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# Polyhydroxyalkanoates production by *Bacillus thuringiensis* HA1 using sugarcane molasses as carbon source

# Producción de polihidroxialcanoatos por *Bacillus thuringiensis* HA1 usando coproductos de la industria azucarera como fuente de carbono

J.D. Castilla-Marroquín[1], N. Pacheco[2], J.A. Herrera-Corredor[1], F. Hernández-Rosas[1], K. Jiménez-Morales[2], M. J. Benítez-Salamanca[1], R. Hernández-Martínez[3]*

[1]Colegio de Postgraduados Campus Córdoba, Carretera Federal Córdoba-Veracruz Km 348, Congregación Manuel León, Municipio Amatlán de los Reyes, 94946 Veracruz, México.

[2]Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C., Subsede Sureste Parque Científico Tecnológico de Yucatán, Km 5.5, Carretera Sierra Papacal Chuburná Puerto, Mérida 97302, Yucatán, México.

[3]CONAHCYT-Colegio de Postgraduados Campus Córdoba, Carretera Federal Córdoba-Veracruz Km 348, Congregación Manuel León, Municipio Amatlán de los Reyes, 94946 Veracruz, México.

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## Abstract

Polyhydroxyalkanoates (PHAs) are a biodegradable alternative to conventional plastics. However, their adoption has been limited by high production costs. The use of cheap carbon sources, like by-products from the sugar cane industry, could reduce PHAs production costs. In the present work, the potential of *Bacillus thuringiensis* HA1 to produce PHAs using commercial sucrose, molasses, and panela as low-cost carbon sources was explored. Single factor experiments were performed to identify the most favorable conditions for PHAs production. Based on the time, temperature and initial pH results, the fixed conditions to evaluate the carbon sources were 36 hours, 33°C and pH 7.5, the carbon sources were tested at 50 to 350 g/L for PHAs production. PHAs recovery ratio was higher when using molasses at 300 g/L (61.6% of PHAs/dry cell weight, 2.28 mg/mL/3.72 mg/mL respectively), followed by sucrose (61.5% of PHAs/dry cell weight, 0.4 mg/mL/0.6 mg/mL respectively) at 50 g/L and 36.4% of PHAs/dry cell weight at 300 g/L, and finally using panela 25.8% of PHAs/dry cell (1.08 mg/mL/4.40 mg/mL respectively) recovery ratio was observed. After conducting FT-IR characterization, the samples exhibited similarity to the standard DL-$\beta$-hydroxybutyric acid sodium salt.

*Keywords*: biopolymers, polyhydroxyalkanoates, *Bacillus thuringiensis*, submerged fermentation, carbon sources.

## Resumen

Los polihidroxialcanoatos (PHAs) son una alternativa biodegradable a los plásticos convencionales. Sin embargo, su adopción ha sido limitada por los costos de producción. El uso de coproductos de la agroindustria azucarera como fuentes de carbono pueden ser una alternativa para reducir los costos de producción de PHAs. Evaluamos el potencial de *Bacillus thuringiensis* HA1 para producir PHAs utilizando azúcar comercial, melaza y panela como fuentes de carbono de bajo costo. Experimentos de un factor fueron realizados para identificar las condiciones para la producción de PHAs. Siguiendo los resultados de tiempo, temperatura y pH, las condiciones para la evaluación de las fuentes de carbono fueron 36 horas, 33°C y pH 7.5, las fuentes de carbono fueron evaluadas a concentraciones de 50-350 g/L para producir PHAs. La recuperación de PHAs fue mayor usando melaza a 300 g/L (61.6% PHAs/peso seco de biomasa, 2.28 mg/mL/3.72 mg/mL respectivamente), después sacarosa (61.5% PHAs /peso seco de biomasa, 0.4 mg/mL/0.6mg/mL respectivamente) a 50 g/L y 36.4% de PHAs/ peso seco de biomasa a 300 g/L, finalmente panela 25.8% PHAs/ peso seco de biomasa (1.08 mg/mL/4.40 mg/mL respectivamente). La caracterización por FT-IR indico que las muestras presentaron bandas similares a las del estándar ácido DL-3-hidroxibutírico sal sódica.

*Palabras clave*: biopolímeros, polihidroxialcanoatos, *Bacillus thuringiensis*, fermentación sumergida, fuentes de carbono.

*Corresponding author. *E-mail*: odracirhema@gmail.com;
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# 1 Introduction

Polyhydroxyalkanoates (PHAs) are intracellular polyesters produced by various microorganisms as a form of energy and carbon storage (Paula *et al*., 2021). PHAs are considered bioplastics because they are obtained from biological sources and can break down into water and carbon dioxide in the environment (Turco *et al*., 2021).

The PHAs have thermoplastic properties like hardness, stiffness, high crystallinity and flexibility and mechanical properties like the ones present in polypropylene (PP) and polyethylene (PE) such as stretchability, toughness, barrier properties, flexibility, manufacturability, and impact resistance making them attractive substitutes for conventional petrochemical-based plastics (McAdam *et al*., 2020; Narancic *et al*., 2020; Boey *et al*., 2021; Naser *et al*., 2021; Riaz *et al*., 2021; Trakunjae *et al*., 2022). For these properties, PHAs have attracted research interest in fields like material science, catalysis and biorefineries (Riaz *et al*., 2021). They have also been studied for their potential use in the development of biomaterials, like 3D-printing scaffolds and biosynthetic terpolyesters, proving their versatility and potential applications (Panaksri and Tanadchangsaeng, 2021; Mok *et al*., 2021).

Polyhydroxyalkanoates production is, currently, limited by expensive production costs and the suitability of carbon sources (de Paula *et al*., 2021). Nowadays, PHAs production ranges from 2 up to 6.6 USD/Kg, in contrast to less than 1 USD/Kg for conventional plastics (Rodrigues *et al*., 2022; Szacherska *et al*., 2021; Srivastava *et al*., 2023). Approximately 50% of the cost associated with PHAs production is derived from the cost of the raw materials used. Therefore, the availability of affordable and consistent carbon sources is critical to the cost-effective production of PHAs (Khatami *et al*., 2021). Efforts to reduce the cost of PHA production are key to improving its economic viability and competitiveness in the plastics market (Mansoor *et al*., 2022).

Nowadays, efforts are focused on cost-effective production methods and the use of alternative carbon sources, such as organic waste and wastewater, to reduce production costs and improve the economic feasibility of PHAs production (Marciniak and Mozejko-Ciesielska, 2021). In recent years, researchers have focused their attention on the potential use of co-products and by-products from agro-industrial processes like those produced in the sugarcane industry as carbon sources for PHAs production (Sirohi *et al*., 2021). These carbon sources comprise commercial sucrose, molasses, and panela, which are produced in a significant amount which make them a valuable resource for bioplastic production (Gayosso-Sánchez *et al*., 2024; Guimarães *et al*., 2022; Santos *et al*., 2020). On the one hand, molasses is a concentrated viscous sugar-rich byproduct generated after sucrose crystals removal in the sugar production process (Eliodório *et al*., 2023; Jung *et al*., 2023). On the other hand, panela or piloncillo is, according to the FAO, a Non-centrifugal Cane Sugar (NCS) obtained by evaporating the water of the sugarcane juice, usually over open fire (Aguilar-Rivera and Olvera-Vargas 2021; Hernández-Martínez *et al*., 2024). Thus, their use represents an alternative to achieve reductions on production costs and improve the sustainability of PHAs production (Aguilar-Rivera *et al*., 2022; Guimarães *et al*., 2022). The use of these carbon sources has the potential to address the challenges associated with the cost and sustainability of PHAs production, thereby contributing to the development of more environmentally friendly bioplastic materials (Mahato *et al*., 2023). Also, using different carbon sources can contribute to the advancement of sustainable bioplastic production and the utilization of agricultural co-products for value-added applications in the biorefinery sector (de Paula *et al*., 2021). In the literature, bacteria from the genres *Burkholderia* sp., *Pseudomonas* sp., *Halomonas* sp., *Ralstonia* sp., and *Bacillus* sp have been reported as PHAs producers (Khatami *et al*., 2022; Guimarães *et al*., 2022) However, to date, *Bacillus thuringiensis* isolated from the sugar production process has not been reported as PHA's producer.

The objective of this research was to investigate the potential of *Bacillus thuringiensis* HA1 to produce PHAs utilizing commercial sucrose, molasses and panela from the sugarcane industry as carbon source.

# 2 Materials and methods

## 2.1 Microorganism and inoculum

The PHAs production was carried out using *Bacillus thuringiensis* HA1, which belongs to the Laboratory of Applied Microbiology at the Postgraduate College Campus Cordoba. The *Bacillus* was inoculated in inclined tubes containing nutrient agar (23 g/L) and was maintained at 37°C for 24 h. The reactivated *Bacillus* was inoculated in a 250 mL Erlenmeyer flask containing nutrient broth (8g/L) and was maintained at 37°C for 24 h at 150 rpm; the produced biomass was considered as inoculum (Vega-Vidaurri *et al*., 2021).

For conservation, additional Erlenmeyer flasks were prepared as described previously, the biomass produced was washed twice with water and resuspended in 5 mL of water. Finally, 1 mL of the suspension was placed in cryovials containing 30% glycerol and sterile glass beads and kept at -4 °C

until use (Gayosso-Sanchez *et al*., 2024). Preserved *Bacillus thuringiensis* HA1 was reactivated prior to single experiments for PHAs production.

## 2.2 Polyhydroxyalkanoates production

PHAs production by *Bacillus thuringiensis* HA1 was carried out using commercial sucrose as carbon sources in submerged fermentation (SF) in 120-mL bottles of uniform geometry. The kinetics for PHAs production was carried out in a formulated medium based on the most frequently reported components containing (g/L): commercial sucrose (100), yeast extract (5), dipotassium phosphate (1), sodium chloride (5) and magnesium sulfide (0.2). The culture conditions were adjusted at initial pH value of 7.5 in sodium phosphate buffer at 0.1 M, $1x10^6$ CFU/mL, 150 rpm and was maintained at 37°C. Samples were obtained at regular intervals of 0, 12, 24, 36, 48, 60, 72, 84 hours (Bosco *et al*., 2021; Choi *et al*., 2021; Vu *et al*., 2021).

## 2.3 Effect of temperature and pH on polyhydroxyalkanoates production

The effect of temperature on PHAs production by *Bacillus thuringiensis* HA1 in SF was carried out at 33, 35, 37, 39, 41 and 43 °C (±0.5 °C) at pH 7. The effect of pH on PHAs production was carried out by *Bacillus thuringiensis* HA1 in SF, was realized by adjusting the medium at initial pH values of 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5 and 9 (±0.1) (Catherine *et al*., 2022).

## 2.4 Effect of carbon source and carbon source concentration on polyhydroxyalkanoate production

The effect of carbon source was carried out by *Bacillus thuringiensis* HA1 in SF using commercial sucrose, molasses and panela, and the concentration of carbon source was analyzed at 50, 100, 150, 200, 250, 300 and 350 g/L (Shah and Kumar 2021).

## 2.5 Variable analysis for polyhydroxyalkanoates production

One factor at a time (OFAT) method was chosen to evaluate the temperature, pH, carbon source and carbon source concentration to get the maximum PHAs production by *Bacillus thuringiensis* HA1 in SF (Sehgal *et al*., 2023).

## 2.6 Polyhydroxyalkanoates recovery

PHAs were recovered by digestion as described by Kojuri *et al* (2021). The biomass produced in SF was recovered by centrifugation at 7500 x *g* for 15 minutes at 4 °C, and lyophilized. Biomass digestion was carried out by adding 5 mL of sodium hypochlorite at pH 12.9 and maintained at room temperature for one hour, followed by two volumes of water being added and the mixture was maintained at room temperature for 4 hours. The mixture was centrifuged at 7500 x *g* for 15 minutes at 4°C, the pellet was washed with 3 mL of isopropyl alcohol, the mixture was centrifuged at 7500 x *g*, after centrifuging the alcohol is discarded as supernatant and the pellet containing the produced PHAs was recovered and lyophilized. Recovered PHAs were quantified by dry weight. The PHA content was calculated between the total dry weight of recovered PHA and the cell dry weight (CDW). The result was expressed as production ratio (Javaid *et al*., 2020).

## 2.7 Sugar profiles by chromatographic analysis of carbon sources

Sugars were quantified using a high-resolution liquids chromatograph (HPLC) Thermo Finnigan Surveyor. The HPLC system consisted of a Surveyor LC plus, an autosampler and a RI Surveyor Plus detector. The separation was carried out using a 300 x 7.8 mm Phenomenex Rezer RNM-Carbohydrate $Na^{+2}$ column and Milli-Q double distilled water as the mobile phase. The column was maintained at 80 °C with a flow rate of 0.4 mL/min, the detector at 37°C. Prior the injection, the samples were diluted and filtered using PHENEX PTFE acrodisc filters (25 mm, 0.20 $\mu$m pore). The sugars concentrations were determined from calibration curves prepared with sucrose, glucose and fructose (Sigma-Aldrich) from 200 to 1000 ppm. Results are reported in g/L (Wilkowska *et al*., 2014; Gayosso-Sanchez *et al*., 2024).

## 2.8 Determination of total phenolic content on carbon sources

The total phenolic content (TPC) of the carbon sources (commercial sucrose, molasses and panela) was determined following the Folin-Ciocalteu method reported by Jiménez-Morales *et al* (2022) with slight modifications. The results were expressed as mg of galic acid equivalent per g (mg GAE/g). Briefly, 250 $\mu$L of Folin-Ciocalteau 1N reagent was added to 250 $\mu$L of the carbon source sample and shaken. After 8 min, 1250 $\mu$L of 7.5% $Na_2CO_3$ and 480 $\mu$L of distilled water were added and kept for 30 min under darkness. The absorbance was measured at 760 nm using a UV-Vis (Thermo Fisher Scientific Biomate 3S UV-Vis, WI, US) spectrophotometer. The TPC was determined from calibration curves prepared with Galic acid (Sigma-Aldrich, St. Louis, MO, USA).

### *2.9 Polyhydroxyalkanoates characterization by Fourier transform infrared spectroscopy*

The PHAs produced by *Bacillus thuringiensis* HA1 in SF were characterized using Fourier-transform infrared spectroscopy in the attenuated total reflectance (ATR) sampling mode using zinc selenide crystal and a resolution of 4 cm-$^{1}$. The measurement region was in the middle of the infrared spectrum, from 400-4000 cm$^{-1}$, DL-$\beta$-hydroxybutyric acid, poly[(R)-3-hydroxybytric] acid and poly (3-hydroxybytyric acid-co-3-hydroxyvaleric acid) were used as standard for PHAs. The obtained spectra were analyzed using the OriginPro 2024 (OriginLab Corporation, USA) software (Gayosso-Sánchez *et al*., 2024).

## 3 Results

### *3.1 Polyhydroxyalkanoates production*

PHAs accumulation through time was evaluated using commercial sucrose (100 g/L). Figure 1. shows that maximum biomass production occurred after 24 h (0.16 mg/mL) and *Bacillus* growth remained constant until 72 h. The PHAs production started after 12 h and reached their maximum after 36 h (0.12 mg/mL) which represents 77.5% of total weight of biomass. The results obtained in the present work were superior than the reported by Vu *et al* (2021) since they reported a maximum production of PHAs (0.093 mg/mL) in 48 h using volatile fatty acids from food waste as carbon source and *Bacillus megatherium* as inoculum. A similar case of Gayosso-Sánchez *et al.* in 2024 reported the highest production of PHAs (0.0666 mg/mL) after 48 h by *Enterobacter soli* using commercial sucrose as carbon source. While *Bacillus thuringiensis* HA1 was capable to produce PHAs (0.12 mg/mL) after 36 h using commercial sucrose as carbon source. However, the obtained results were lower than the reported by Tohme *et al* (2018) were a maximum PHAs production (48 mg/mL) after 144 h of culture using *Halomonas smyrnensis* as inoculum and glucose as carbon source was achieved. As can be seen in Figure 1. PHAs production dropped at 48 h (0.07 mg/mL) and then increased at 60 and 72 h (0.09 and 0.10 mg/mL respectively) to finally decrease at 84 h (0.09 mg/mL), while biomass remained constant. This may be due to the prolonged fermentation time, leading to the diminution of the carbon source and therefore the consumption of PHAs for bacterial growth, (Brojanigo *et al*., 2020).

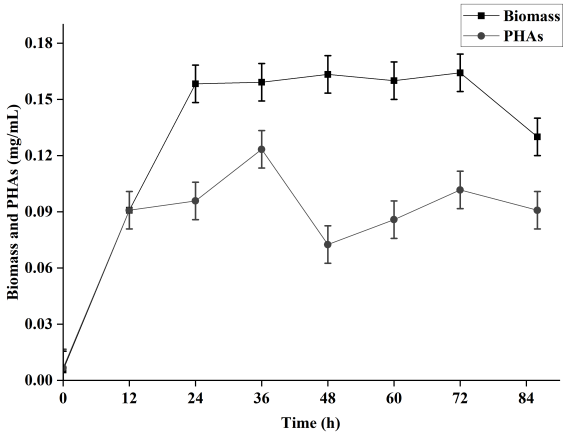


Figure 1. Kinetics of PHA production by *Bacillus thuringiensis* HA1 in submerged fermentation using commercial sucrose as carbon source. (Error bars indicates standard error).

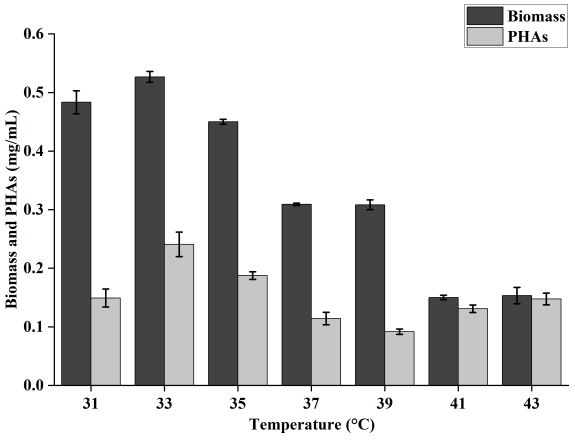


Figure 2. Effect of temperature on PHA production by *Bacillus thuringiensis* HA1. (Error bars indicates standard error).

### *3.2 Temperature effect on polyhydroxyalkanoates production*

The results shown in Figure 2 present the profile of the effect of temperature on biomass and PHAs production by *Bacillus thuringiensis* HA1 in SF indicated that the highest biomass production was achieved at 33 °C with 0.53 mg/mL, then *Bacillus thuringiensis* HA1 biomass decreased to its minimum at 41 and 43 °C with 0.15 mg/mL. The PHAs production reached its maximum at 33°C recovering 0.24 mg/mL being a 45.7% recovery ratio of total biomass, and the lowest PHAs production was observed at 39 °C (0.09 mg/mL). The results obtained in the present work were superior to the reported by Cai *et al* (2021) where they reached a maximum PHAs production of 0.08 mg/mL at 37 °C using *Natrinema altunense* as inoculum and silkworm excrement as carbon source, in the same research a similar result was observed with a maximum PHAs production of 0.23 mg/mL at 37 °C when *Haloarcula hispanica* was used as inoculum.

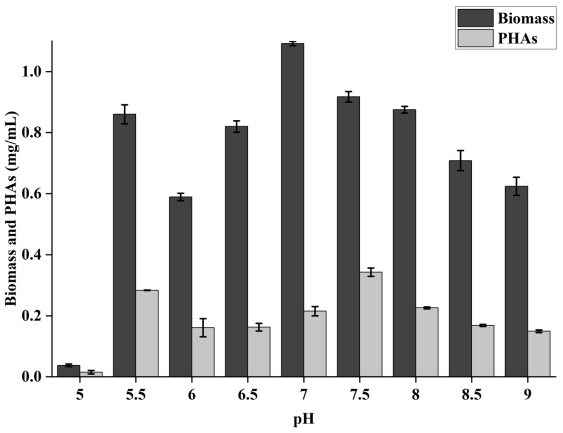


Figure 3. Effect of pH on PHAs production by *Bacillus thuringiensis* HA1 by SF. (Error bars indicates standard error).

Our results were lower than the reported by Bosco *et al* (2021) since they were able to recover 0.42 mg/mL at 30°C using *Leuconostoc mesenteroides* as inoculum and scotta milk whey as carbon source. As can be seen, the proper temperature for PHAs production can ensure higher PHAs accumulation, as was reported by Pérez *et al* (2019), where a fundamental role of the temperature was recorded showing that an increment from 30 to 37 °C increased PHAs production up to 30% regardless of the inoculum.

### *3.3 Effect of pH on polyhydroxyalkanoates production*

The results shown in Figure 3 present the profile of the pH effect on PHAs production by *Bacillus thuringiensis* HA1 in SF. The results showed that the maximum biomass production was achieved at pH 7 (1.092 mg/mL), while the minimum biomass production was observed at pH 5 (0.038 mg/mL). The PHAs production reached its maximum at pH 7.5 (0.34 mg/mL, representing 37.3% of total biomass), while the lowest PHAs production was observed at pH 5 (0.015 mg/mL). On the one hand, the ratio results was higher than those reported by Catherine *et al* (2022) where they reported a maximum PHAs recovery ratio of 20% of total biomass at pH 8.8, while we had a recovery ratio of 37.3 % of PHAs at pH 7.5. On the other hand, when measuring in weight, our results were lower to those reported by Muneer *et al* (2022) where it was shown that the maximum PHAs production of 2 mg/mL was achieved at pH 9 using *Pseudomonas* sp AK3 as inoculum. The different pH reported indicates that PHAs production depends not only on conditions like pH but also on the selected bacterial strain for such purpose (Bosco *et al*., 2021).

### *3.4 Effect of carbon sources and its concentration on PHAs production*

The effect of carbon sources such as commercial sucrose, molasses and panela and their concentration were evaluated for biomass and PHAs production.

Figure 4 shows that molasses as carbon source presented the maximum PHAs production (2.28 mg/mL) at 300 g/L. When using panela as carbon source, the maximum PHAs production (1.08 mg/mL) occurred at 300 g/L. When using sucrose as carbon source two maximum PHAs production (0.40 mg/mL) occurred at 50 g/L and 300 g/L. Furthermore, when using molasses the maximum biomass production (3.86 mg/mL) occurred at 250 g/L. When using panela the highest biomass production (4.18 mg/mL) was observed at 300 g/L. Finally, when using sucrose as carbon source, the maximum biomass production (1.06 mg/mL) occurred at 300 g/L. PHAs recovery ratio was higher when using molasses (61.6% of PHAs/dry cell weight), followed by sucrose (61.5% of PHAs/dry cell weight) at 50 g/L and 36.4% of PHAs/dry cell weight at 300 g/L, and finally using panela 25.8% of PHAs/dry cell weight were recovered. The maximum PHAs productivity was observed when using molasses (0.0633 mg/mL/h), followed by panela (0.03 mg/mL/h) and the lower productivity was observed in sucrose (0.011 mg/mL/h). As can be seen, *Bacillus thuringiensis* HA1 produced PHAs using the three carbon sources at high concentrations (300 g/L) and at 50 g/L using sucrose as carbon source. However, using molasses *Bacillus thuringiensis* HA1 achieved the maximum PHAs production in SF. On the one hand, the results obtained in this work were higher compared to the results obtained by Tohme *et al* (2018) where they produced 0.48 mg/mL of PHA using food-grade sucrose as carbon source and *Halomonas smyrnensis* AAD6 as inoculum. Our results were also higher to the ones reported by Kourmentza *et al* (2018) where a production of 0.6 mg/mL of PHAs was achieved using used kitchen oil as carbon source and *Burkholderia thailandensis* as inoculum. On the other hand, the results obtained in this research were like the reported by Sen *et al* (2019) where they produced 2.86 mg/mL of PHAs using molasses as carbon source and *Cupriavidus necator* as inoculum. However, other researchers have reported higher PHAs productions, for instance, Kingsly *et al* (2022) showed that *Enterobacter cloacae* produced 4.13-4.98 mg/mL of PHAs using molasses as carbon source. Aside from the production levels, *Bacillus thuringiensis* HA1 produced PHAs using the three carbon sources, however, had better adaptability to use molasses as carbon sources for PHAs production. According to Ferrari *et al*. (2022), the physiological behavior of bacteria is influenced by nutrient availability, such as nitrogen, phosphorus, magnesium, and carbon.

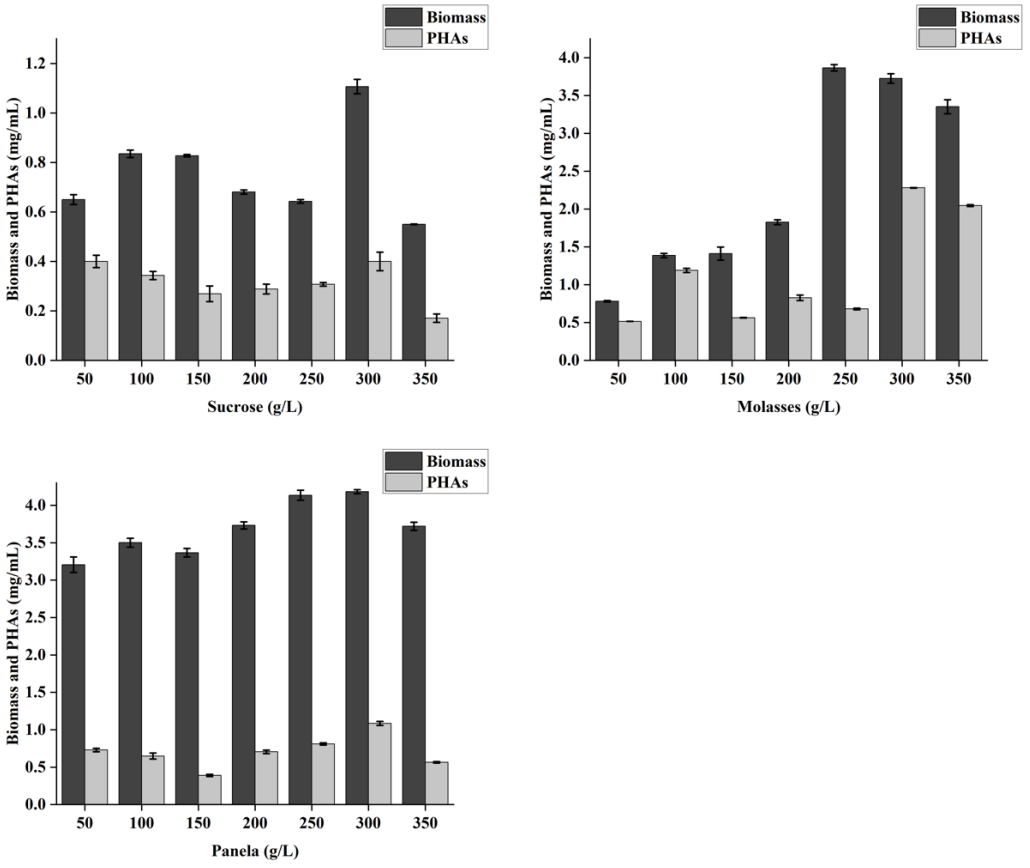


Figure 4. Effect of carbon source and its concentration on PHAs production by *Bacillus thuringiensis* HA1. (Error bars indicates standard error).

This could be the result of *Bacillus thuringiensis* HA1 physiological reaction to the carbon supply. Furthermore, under situations of nutritional limitation, certain bacteria accumulate polyhydroxyalkanoates (PHAs) during their stationary phase, which was observed in this work, while other bacteria accumulate PHAs during the growing phase without nutrient limitation (Bedade *et al*., 2021). Furthermore, some bacteria have been reported as PHAs producers using different carbon sources, for instance *Haloarchea halolamina* spp. produced PHAs using starch as carbon source (Hagagy *et al*., 2022). *Natrinema altunense* produced PHAs using silkworm excrement as carbon source. (Cai *et al*., 2021). *Leuconostoc mesenteroides* produced PHAs using scotta milk whey as carbon source (Bosco *et al*., 2021). At the time of writing, there was not any information about *Bacillus thuringiensis* HA1 as PHAs producer.

### *3.5 Total phenolic contents and sugars profile determination of carbon sources*

Total phenolic content and sugars profile were determined to understand how *Bacillus thuringiensis* HA1 responded to the carbon sources. In the case of commercial sucrose, 94% of the disaccharide was quantified and no phenolics compounds were found in this carbon source. In the case of panela, the composition was 69% sucrose, 33% glucose, 50% fructose, while the phenolic content was 2.4 mg GAE/g. In the case of molasses, the soluble sugars composition showed that 18% was sucrose, 1% was glucose and 6% was fructose, while the phenolic content was 19.5 mg GAE/g. In contrast to the sugar profiles, the best PHAs production was observed in the carbon source where the lower disaccharide content was observed, this may be caused by a stress response from *Bacillus thuringiensis* HA1 when lower metabolizable sugars (monosaccharides) were present in the SF. In the other hand, the production of PHAs using molasses showed the highest outcome, even when there was a high presence of phenolic compounds, which are known for their interactions with bacteria by interfering with essential cellular process like adhesion to surfaces, biofilm formation and metabolic activities (Balada *et al*., 2022). For instance, Pérez-Burillo *et al* (2021) showed that phenolic compounds of green tea presented inhibitory effects in bacteria like *Bacillus cereus*, *Enterobacter coli*, *and Helicobacter pylori*. However, many bacteria can degrade phenol into $CO_2$ and $H_2O$ during metabolic processes,

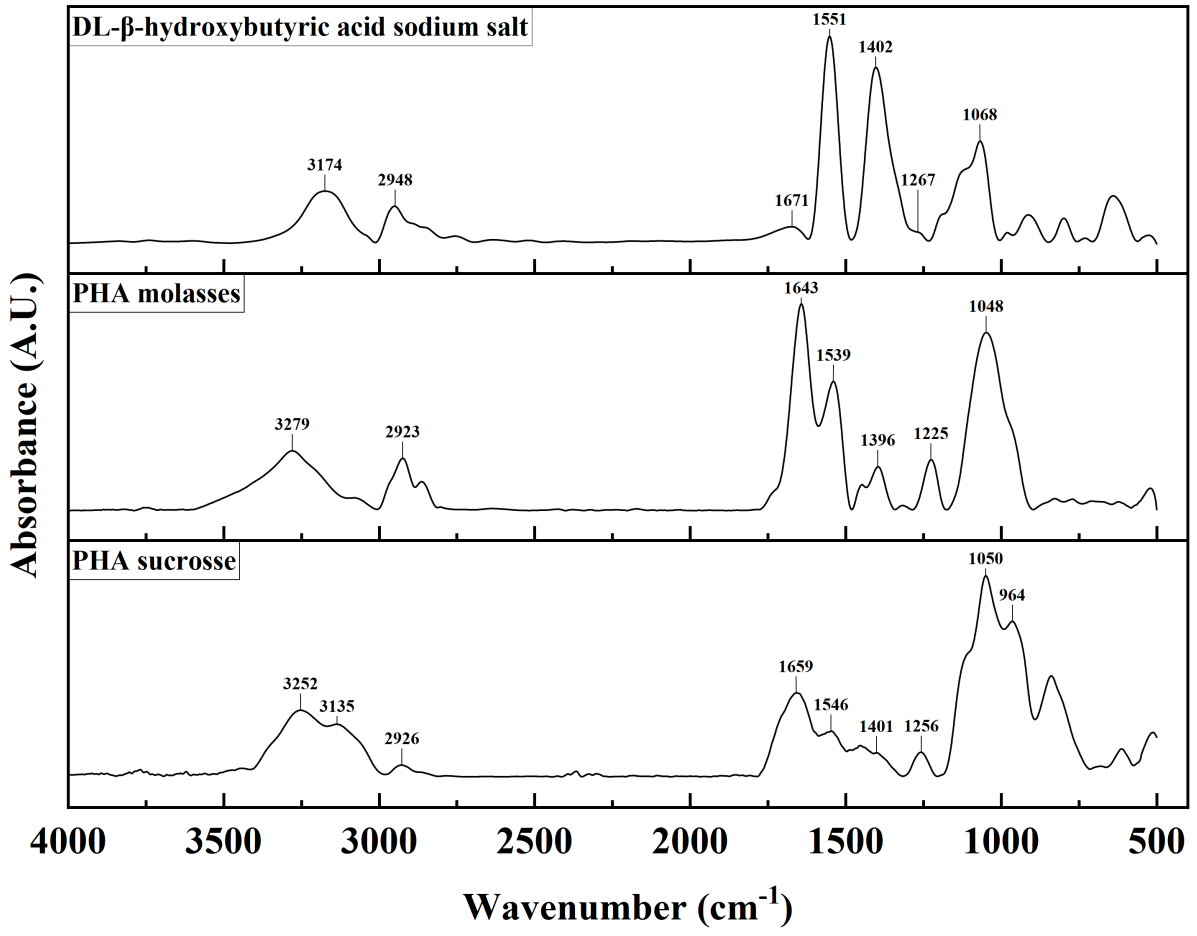


Figure 5. Fourier transform infrared spectra of the polyhydroxyalkanoates produced by *Bacillus thuringiensis* HA1 compared to DL-*β*-hydroxybutyric acid sodium salt as standard reference.

utilizing it as a carbon source for their development (Mahgoub *et al*., 2023). Furthermore, the inhibitory effect of phenolic compounds on bacteria can inhibit the expression of enzymes and the synthesis of crucial metabolic compounds, stressing the cell and potentially promoting the metabolism towards PHAs production (Demir, 2022; Idris *et al*., 2022, Chaiwarit *et al*., 2021).

### *3.6 Polyhydroxyalkanoate characterization by Fourier-transform infrared spectroscopy*

The results of PHA characterization by FT-IR are presented in Figure 5. The spectra obtained from different carbon sources were compared to those of poly (R)-3-hydroxybutyric acid (PHB), poly (3-hydroxybutyric acid-co-3-hydroxyvaleric acid) (PHBV), and DL-*β*-hydroxybutyric acid sodium salt. The most closely related spectrum was observed to be that of DL-*β*-hydroxybutyric acid sodium salt. Characteristic spectra were observed at 3200-2500 $cm^{-1}$ for the O-H functional group of the carboxylic acid, and the hydroxyl groups exhibited distinctive peaks at 3279, 3174, and 2948 $cm^{-1}$. Additionally, peaks were observed at 1659 $cm^{-1}$, which corresponded to the carbonyl stretching vibration. Finally, peaks were observed at 1551, 1402, and 1060 $cm^{-1}$, which corresponded to the stretching vibration of the carboxylate group. The observed spectra were like the ones archived in the National Center for Biotechnology Information (2024) for the DL-*β*-hydroxybutyric acid sodium salt with the distinctive peaks at 3210, 2960, 2940, 1660, 1560, 1408, and 1065 cm. To date, there is not any report of a DL-*β*-hydroxybutyric acid sodium salt produced by *Bacillus thuringiensis*. Other researchers have reported the characterization of other types of PHAs, is important to highlight that those PHAs presented differently bands, since they were a different product and intense peak was observed at 1730-1700 $cm^{-1}$ being a characteristic band of P(3HB) (Trakunjae *et al*., 2022; Munner *et al*., 2022).

## Conclusion

*Bacillus thuringiensis* HA1 efficiently produced PHAs using molasses as carbon source in short-time period, this could be attractive for potential industrial applications in bioplastics production due to the potential on production cost-reduction and efficiency on PHAs, also the production processes may be improved. At date, there is no report of DL-$\beta$-hydroxybutyric acid sodium production by any sugarcane native *Bacillus thuringiensis*.

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