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Poly-β-hydroxybutyrate and Exopolysaccharide Biosynthesis by Bacterial Isolates from Pigeonpea [*Cajanus cajan* (L.) Millsp] Root Nodules

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Abstract The bacterial strains that are able to produce biopolymers that are applied in industrial sectors present a source of renewable resources. Some microorganisms are already applied at several industrial sectors, but the prospecting of new microbes must bring microorganisms that are feasible to produce interesting biopolymers more efficiently and in cheaper conditions. Among the biopolymers applied industrially, polyhydroxybutyrate (PHB) and exopolysaccharides (EPS) stand out because of its applications, mainly in biodegradable plastic production and in food industry, respectively. In this context, the capacity of bacteria isolated from pigeonpea root nodules to produce EPS and PHB was evaluated, as well as the cultural characterization of these isolates. Among the 38 isolates evaluated, the majority presented fast growth and ability to acidify the culture media. Regarding the biopolymer production, five isolates produced more than 10 mg PHB per liter of culture medium. Six EPS producing bacteria achieved more than 200 mg EPS per liter of culture medium.

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Evaluating different carbon sources, the PHB productivity of the isolate 24.6b reached 69% of cell dry weight when cultured with starch as sole carbon source, and the isolate 8.1c synthesized 53% PHB in dry cell biomass and more than 1.3 g L^{-1} of EPS when grown using xylose as sole carbon source.

Index terms Biopolymers · Rhizobia · UV-HPLC · Technological innovation · Inoculant technology · Strain selection

Introduction

Microbial biopolymers are potentially useful for new industrial applications where sources of new material for industrial application and technological innovation are sought. The use of microbial biopolymers has some advantages over biopolymers from other sources, such as plant molecules, principally regarding the space and time needed to grow microorganisms, the cost of their cultivation, and the ratio between growth rate and production [1]. Among the microbial molecules applied in industrial processes, exopolysaccharides (EPS) and polyhydroxyalcanoates (PHA) stand out.

EPS are produced by several bacterial isolates and have important ecological functions such as cell aggregation and protection and quorum sensing behavior [2]. The main biotechnological applications of EPS are in their use as a thickening agent in the food and cosmetics industry. EPS produced by rhizobial strains also is important to the recognition by the host plant at the infection process [3] and by the biofilm formation [4].

Polyhydroxybutyrate (PHB) have several uses in the medical and pharmaceutical industry [5], but the main application of this biopolymer is in the plastic industry due to the characteristics similar to those of polypropylene that allow the application of these polymer to produce of biodegradable plastics [6, 7]. Root nodule bacteria are also able to produce PHB in root nodules, and investigations have shown the importance of these polymers on the biological nitrogen fixation [8].

The two genera industrially applied to produce either EPS and PHB (*Xanthomonas* and *Ralstonia*, respectively) include several phytopathogenic species, which is disadvantageous due to the health laws of some countries. The search for new beneficial microorganisms able to synthesize these polymers must produce more efficient resources mainly for the microbial industry, for example, for rhizobial inoculant producers. The Brazilian rhizobial inoculant industry is the largest inoculant-producing industry in the world, but it is very dependent on soybean production [9]. Soybean sowing in Brazil occurs from August to December, and it is in this period that inoculants are sold. Outside this period, the activity of inoculant producers is very low. The possibility to provide bacterial strains that are able to produce interesting polymers under the same conditions of rhizobial batch should provide another activity for the rhizobial inoculant industry.

In addition, the prospecting for bacterial strains able to produce PHB and/or EPS should uncover unexploited genetic resources, as already shown in Brazil [7, 10]. Therefore, the aim of this work was to evaluate the production and the productivity of PHB and EPS by bacterial strains that present different cultural characteristics, isolated from root nodules of pigeonpea, and select bacterial isolates able to produce these polymers at routine laboratory conditions using low expensive carbohydrates as carbon sources.

Materials and Methods

Bacterial Strains and Growth Conditions

A total of 38 bacterial isolates from pigeonpea [*Cajanus cajan* (L.) Millsp.] root nodules were evaluated, related to their cultural characteristics in yeast extract–mannitol–agar medium and in their ability to produce PHB and EPS in yeast extract–mannitol (YM) medium [12]. The following were also evaluated: the EPS and PHB production by *Bradyrhizobium* sp. (BR 3267), which presents moderate growth; *Bradyrhizobium elkanii* (BR 96 and BR 29), which presents slow growth; *Rhizobium tropici* (BR520); *R. leguminosarum* bv. *phaseoli* (BR7606); and *Sinorhizobium terangae* (BR527) with fast growth. These six rhizobial strains are recommended to produce rhizobial inoculants to different legumes in Brazil. At the "Supplementary Material", some cultural characteristics and isolates origins are shown.

The bacterial strains/isolates were grown in 250-mL Erlenmeyer flasks containing 120 mL of YM medium in an orbital shaker set at 200 rpm and 28 °C, for 3 days to fast growth, 5 days to moderate growth, and 7 days to slow growth strains. The growth of bacteria in media containing different carbon sources was carried out with the addition of 1% (wt/vol) fructose, glucose, xylose, sucrose, or starch to YM medium replacing mannitol as the sole carbon source.

Cultural Characterization of the Bacterial Isolates

To the phenotypical (cultural) characterization, bacteria were grown in Petri dishes with yeast extract–mannitol–agar medium [12], and the parameters considered were growth period, pH alteration of culture medium (acidification or alkalinization), colony color, colony edge aspect (smooth or rough), uniformity of the culture in the dish (homogeneous or heterogeneous), colony diameter, and gum production (low, intermediate or high production) and elasticity (with or without elasticity) [13, 14]. Based at the phenotypical characterization, a binary matrix was built, and the clustering analyses were carried out at software PaSt [15] with unweighted pair group method with arithmetic mean (UPGMA) clustering method and applying the Dice coefficient to calculate the similarity between the bacterial isolates.

Dry Cell Biomass Determination

The bacterial broth was centrifuged at 8,000×*g* for 30 min at 4 °C. The pellet was resuspended in 120 mL of distilled water. A 1-mL cell suspension aliquot was transferred to preweighed tubes, dried overnight, and weighed to determine the dry cell biomass (DCB) [10].

EPS Extraction and Determination

EPS was precipitated with the addition of three volumes of cold ethanol (95%) to the supernatant of the first centrifugation as described before [11, 16, 17]. The extract was centrifuged again at 10,000×*g* for 30 min. The pellet was washed several times with cold ethanol and vacuum dried for storage at room temperature [16].

Ethanolic EPS suspension was filtered through a nitrocellulose membrane (0.45 μm). An aliquot (1 mL) was transferred to preweighed tubes, dried overnight, and weighted [10, 16].

PHB Extraction and Concentration Measurement

To extract the intracellular PHB content, 1 mL aliquots of cell suspension obtained after the first centrifugation were transferred to test tubes and dried overnight. The cell contents were digested with 1 mL sulfuric acid (98%) in a water bath for 45 min at boiling temperature. The digested suspension was diluted with 4 mL 0.004 M sulfuric acid, mixed and cooled on ice [18, 20].

The sulfuric acid-digested solution was further diluted tenfold with 0.004 M sulfuric acid. The PHB concentration was determined by high-performance liquid chromatography (HPLC). The column and column guard used was the Aminex HPX-87H column measuring 300×1.8 mm (BioRad, Richmond, CA). The isocratic elution was carried out, with a flow rate of 0.5 mL min^{-1} of mobile phase (0.004 M sulfuric acid; adapted from Karr et al. [18]). The PHB concentration was detected with a UV detector (10 AC) at 210 nm [18]. The sample injection volume was 50 μL, and the total detection time was 45 min. To determine the concentration of PHB in the samples, positive controls with pure PHB were used as standards (Sigma-Aldrich, St. Louis, MO). Samples of 0, 1, 2.5, 10, 20, 35, 40, and 50 mg of PHB were also digested, diluted and injected as described above. The digestions of positive controls also were carried out with three replications. The PHB concentration in each sample was determined comparing the peak area with the control peak using regression analysis.

EPS and PHB Production Data Analysis

All experiments were conducted in triplicate using a completely random design. The data of cell biomass production, PHB production end EPS production were correlated with Spearman's rank correlation test [19].

Results and Discussion

The clustering analyses based at the phenotypical features formed mainly three clusters (Fig. 1). The major cluster (cluster A) encompassed 27 bacterial isolates and the rhizobial strains BR 520, BR 526, BR 7606, and BR 3267, with about 75% of similarity (Fig. 1). This cluster presents mainly bacterial isolates that have fast-growing and acidify the culture media pH (Fig. 1). The cluster A also presents subclusters. Among then, the subclusters A1, A2, and A3 stand out (Fig. 1). The cultural characteristic that is mainly contributed to this subdivision is the production of gum at solid medium. The subclusters A1, A2, and A3 showed intermediate, high, and low gum production, respectively (Fig. 1). The cluster B presents ten bacterial isolates with approximately 72% of similarity and principally, as the previous cluster, also showed fast-growing bacteria that decrease the pH of culture media. This cluster differs from cluster A by characteristics such as colony color, colony edge aspect, and the uniformity of the culture in the dish. Cluster C encompassed both *B. elkanii* strains and six bacterial isolates with approximately 68% of similarity (Fig. 1). All these bacteria are able to alkalinize the culture media and present slow growth (except isolate 29.5b that presents moderate growth).

Analyzing the clustering based at the cultural features, groups with only bacteria that present fast growth and acidification of the culture media are very common in studies with root nodule bacteria from tropical soils [14, 20]. In this study, the high gum production in solid media was also observed among the isolates that acidified the culture media, in agreement with previous studies [13, 14, 20].

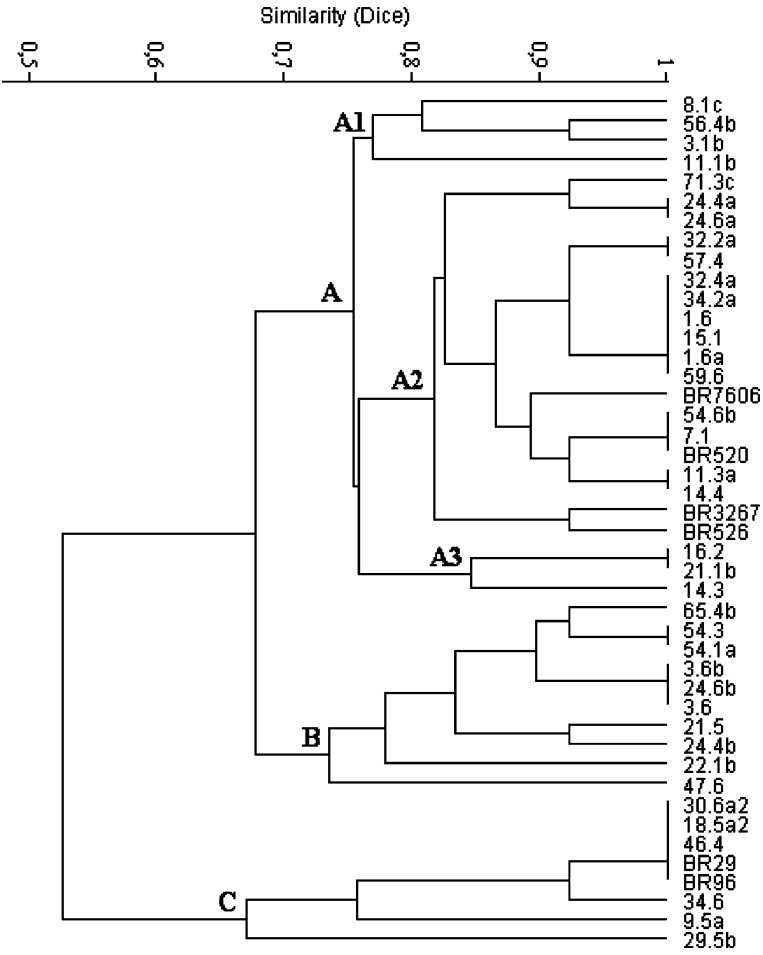

Fig. 1 Similarity dendrogram obtained from phenotypical characterization of 38 bacterial isolates from pigeonpea root nodules and reference rhizobial strains of *B. elkanii* (BR96 and BR29), *Bradyrhizobium* sp. (BR3267), *R. tropici* (BR520), *Rhizobium leguminosarum* bv. *phaseoli* (BR7606), and *S. terangae* (BR527). Dendrogram was built with UPGMA clustering method and Dice coefficient of similarity

The fast growing and the acidification of culture medium are identified as adaptations of bacterial isolates to the tropical soil characteristics such as low pH, low organic matter content, and high Al^{3+} content [21]. Bacteria that present slow growth and alkalinization of medium pH introduced to tropical soils must reduce the time of growth or increase the gum production as adaptations to tropical soil environmental conditions [22]. In the present study, only six isolates had the cultural characteristics similar to that showed by typical *Bradyrhizobium* strains (mainly, alkalinization of the culture media and slow growth). Recent studies in which rhizobia from green manures such as cowpea [20], velvet bean [23], and pigeonpea [24] root nodules were evaluated also presented few isolates with similar features to the typical *Bradyrhizobium* strains.

Regarding the biopolymer production, some root nodule bacterial isolates evaluated were able to produce high amounts of EPS, growing in YM medium (Table 1). Six isolates produced more than 200 mg L^{-1} EPS. The other 23 isolates produced between 100 and

Table 1 PHB, EPS and DCB production and productivity by bacterial isolates from root of pigeonpea and by strains recommended for inoculant production in Brazil (data are means of three replications)

Bacterial isolate	PHB	DCB	EPS	PHB	EPS
	Production (mg L^{-1})			Productivity (%)	
24.6b	36.6	76	33	48	43
30.6a2	28.4	188	83	15	44
9.5a	28.0	209	101	13	48
29.5b	26.0	229	90	11	39
24.6a	18.9	65	34	29	52
8.1c	18.3	48	125	38	260
24.4b	14.7	70	85	21	121
34.6	10.1	102	156	10	153
54.6b	9.8	120	130	8	108
56.4b	9.1	105	73	9	70
71.3b	7.2	92	223	8	242
54.3	6.8	80	120	9	150
11.3a	6.2	92	244	7	265
3.6	6.1	97	198	6	204
65.4b	4.6	99	133	5	134
18.5a2	4.5	201	121	2	60
32.2a	3.6	110	135	3	123
32.4a	3.0	65	168	5	259
46.4	2.2	193	99	1	51
54.1a	1.9	130	56	1	43
24.4a	1.7	70	75	2	107
22.1b	1.6	51	81	3	159
16.2	1.3	89	156	2	175
21.1b	1.2	27	78	5	289
34.2a	0.9	101	78	1	77
14.3	0.7	98	89	1	91
1.6	0.6	120	126	1	105
3.6b	0.5	77	199	1	258
3.1b	0.4	17	25	3	147
15.1	0.3	100	225	0	225
1.6a	0.2	100	203	0	203
57.4	0.1	62	100	0	161
14.4	0.1	60	100	0	167
21.5	nd	43	113	nd	263
11.1b	nd	105	120	nd	114
47.6	nd	32	56	nd	175
59.6	nd	85	101	nd	119
7.1	nd	103	222	nd	216
BR29	40.9	199	100	21	50
BR96	38.5	210	105	18	50
BR7606	0.2	89	138	0	155
BR3267	0.1	56	156	0	279
BR520	0.1	102	229	0	225
BR527	0.6	98	155	1	158
Spearman's correlation coefficient				−0.89	−0.83

nd not determined

200 mg L^{-1} EPS. Poor production of EPS was observed only in nine isolates. EPS productivity based on microbial biomass (wt/wt) was about 290% for the isolate 21.1b. Ten other isolates also reached productivity higher than 200% (Table 1). The production of EPS between 100% and 200% of dry cell biomass was found in 17 isolates (Table 1). The production of high amounts of EPS by some rhizobial strains was also achieved when these bacteria were cultured in YM media, which is different to the other isolates that were not able to produce great quantities of EPS at the same media [16]. This suggests differences among strains in how they use the same carbon and nitrogen resources to produce EPS.

The detection of PHB by UV-HPLC showed that the digested PHB eluted up to approximately 36 min. Another peak was also observed at about 7.5 min, and it must be due to the solvent elution, and in cell-digested samples, the peak at 7.5 min must also contain the digested cell debris and other molecules that do not bind to the HPX87H column (Fig. 2), as already pointed out by Karr et al. [18]. The regression analysis between the different concentrations of synthetic PHB and the 36-min peak area showed a high correlation coefficient (R^2) of 0.994 (Fig. 3), which suggests very good digestion and detection over the range of concentrations used and the high accuracy of the method. A similar regression coefficient was found by the authors that developed the method [18], which shows the reproducibility of the results obtained. The retention time of digested PHB was around 36 min, while Karr et al. [18] found 29 min. This difference is likely due to the changes in method conditions such as flow rate (0.7–0.5 mL min^{-1}) and the concentration of mobile phase (H_2SO_4 0.028 to 0.004 M). A little peak was observed at 33 min in all samples, including the standard samples, and the same noise has been observed by the authors who developed this technique [18].

The isolates 24.6b, 24.6a, 8.1c, 24.4b, 34.6, 30.6a2, 9.5a, and 29.5b were able to produce more than 10 mg L^{-1} PHB, noting that the isolate 24.6b produced 36.6 mg L^{-1}, attaining a productivity of 48% (Table 1). Other isolates also had elevated productivity, particularly isolates 8.1c and 24.6a, which produced 38% and 29% PHB, respectively (Table 1). Note that these isolates are present in different clusters when evaluated the phenotypical characteristics (Fig. 1), showing that divergent isolates may produce similar PHB amounts as already showed [26]. Only five isolates did not produce PHB or produced amounts below the detection level with UV-HPLC. Among the strains recommended to produce inoculant in Brazil, the slow-growing rhizobia were able to produce high quantities

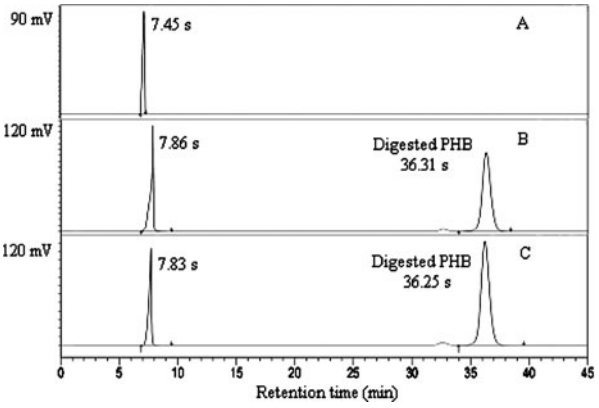

Fig. 2 Detection of digested PHB by UV-HPLC using an Aminex HPX87H column and a UV detector at 210 nm. **a** Negative control. **b** Sample of 8.1c isolate. **c** Sample of 5 mg PHB

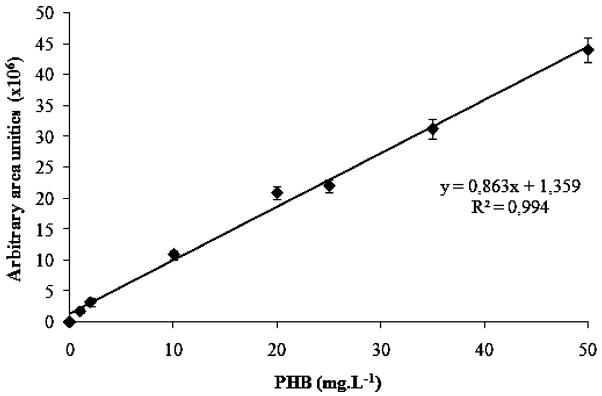

Fig. 3 Regression analysis between the concentration of PHB digested (axis *x*) and the peak area at 36 min of retention time obtained from chromatograms. Different concentrations of PHB were digested, diluted, and injected as described in the text. Data are means of three replications. Bars present the error mean deviation

of PHB, which was not observed in fast-growing rhizobia, showing the potential of the fast-growing pigeonpea root nodule bacteria regarding the production of PHB or EPS.

Bacteria can concomitantly produce different polymers (e.g., EPS and PHB) in different amounts [25]. Bacterial isolates that are able to present fast growth to acidify the culture medium usually show a capacity to produce high amounts of EPS in YM medium; this capacity was already shown in root nodule bacteria such as *Rhizobium* sp. [26]. Root bacteria differ on EPS production and in the rheological characteristics of this polymer such as apparent viscosity. Some bacterial isolates evaluated achieved EPS productivity of more than 290%. The *R. tropici* strain BR 520 is used to produce common bean inoculants in South America. Some isolates evaluated here, for example, the isolate 11.3a that presented high EPS production and productivity (Table 1), showed cultural characteristics very close to those of this strain, belonging to the same cluster at the phenotypical characterization analysis (Fig. 1). EPS production by some root nodule bacteria is very important for nodule formation as already shown for root nodule bacteria [4, 27]. Studies on EPS production by recently isolated bacteria should show a new source of biopolymers industrial interest and also should indicate important characteristics related to nodulation and its efficiency.

The bacterial isolates 24.6b, 24.6a, and 8.1c showed an ability to produce PHB after growth on YM medium supplemented with different carbon sources producing cell biomass above that was achieved when cultured in original YM media (with mannitol as carbon source; Table 2). Despite the low cell biomass, the PHB productivity was higher in the isolate 24.6b grown in YM with starch or xylose as carbon source, reaching 69% and 54% of dry cell biomass, respectively. The isolate 8.1c also accumulated a high PHB content when grown in YM supplemented with starch, reaching a productivity of 49%, but this isolate was able to produce more quantities of PHB growing in medium with fructose, with 53% of PHB compared with dry cell biomass.

Regarding EPS production, the isolates 24.6a and 24.6b that did not produce high amounts of EPS when grown in original EPS medium had increased EPS production and productivity using other carbon sources. The isolate 24.6 reached 153% EPS productivity using fructose as sole carbon source, and the isolate 24.6a reached 192% when grown in YM supplemented with sucrose (Table 2). The isolate 8.1c that showed high productivity of EPS when grown in original YM medium had increased productivity after growth in medium containing xylose, yielding 13.4-fold more EPS.

Table 2 PHB, EPS, and DCB production and productivity by 24.6b, 8.1c, and 24.6a grown in YM medium supplemented with different carbon sources (data are means of three replications)

Isolate	Carbon source	PHB	DCB	EPS	PHB	EPS
		Production (mg L^{-1})			Productivity (%)	
24.6b	Fructose	13.53	32	49	42	153
	Glucose	2.86	30	19	10	63
	Sucrose	8.09	17	15	48	88
	Xylose	10.80	20	23	54	115
	Starch	8.29	12	7	69	58
	Mannitol	36.69	73	42	50	58
8.1c	Fructose	8.95	17	44	53	259
	Glucose	3.22	44	33	7	75
	Sucrose	5.64	19	39	30	205
	Xylose	4.08	10	134	41	1340
	Starch	6.91	14	35	49	250
	Mannitol	14.61	92	260	16	283
24.6a	Fructose	0.64	3	2	21	67
	Glucose	1.05	5	5	21	100
	Sucrose	1.68	12	23	14	192
	Xylose	0.52	14	11	4	79
	Starch	2.73	10	6	27	60
	Mannitol	6.20	34	20	18	59
Spearman's correlation coefficient					−0.93	−0.91

PHB production has already been shown for soil-borne bacteria such as *Azotobacter* [25, 28, 35], *Bacillus* [29], *Cupriavidus* [30], *Sinorhizobium* [31], *Pseudomonas* [32], *Rhizobium* and *Bradyrhizobium* [26]. PHB produced by soil bacteria is also important in several ecological processes such as biological nitrogen fixation [8]. Differences in PHB production among rhizobial strains grown under the same conditions have been shown [26]. Our results also showed that PHB production in different rhizobial strains varied greatly from near zero to more than 36 mg L^{-1}. This production was easily achieved under standard laboratory conditions without the need for expensive bioreactors and controlled processes. The production of biopolymers under routine laboratory conditions allows the production of this polymer by inoculant producers in Brazil and contributes to reduce the cost of PHB produced due to the low expensive conditions adopted.

The PHB productivity of the 24.6b isolate ranged from 10% to 69% when grown in the presence of glucose and starch as carbon source, respectively. Strain 8.1c showed a variation from 7% to 53% PHB productivity when grown in glucose and fructose, respectively. The isolate 24.6a showed a low production of PHB, reaching only 27% of cell dry weight using starch as sole carbon source. Strains of *Azotobacter chroococcum* in the presence of starch as the carbon source were not able to grow abundantly, but they did accumulate substantial amounts of PHB per cell [28]. Our results show that PHB production also varies according to the carbon source used for the cultivation of root nodule bacteria. In evaluating the different carbon sources used, starch stands out because isolates 24.6b and 8.1c accumulated great amounts of PHB with this substrate. High PHB accumulation using starch as sole carbon source has already been shown for several strains

of *Bacillus* spp. [29, 33], *Ralstonia eutropha* [34], and *Azotobacter* spp. [28, 35]. The production of PHB by root nodule bacteria using starch as the carbon source is poorly reported in literature, and this study shows the ability of these bacteria to produce PHB with starch, as carbon source.

Isolate 24.6b also achieved an elevated productivity for PHB (54%) using xylose as sole carbon source. The production of PHB in soil bacterial cultures in medium with this carbon source has been shown for *Burkholderia sacchari* [36]. Xylose is the main monosaccharide resulting of the obtained by hydrolysis of sugarcane bagasse, making this sugar a cheap carbon source for microbial growth [36]. High productivity for PHB using starch as sole carbon source is also interesting at an industrial scale because starch is an inexpensive substrate, which can be extracted from various crops in marginal lands of Brazil, such as cassava, pech palm, araruta, and yam bean.

We also found that cell proliferation in the strains evaluated was negatively affected by the carbon source of the medium, while control grown in original YM medium displayed abundant growth. Other bacteria that grew poorly in culture media were also able to produce elevated quantities of PHB per cell [35]. PHB plays a role in cell protection during stress periods, such as salt stresses [37], starvation [38], and low mineral salt availability [39]. The poor proliferation of bacterial cells in culture medium without mannitol must be due to a bacterial stress caused by the absence of a more compatible carbon source, which should trigger the storage of more carbon reserves. In the strain 24.6b, PHB productivity was increased with starch and xylose, and in isolate 8.1c starch and fructose yielded higher productivities. In spite of the possible starvation due to the low carbon use, the results suggest that the differences in the accumulation of PHB in bacteria grown in media supplemented with different sole carbon sources have strong influences on cell biochemistry. Mannitol is a carbon source used in the media to isolate the rhizobial strains from soil or nodules [12]. Thus, it is expected that this carbon source must be well metabolized by rhizobial strains. Other components of culture media must influence the production of PHB by rhizobial strains. As the carbon source, the nitrogen supplied also presents an important factor in PHB production [26]. In YM media, the nitrogen source is the yeast extract, a low expensive nitrogen source. In this study, we show the increasing of productivity changing only the most expensive compound at the media, the mannitol, maintaining the yeast extract as a feasible nitrogen source to PHB production by rhizobial strains.

Regarding EPS production, the strains that produced low amounts of EPS when grown originally in YM medium with mannitol showed a more than threefold increase in productivity when grown in medium with sucrose (24.6a), and more than twofold when cultures were in fructose-supplemented medium (24.6b; Table 2). The isolate 8.1c showed an elevated production of EPS in mannitol but produced more than fourfold higher levels when grown in xylose-supplemented medium, which were higher than 1.3 g L^{-1}. Carbon source has been described as affecting EPS production in fast-growing rhizobia. Kuçuk and Kivanc [40] evaluated EPS production by *Rhizobium ciceri* grown in culture media with different carbon sources and found that mannitol provided the best production, but that fructose and glucose also provided high EPS production.

The production of EPS by bacteria that differ phenotypically and genotypically has been shown for the genera *Xanthomonas* [10] and *Rhizobium* [41], among others, as well as was found in the present study. The production of EPS by soil bacteria (e.g., *Rhizobium*) involves complex biochemical pathways, interacting with several other primary and secondary metabolic pathways [3]. The production of EPS using different carbon sources must be surely related with the efficiency of carbon use, which requires regulation of the

metabolic pathways that usually compete for the carbon skeleton. Culture medium supplemented with different carbon sources should affect EPS production and its chemical characteristics [16], possibly affecting the ecological role of these polymers and other characteristics of industrial interest, for example, broth viscosity [40].

Coproduction of EPS and PHB requires a regulation between metabolic pathways and cell growth stage. Wang and Yu [42] studied EPS and PHB coproduction in *R. eutropha* and showed that in the early exponential phase, the PHB in the biomass was very high, while the concentration of EPS present in the culture broth was very low. In the late stationary phase, the EPS concentration increased while the initially stored PHB decreased. The production of these two biopolymers was not high in two strains of *Rhizobium* [43]. On the other hand, *A. chroococcum* grown with different carbon and nitrogen sources showed high amounts of both polymers, principally in the presence of sucrose and ammonium nitrate [25]. Our results show that in 8.1c strain, the production of large quantities of EPS did not negatively affect the accumulation of PHB, thereby leading to an increment in PHB accumulation reaching 49% in starch-supplemented medium.

The strain 8.1c showed the potential to be used for the studies in regulation of metabolic pathways of EPS and PHB due to the high production of the both polymers in some cultural conditions. For industrial application of this strain where PHB is desired, the strain 24.6b also should be evaluated in order to improve culture conditions. Further studies must be carried out to evaluate the properties of the PHB and EPS produced by particular bacterial strains.

Conclusions

The pigeonpea root nodule isolates showed large variation in regard to their ability to produce the biolpolymers evaluated. The productivities ranged from zero to 69% of cell biomass for PHB and until 1340% to EPS. Some isolates to the production of PHB or EPS stand out, mainly when inexpensive carbon sources, as starch or xylose, were used. Furthermore, the isolate 8.1c presents the capacity of produce both polymers in high amounts, which suggests a very interesting biochemical regulatory mechanism, which must be investigated.

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