with a RID-10A refractive index detector. The stationary and mobile phases were an Aminex HPX-87H column (300 × 7.8 mm) (Bio-Rad, USA) and 50 mmol L^{-1} H_2SO_4 solution, at 0.6 mL min^{-1} respectively. The column temperature was controlled at 45 °C. Glutamic acid was quantified as its α-hydroxy acid derivative, being possible to use the same column, according to Pleissner et al. [20]. For the derivatization reaction, 1 mL of the filtrated sample was transferred to a 2.5 mL Eppendorf with 0.2 mL of 1 M KNO_3 and 0.04 mL of 12 M HCl. This mixture was maintained at 45 °C for 90 min. The reaction was stopped with the addition of 0.43 mL of NaOH 2 M [20].

Determination of oxygen volumetric mass transfer coefficient (k_La)

The oxygen volumetric mass transfer coefficient (k_La) was determined following the dynamic method described by Doran [8]. This coefficient was measured using the dissolved oxygen data of cultures (given in % of saturation under the conditions of the experiments), and always conducted at mid-exponential growth phase in order to compare cells at the same physiological status.

Results and discussion

Effects of glutamic acid in the pre-inocula media on the γ-PGA production

Figure 1 shows the kinetics of the cultivations in the three different pre-inocula media (LB, E, and LBGlu) used to evaluate the production of γ-PGA. It is possible to identify the consumption of glycerol, citric acid, and glutamic acid, indicating that the cells were using the TCA metabolic pathway to form γ-PGA. Although the results obtained when the pre-inoculum was in medium E, Fig. 1b shows a faster growth and higher biomass production, the γ-PGA production was approximately 40 % lower than that when LB was used for the pre-inoculum (Fig. 1a). Usually, the use of the same medium for pre-inoculum and subsequent culture produces better results in terms of cell formation and product yield, because cell adaptation is faster, reducing or suppressing the lag phase. *Bacillus* produces γ-PGA as a glutamate source in starvation during stationary phase,

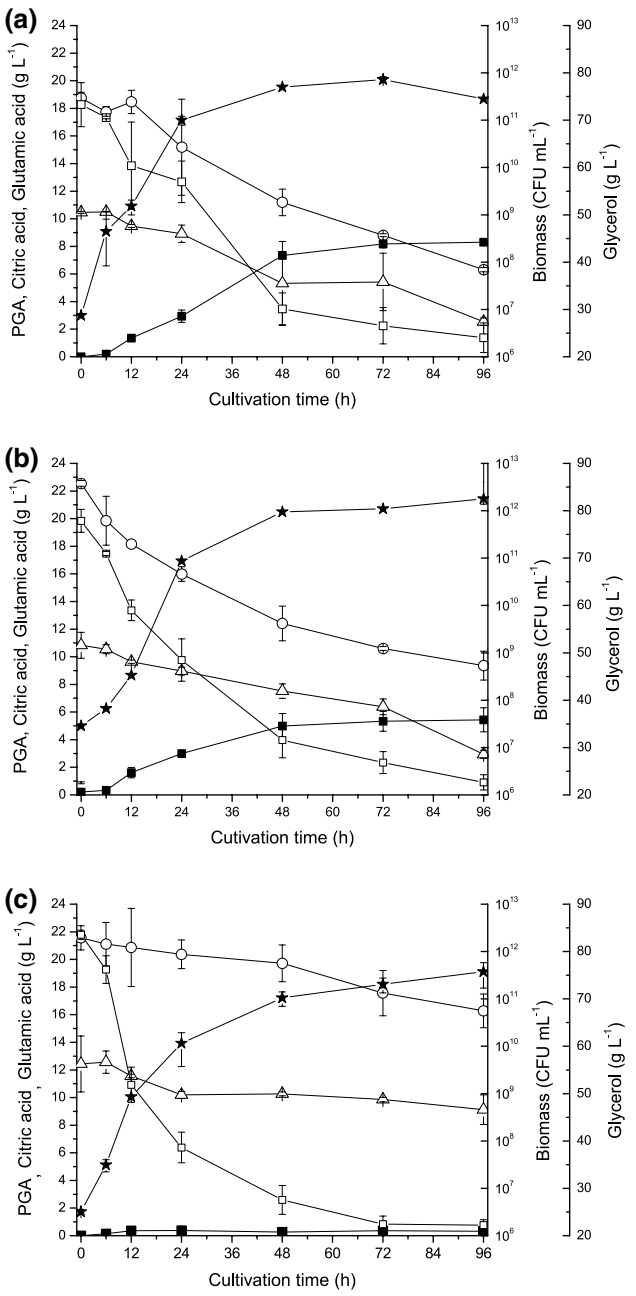

Fig. 1 1 Culture kinetics of *Bacillus subtilis* BL53 carried out in rotatory shaker at 37 °C and 180 rpm, using cultivation medium E and different inocula media: **a** LB, **b** E, and **c** LBGlu. γ-PGA concentration (*filled square*), number of viable cells (*filled star*), L-glutamic acid concentration (*open circle*), citric acid concentration (*open triangle*), and glycerol concentration (*open square*). Results are the mean of duplicate runs

or under stressed conditions [5], and, therefore, a change from LB (inoculum) to medium E (cultivation medium) might have stimulated higher γ-PGA yields. Other authors reported the use of a modified LB pre-inoculum by replacing the yeast extract for beef extract, inducing the spore formation [24, 28].

Figure 1c shows the kinetics for cultures pre-inoculated with LBGlu. The glutamic acid was added as a precursor of γ-PGA, and we hypothesized that it could stimulate the production of the biopolymer. Results, however, showed that the cell metabolism was directed toward biomass formation, whereas γ-PGA production was completely suppressed. Kambourova et al. [13] reported that glutamic acid could induce different responses for specific strains of *B. subtilis and B. licheniformis*, either repressing the expression of γ-PGA biosynthetic enzymes' genes when added to early log phase cultures of some strains, while inducing the expression for others.

According to these results, the pre-inoculum medium chosen for the next experiments was LB.

Effects of metabolic precursors in culture media on the γ-PGA production

In Fig. 2 are shown the kinetics of the cultivations in the three different media: LBGlu, E(-glu), and E(+prec). When LBGlu was used, γ-PGA was not produced and only a small amount of glutamic acid was consumed (Fig. 2a). According to Kamborouva [13], in some strain of *B. licheniformis* and *B. subtilis,* exogenous glutamate could repress the expression of γ-PGA biosynthetic enzymes' genes if $(NH_4)_2SO_4$ or glutamine were not provided as additional nitrogen sources, which appears to be the case for *B. subtilis BL53* growing in LBGlu. Our results also suggest that the absence of readily available metabolic pathway intermediates prevented the production of γ-PGA [23] from endogenous glutamate. The complete metabolic pathway for endogenous glutamic acid synthesis and all the enzymes involved was well described by Shih and Van [25]. In short, glucose is converted to acetyl-CoA through glycolysis, entering in the TCA cycle and producing endogenous glutamic acid from citric acid and α-ketoglutaric acid. The pyruvate dehydrogenase (PDH) mediates the glycolysis pathway to the TCA cycle, and finally glutamate dehydrogenase (GDH) converts α-ketoglutarate (formed from citrate) into glutamic acid. Both enzymes are stimulated by the presence of citric acid in the medium, which was not added to LBGlu in this work, consequently preventing the production of endogenous glutamate and γ-PGA [33].

Medium E(-glu) was tested to evaluate the dependence of γ-PGA production on exogenous glutamic acid by *B. subtilis* BL53, and the results are shown in Fig. 2b. The γ-PGA production was very small compared to the medium comprising glutamic acid (Fig. 1a), characterizing *B. subtilis* BL53 as an exogenous glutamic acid-dependent strain. Glutamic acid-dependent strains cannot produce γ-PGA in the absence of this amino acid, although it is not always consumed in large amounts. Glutamic acid units forming γ-PGA are mainly produced from citric acid through the TCA

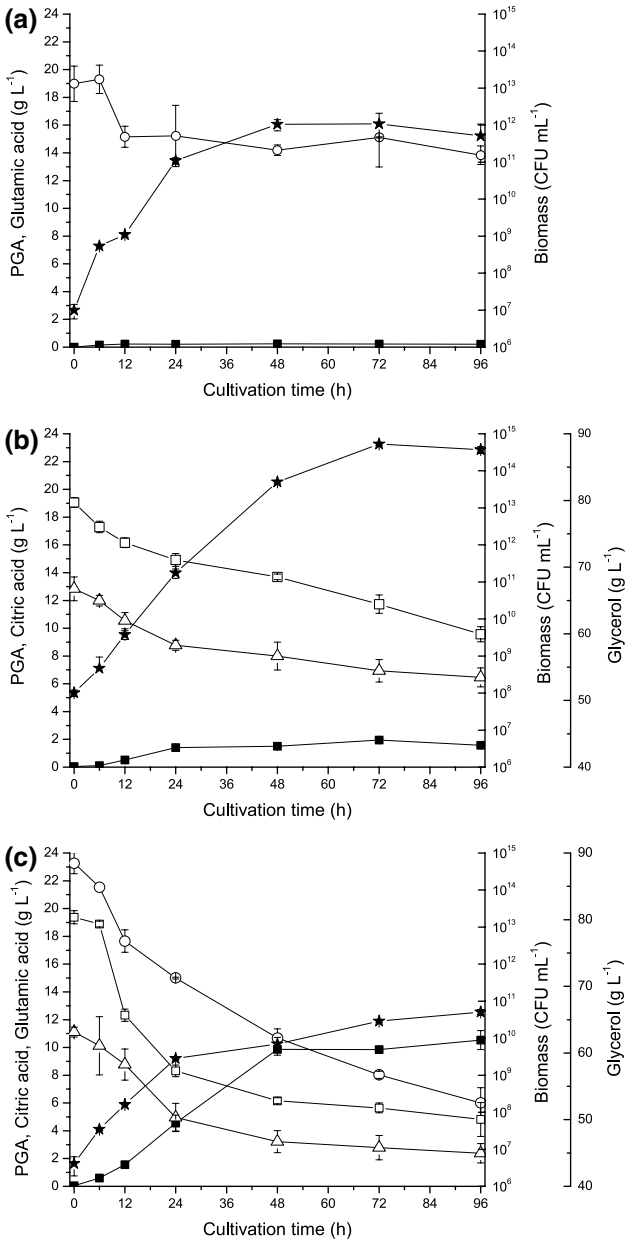

Fig. 2 Culture kinetics of *B. subtilis* BL53 carried out in rotatory shaker at 37 °C and 180 rpm, using LB as pre-inoculum and: **a** LBGlu, **b** E(-glu), and **c** E(+prec) as production media. γ-PGA concentration (*filled square*), number of viable cells (*filled star*), L-glutamic acid concentration (*open circle*), citric acid concentration (*open triangle*), and glycerol concentration (*open square*). Results are the mean of duplicates

cycle and the addition of glutamic acid to the medium acts as an activator of enzymes in the pathway of the biopolymer synthesis [10, 12, 17, 30, 33]. According to Zhang et al. [33], α-ketoglutaric acid catalyzed by 2-oxoglutarate dehydrogenase complex can deviate the carbon flux from biopolymer synthesis, producing energy and biomass. The consumption of glycerol and the higher biomass formation, especially compared with the results shown in Fig. 1a, suggests that the carbon flux was indeed deviated from γ-PGA production.

In Fig. 2c is depicted the culture kinetics when *B. subtilis* BL53 was grown in medium E(+prec). This experiment was carried out to evaluate the effects of metabolic pathway precursors of γ-PGA synthesis (L-glutamine and α-ketoglutaric acid) on the production of the biopolymer. Results show that glutamic and citric acids were rapidly consumed, along with glycerol, and the production of γ-PGA was high, reaching 10 g L^{-1}. Citric acid is converted to α-ketoglutaric acid via isocitric acid in the TCA cycle [25]. Glutamic acid can be produced either from endogenous or exogenous sources of α-ketoglutaric through two different pathways, which are well described by Bajaj and Singhal [2]. Glutamate can be formed from α-ketoglutaric acid and ammonium sulfate via GDH pathway or from α-ketoglutaric acid and glutamine via glutamine-2-oxoglutarate aminotransferase (GOGAT) catalysis. GDH and GOGAT activities are induced by L-glutamine and α-ketoglutaric acid present in the cultivation medium. Glutamine may be regenerated from glutamate and ammonium sulfate by glutamine syhthetase (GS) [2, 25].

The positive effects of the addition of the TCA cycle precursors on γ-PGA production have been reported for other *Bacilli* strains. Kunioka [16] observed higher yields of γ-PGA without the generation of any by-products in cultures of *B. subtilis* IFO3335 when L-glutamine was used instead of glutamic acid. Bajaj and Singhal [2] observed that the combined addition of L-glutamine (0.5 mM) and α-ketoglutaric acid (10 mM) enhanced γ-PGA production by *B. licheniformis* NCIM 2324 as compared to the medium without these precursors.

Effects of the addition of oxygen carrier on the γ-PGA production in bioreactor cultures

The best culture media for γ-PGA production [medium E and medium E(+prec)] were tested with the addition of PDMS working as an oxygen carrier in bioreactor cultures, in order to observe the effects of this chemical on γ-PGA production and on the oxygen mass transfer coefficient (k_La) of the system. Results are depicted in Fig. 3a (γ-PGA) and Fig. 3b (biomass), clearly showing the enhanced production of γ-PGA in both culture media in the presence of PDMS. For medium E(+prec), a maximum production of 19.7 g L^{-1} of γ-PGA and a productivity of 0.82 g L^{-1} h^{-1} were observed in the absence of PDMS, whereas in the presence of the oxygen carrier, production was 23.5 g L^{-1} and productivity was 0.98 g L^{-1} h^{-1}.

Medium E, without PDMS, showed γ-PGA production of 14.8 g L^{-1} and productivity of 0.30 g L^{-1} h^{-1}, compared

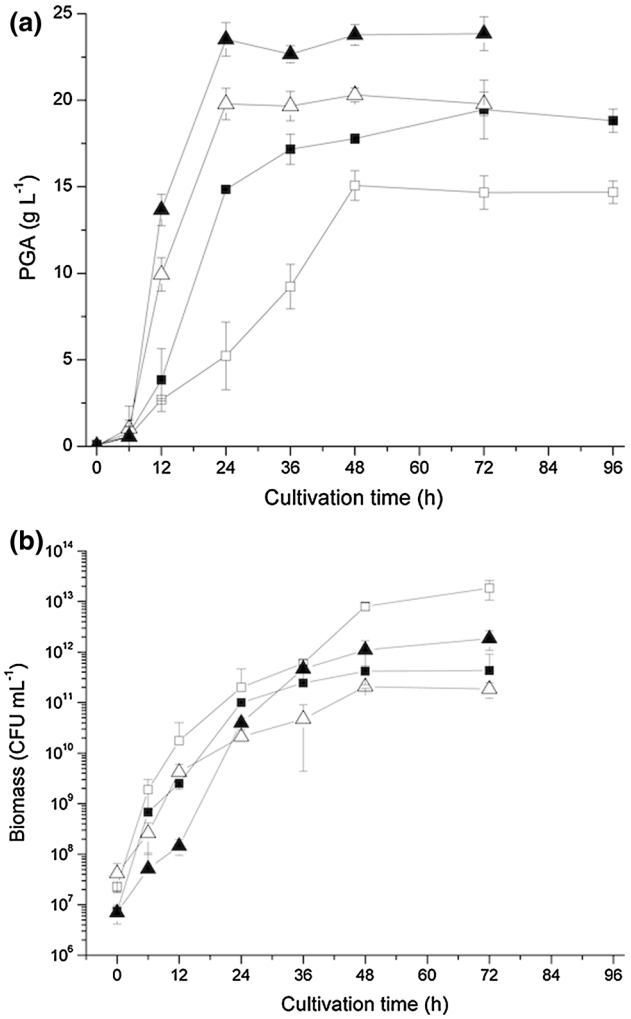

Fig. 3 Kinetics of γ-PGA production **a** and biomass formation **b** using different media: (*open square*) medium E; (*filled square*) medium E plus PDMS; (*open triangle*) medium E(+prec); (*filled triangle*) medium E(+prec) plus PDMS. Results are the mean of duplicates

to 17.2 g L^{-1} and 0.48 g L^{-1} h^{-1}, when the oxygen carrier was added to the culture.

Biomasses (measured as CFU mL^{-1}) in both media were slightly reduced when PDMS was added to the culture (Fig. 3b), which might be explained by the diversion of carbon sources from biomass formation toward the synthesis of γ-PGA. Another possibility is the fact that the enhanced oxygen supply to cells might have caused some oxidative stress of cells.

In aerobic cultivations, two main resistances may control the overall oxygen supply to cells: the physical mass transfer resistances from gas to the liquid phase, and the biochemical reactions linked to respiration of the cell. When the biochemical reactions occur faster than the physical mass transfer rate, oxygen availability to the cell will be controlled by the latter, consequently limiting cell

Table 1 Volumetric oxygen transfer coefficient (k_La) for *Bacillus subtilis* BL53 cultures performed at 2 vvm, and 1,000 rpm as function of PDMS addition (10 % volume fraction) measured at the physiological state of mid-exponential growth phase

Medium	k_La (h^{-1})	Increase in k_La
E	33.0 (±2.3)	
E + PDMS	205 (±2)	521 %
E(+prec)	61.4 (±3.1)	
E(+prec) + PDMS	222 (±2)	262 %

Results are the mean of duplicate runs

metabolism [3]. Therefore, oxygen transfer rate is vitally important, particularly in the production of biopolymers such as γ-PGA from *B. subtilis* BL 53, which has a high molecular mass (about 1.5 × 10^6 g mol^{-1}) and produces culture broths with high viscosity [6]. This aspect is increasingly important as the cultivation proceeds and the concentration of γ-PGA increases, resulting in oxygen limitations [22].

Table 1 shows the volumetric mass transfer coefficient (k_La) for media E and E(+prec) with and without the additions of PDMS, measured at the physiological mid-exponential growth phases. Although a small increase in the k_La value of E(+prec) in comparison with medium E even without the addition of PDMS could be observed, probably as a result of some change in the medium viscosity or salinity (not measured), results clearly show that the addition of PDMS greatly enhanced k_La of cultures, explaining the enhanced γ-PGA production shown in Fig. 3a. Moreover, k_La decreased over time when PDMS was not added due to the increase of medium viscosity, effect that was not observed when the oxygen carrier was added (results not shown).

This work is the first report on the literature on the use of PDMS in *B. subtilis* cultures in order to enhance γ-PGA production. Dey et al. [7] tested the addition of PDMS in cultures of *B. thuriginensis* A3 for biomass formation and reported an increase of about 50 % in biomass production. Zhang et al. [32] observed that the addition of n-hexane, n-heptane or n-hexadecane increased γ-PGA concentration and its molecular weight. Rech et al. [21] studied the effects of the addition of emulsified PMDS on k_La of submerged cultures of *Staphylococcus warneri* EX17 for lipase production. The addition of 10 % of PDMS improved the k_La by 160 % and lipase activity was approximately two times higher than in the medium without the oxygen carrier.

PDMS, being immiscible with water, will form droplets in the bulk of culture, originating a liquid biphasic system, dynamically interacting at the liquid boundary layer around the gas bubbles (provided by air supply and agitation), increasing the turbulence or mixing in this layer [21]. The resistance in the liquid layer and the gas bubble

is decreased when PDMS was used, greatly enhancing the transference from the gas phase to the liquid phase. Additionally, because the oxygen solubility in oxygen carriers such as PDMS is higher than in water, oxygen permeability within the biphasic liquid boundary layer is enhanced [21]. Because oxygen plays an important role in the metabolic pathway of γ-PGA synthesis through glycolysis and the TCA cycle, any strategy that can increase the oxygen transfer rate (k_La) would positively impact γ-PGA production. According to Zhang et al. [32], oxygen carriers can also stimulate the aerobic respiratory pathways in *B. subtilis* NX-2 regulating the NADH/NAD^+ ratio and the ATP concentration. Oxygen carriers may also influence the activity of enzymes that require ATP, such as PGA synthetase [25], responsible for the molecule polymerization. Cofactors such as NADH and NAD^+ have major roles in microbial catabolism. The cofactor pair NADH/NAD^+ is crucial for cell growth, during which NADH is oxidized to NAD^+ and a redox balance is achieved [32].

Conclusions

Bacillus subtilis BL53 is a new and promising γ-PGA producer, showing to be an exogenous glutamic acid-dependent strain. Medium E added of metabolic pathway precursors glutamine and α-ketolgutaric acid proved to be an ideal culture medium in order to enhance γ-PGA production by this bacterium. PDMS, added to the bioreactor as an oxygen carrier, enhanced the oxygen mass transport in the medium, increasing k_La for both cultivation media, E and E(+prec), improving γ-PGA production and productivity. The results presented in this work could be a promising technology for γ-PGA production by new bacteria strains and could be extended to other bioproducts.

Acknowledgments The authors wish to thank CNPQ, FAPERGS, and CAPES (Brazil) for their financial support for this project and the scholarship for the first author.

REFERENCES

1. Bajaj I, Singhal R (2011) Poly (glutamic acid): an emerging biopolymer of commercial interest. Bioresour Technol 102:5551–5561
2. Bajaj IB, Singhal RS (2009) Enhanced production of poly (gamma-glutamic acid) from *Bacillus licheniformis* NCIM 2324 by using metabolic precursors. Appl Biochem Biotechnol 159:133–141
3. Bandaiphet C, Prasertsan P (2006) Effect of aeration and agitation rates and scale-up on oxygen transfer coefficient, kLa in exopolysaccharide production from *Enterobacter cloacae* WD7. Carbohyd Polym 66:216–228
4. Buescher JM, Margaritis A (2007) Microbial biosynthesis of polyglutamic acid biopolymer and applications in the biopharmaceutical, biomedical and food industries. Crit Rev Biotechnol 27:1–19
5. Candela T, Fouet A (2006) Poly-gamma-glutamate in bacteria. Mol Microbiol 60:1091–1098
6. Cesaro A, Silva SB, Ayub MAZ (2014) Physico-chemical and rheological characterization of poly-gamma-glutamic acid produced by a new strain of *Bacillus subtilis*. Eur Polym J 57: 91–98
7. Dey E, Norrlöw O, Liu Y (2004) Artificial carrier for oxygen supply in biological systems. Appl Microbiol Biotechnol 64:187–191
8. Doran PM (1995) Bioprocess engineering principles. Academic Press, London
9. Du G, Yang G, Qu Y, Chen J, Lun S (2005) Effects of glycerol on the production of poly([gamma]-glutamic acid) by *Bacillus licheniformis*. Process Biochem 40:2143–2147
10. Goto A, Kunioka M (1992) Biosynthesis and hydrolysis of poly(gamma-glutamic acid) from *Bacillus subtilis* IFO3335. Biosc Biotechnol Biochem 56:1031–1035
11. Graiver D, Farminer K, Narayan R (2003) A review of the fate and effects of silicones in the environment. J Polym Environ 11:129–136
12. Jung DY, Jung S, Yun JS, Kim JN, Wee YJ, Jang HG, Ryu HW (2005) Influences of cultural medium component on the production of poly (γ-glutamic acid) by *Bacillus* sp. RKY3. Biotechnol Bioprocess Eng 10:289–295
13. Kambourova M, Tangney M, Priest FG (2001) Regulation of polyglutamic acid synthesis by glutamate in *Bacillus licheniformis* and *Bacillus subtilis*. Appl Environl Microbiol 67:1004–1007
14. Kanno A, Takamatsu H (1995) Determination of polyglutamic acid in "Natto" using cetyltrimethylammonium bromide (Studies on "Natto" part V). Nippon Shokuhin Kagaku Kogaku Kaishi 42:878–886
15. Kunioka M (1997) Biosynthesis and chemical reactions of poly (amino acid) s from microorganisms. Appl Microbiol and Biotechnol 47:469–475
16. Kunioka M (1995) Biosynthesis of poly (γ-glutamic acid) from L-glutamine, citric acid and ammonium sulfate in *Bacillus subtilis* IFO3335. Appl Microbiol Biotechnol 44:501–506
17. Kunioka M, Goto A (1994) Biosynthesis of poly (γ-glutamic acid) from L-glutamic acid, citric acid, and ammonium sulfate in *Bacillus subtilis* IFO3335. Appl Microbiol Biotechnol 40:867–872
18. Leonard CG, Housewright RD, Thorne CB (1958) Effects of some metallic ions on glutamyl polypeptide synthesis by *Bacillus subtilis*. J Bacteriol 76:499–503
19. Li C, Yu DF, Newman RA, Cabral F, Stephens LC, Hunter N, Milas L, Wallace S (1998) Complete regression of well-established tumors using a novel water-soluble poly (L-glutamic acid)-paclitaxel conjugate. Cancer Res 58:2404–2409
20. Pleissner D, Wimmer R, Eriksen NT (2010) Quantification of amino acids in fermentation media by isocratic HPLC analysis of their α-hydroxy acid derivatives. Anal Chem 83:175–181
21. Rech FR, Volpato G, Ayub MAZ (2012) The effects of emulsified polydimethylsiloxane FG-10 on the oxygen transfer coefficient (kLa) and lipase production by Staphylococcus warneri EX17. J Chem Technol Biotechnol 87:990–995
22. Richard A, Margaritis A (2003) Rheology, oxygen transfer, and molecular weight characteristics of poly(glutamic acid) fermentation by *Bacillus subtilis*. Biotechnol Bioeng 82:299–305
23. Shi F, Xu Z, Cen P (2006) Optimization of γ-polyglutamic acid production by *Bacillus subtilis* ZJU-7 using a surface-response methodology. Biotechnol Bioprocess Eng 11:251–257
24. Shih IL, Van YT, Chang YN (2002) Application of statistical experimental methods to optimize production of poly(gamma-glutamic acid) by *Bacillus licheniformis* CCRC 12826. Enz Microbial Technol 31:213–220

25. Shih IL, Van YT (2001) The production of poly-(gamma-glutamic acid) from microorganisms and its various applications. Bioresour Technol 79:207–225
26. Shih IL, Van YT, Yeh LC, Lin HG, Chang YN (2001) Production of a biopolymer flocculant from *Bacillus licheniformis* and its flocculation properties. Bioresour Technol 78:267–272
27. Silva SB, Cantarelli VV, Ayub MAZ (2014) Production and optimization of poly-γ-glutamic acid by *Bacillus subtilis* BL53 isolated from the amazonian environment. Bioprocess Biosyst Eng 37(3):469–479
28. Sung MH, Park C, Kim CJ, Poo H, Soda K, Ashiuchi M (2005) Natural and edible biopolymer poly-gamma-glutamic acid: synthesis, production, and applications. Cheml Rec 5:352–366
29. Troy FA (1973) Chemistry and biosynthesis of the poly (γ-d-glutamyl) capsule in *Bacillus licheniformis*-I. properties of the membrane-mediated biosynthetic reaction. J Biol Chem 248:305–315
30. Wu Q, Xu H, Shi N, Yao J, Li S, Ouyang P (2008) Improvement of poly (γ-glutamic acid) biosynthesis and redistribution of metabolic flux with the presence of different additives in *Bacillus subtilis* CGMCC 0833. Appl Microbiol Biotechnol 79:527–535
31. Xu H, Jiang M, Li H, Lu D, Ouyang P (2005) Efficient production of poly([gamma]-glutamic acid) by newly isolated *Bacillus subtilis* NX-2. Process Biochem 40:519–523
32. Zhang D, Feng X, Li S, Chen F, Xu H (2012) Effects of oxygen vectors on the synthesis and molecular weight of poly (γ- glutamic acid) and the metabolic characterization of *Bacillus subtilis* NX-2. Process Biochem 47:2103–2109
33. Zhang H, Zhu J, Zhu X, Cai J, Zhang A, Hong Y, Huang J, Huang L, Xu Z (2012) High-level exogenous glutamic acid-independent production of poly-(γ-glutamic acid) with organic acid addition in a new isolated *Bacillus subtilis* C10. Bioresour Technol 116:241–246