

Co-production of polyhydroxyalkanoates and levan by *Halomonas smyrnensis* AAD6^T

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Abstract

The simultaneous production of microbial polymers levan and poly[3-hydroxybutyrate] (PHB), a type of polyhydroxyalkanoates, was investigated in this work. The study involved the fermentation of sucrose and molasses by *H. smyrnensis* AAD6^T (BAE2 strain) to produce PHB (intracellular) and levan (extracellular). Both polymers were isolated and characterized by FTIR. Levan was also characterized by thin-layer chromatography (TLC) and viscosimetric analysis. The amount of biomass was 25 g until the end of fermentation. The PHB rate was 0.015 g in both media and the average PHB productivity was 6.0×10^{-4} g PHB/g biomass. The highest rate of levan was 9 g/L in the range of 72–80 h, in the molasses-based medium. The FTIR spectra showed specific signals for each of the polymers, such as the peak at 1700 for the carbonyl group of esters for the PHB and signals at 900 and 800, which are typical signals for levan fructose rings. Furthermore, acid hydrolysis of levan revealed that it was formed only by fructose, as confirmed by TLC. With this study, *H. smyrnensis* AAD6^T BAE2 co-produced PHB and levan using a low-cost carbon source, showing great potential in reducing biopolymer manufacturing costs.

Keywords: Halófilas; *Halomonas smyrnensis*; Polyhydroxyalkanoates; Levan; Co-production.

Resumo

A produção simultânea dos polímeros microbianos levana e poli[3-hidroxibutirato] (PHB), um tipo de polihidroxialcanoatos, foi investigada neste trabalho. O estudo envolveu a fermentação de sacarose e melação por *H. smyrnensis* AAD6^T (cepa BAE2) para produzir PHB (intracelular) e levana (extracelular). Ambos os polímeros foram isolados e caracterizados por FTIR. Levana também foi caracterizada por cromatografia em camada delgada (CCD) e análise viscosimétrica. A quantidade de biomassa foi de 25 g até o final da fermentação. A taxa de PHB foi de 0,015 g em ambos os meios e a produtividade média de PHB foi de $6,0 \times 10^{-4}$ g de PHA/g de biomassa. A maior taxa de levana foi de 9 g/L no intervalo de 72–80 h, no meio à base de melação. Os espectros de FTIR mostraram sinais específicos para cada um dos polímeros, como o pico em 1700 para o grupo carbonil de ésteres para o PHB e sinais em 900 e 800, que são sinais típicos de anéis de frutose de levana. Além disso, a hidrólise ácida de levana revelou que esta era formada apenas por frutose, como foi confirmada por CCD. Com este estudo, *H. smyrnensis* AAD6^T BAE2 coproduziu PHB e levana usando uma fonte de carbono de baixo custo, mostrando ter um grande potencial na redução de custos de fabricação de biopolímeros.

Palavras-chave: Halófilas; *Halomonas smyrnensis*; Polihidroxialcanoatos; Levana; Coprodução.

Resumen

En este trabajo se investigó la producción simultánea de polímeros microbianos levan y poli[3-hidroxibutirato] (PHB), un tipo de polihidroxialcanoatos. El estudio involucró la fermentación de melaza de sacarosa por *H. smyrnensis* AAD6^T (cepa BAE2) para producir PHB (intracelular) y leván (extracelular). Ambos polímeros fueron aislados y caracterizados por FTIR. Levana también se caracterizó mediante cromatografía en capa fina (CCD) y análisis viscosimétrico. La cantidad de biomasa fue de 25 g hasta el final de la fermentación. La tasa de PHB fue de 0,015 g en ambos medios y la productividad promedio de PHB fue de $6,0 \times 10^{-4}$ g de PHA/g de biomasa. La tasa más altas de leván fue de 9 g/L en el rango de 72 a 80 h, en el medio a base de melaza. Los espectros FTIR mostraron señales específicas para cada uno de los polímeros, como el pico a 1700 para el grupo carbonilo de ésteres para el PHB y señales a 900 y 800, que son señales típicas para los anillos de fructosa de levano. Además, la hidrólisis ácida del leván reveló que estaba formado únicamente por fructosa, como lo confirmó la CCD. Con este estudio, *H. smyrnensis*

AAD6^T BAE2 coprodujo PHB y levan utilizando una fuente de carbono de bajo costo, mostrando un gran potencial para reducir los costos de fabricación de biopolímeros.

Palabras clave: Halófilos; *Halomonas smyrnensis*; Polihidroxicanoatos; Levana; Coproducción.

1. Introduction

Synthetic plastics have become an integral part of contemporary society and are present in almost all utensils and products used in everyday life. They were designed with high-performance and quality materials and, therefore, have a very long service life (Alves et al., 2022). However, the non-biodegradable nature of these plastics favors their accumulation in difficult-to-handle amounts, generating serious environmental problems (Tohme et al., 2018; Castro et al., 2022).

Conventional plastics have low rates of degradation in the environment, with a half-life of more than 500 years (Samui & Kanai, 2019). Therefore, the expansion of production of these plastics favors their accumulation mainly in sanitary landfills. In 2020, the production of conventional plastics was approximately 367 million tons (PlasticsEurope, 2021). The waste generated by these plastics represents about 8% by weight and 25% by volume of total municipal solid waste (Samui & Kanai, 2019). In this sense, there is a permanent need to replace conventional plastics with biodegradable plastics (Albuquerque & Malafaia, 2018; Tohme et al., 2018; Alves et al., 2022).

Currently, bio-based polymers have been considered one of the main alternatives to petrochemical plastics (Samui and Kanai, 2019; Alves et al., 2022). Among the biopolymers, polyhydroxyalkanoates (PHAs) have been gaining importance among researchers due to their non-toxic, biodegradable, and biocompatible nature (Alves et al., 2022). They are classified into three different groups according to their monomeric units; short, medium, and long-chain-length PHAs. Most PHAs are short chain, like poly(3-hydroxybutyrate), or PHB. The PHAs have recognized applications in the food, pharmaceutical, and biomedical sectors. Although PHAs are considered an alternative to petroleum-based polymers, the high cost of production limits their availability on a commercial scale (Amaro et al., 2019; Alves et al., 2022).

On the other hand, levan is a fructan (a fructose homopolysaccharide) with great biotechnological potential. It is produced in a medium containing sucrose under the action of the enzyme levansucrase (EC 2.4.1.10), an enzyme present in several microbial species, mainly bacteria (Öner, Hernández & Combie, 2016). Due to its multifunctional characteristics, such as low viscosity, thermal stability, and water solubility, levan offers a variety of applications in the cosmetics, food, and pharmaceutical sectors (Siqueira et al., 2020). In addition, levan is used as a blood plasma extender, hypocholesterolemic agent, antitumor agent, emulsifier, stabilizer, thickener, and encapsulating agent in a controlled drug delivery system (Öner, Hernández & Combie, 2016; Lima et al., 2020; Siqueira et al., 2020).

Recently, the integrated biosynthesis of microbial polymers has been gaining importance as an interesting strategy to reduce the total costs of its production. Tohme et al. (2018) studied the co-production of levan and PHB by *Halomonas smyrnensis* AAD6^T using 50 g/L of sucrose and obtained 0.48 g/L of PHB and 15.3 g/L of levan. Erkorkmaz et al. (2022), working with the same strain and the same sucrose concentration, obtained 3.15 g/L of PHB and 17.15 g/L of levan. Finally, Vega-Vidaurre et al. (2022) isolated two bacterial strains coproducing PHB and levan. The strains were identified as *Bacillus thuringiensis* HA1 and *Suhomyces kilbournensis* HD1. After 72 h of fermentation, *B. thuringiensis* HA1 produced 0.058 g/L of PHA and 16.5 mg/L of levan, while *S. kilbournensis* HD1 synthesized 0.148 g/L and 86 mg/L of PHA and levan, respectively.

The integrated approach is an interesting strategy to make microbial cells efficient biofactories, accumulating PHA inside the cell while releasing levan to the outside (Tohme et al., 2018). So far, this strategy has been investigated using only analytical standard substrates as carbon sources (Tohme et al., 2018; Erkorkmaz et al., 2022; Vega-Vidaurre et al., 2022). In this sense, the present study was designed to determine the viability of sugarcane molasses as an alternative substrate in the co-production of PHA and levan.

2. Methodology

2.1 Microorganism and reagents

The bacterium *H. smyrnensis* AAD6^T BAE2 isolated from Çamalti Saltern area (Aegean Region of Turkey) and was kindly provided by the Research Group in Industrial Biotechnology and Systems Biology (IBSB) at Marmara University, Istanbul. *H. smyrnensis* AAD6^T BAE2 whole genome shotgun sequence was taken from NCBI with GenBank accession numbers AJKS02000001 to AJKS02000034 (Tohme et al., 2018). The molasses used in the production of PHA and levan was supplied by the Pernambuco firm Empermel. The other reagents and inputs used for the culture media had purity and analytical grade. All reagents and supplies will be described along with the experimental procedures.

2.2 Reactivation of the microorganism and preparation of the pre-inoculum

The *H. smyrnensis* AAD6^T BAE2 was provided in sterile filter paper wrapped in aluminum foil and this, in turn, was deposited in a paper envelope. After receiving it, the filter paper smear was reactivated in DSMZ 1158 medium, composed by 5.0 g/L yeast extract, 3.0 g/L MgSO₄·7H₂O, 2.0 g/L KCl, 100 g/L NaCl and 3.0 g/L trisodium citrate, with pH adjusted to 6.9. The minimum concentration of NaCl used should be 5%. The medium can also be solidified by adding 15.0 g/L of agar (Kirtel et al., 2019). The bacteria were inoculated in 50 mL of DSMZ 1158 medium and cultured in Erlenmeyer flask (250 mL) at 37 °C, 180 rpm for 24 h and 1% of this pre-culture at a volume fraction of 1% was used as inoculum.

2.3 Inoculum preparation and cultivation conditions

The optimal medium (OM) used for *H. smyrnensis* AAD6^T BAE2 cultures were established by Tohme et al. (2018) and consisted of a semi-synthetic medium, containing trace elements (1 mL/L), 5 g/L peptone, 137.2 g/L NaCl, sucrose (2%) and molasses (2 °Brix) as a carbon source. The shake flask cultures were incubated at 37 °C, pH 7.0, 180 rpm for 168 h and 1% of inoculum. All materials and culture media were sterilized at 121 °C for 20 min.

2.4 Extraction of PHA and levan

Initially, the biomass was isolated by centrifugation at 8000 g at 4 °C for 20 min. The extraction of PHAs from the interior of the cells of *H. smyrnensis* AAD6^T BAE2 was performed according to Albuquerque et al. (2018). (2013), using combined chloroform and sodium hypochlorite (2:1 v/v), followed by evaporation of the solvent in an oven at 40 °C for 24 h. PHA productivity was calculated by gravimetric method for the biopolymer obtained after extraction. The accumulation percentage was determined by the relationship between the amount of PHA produced and the amount of biomass (Albuquerque et al., 2018).

For the isolation of levan, the fermented broth was centrifuged (18500 g, 20 min at 4°C) to remove cells. Then, heated in a water bath (100 °C, 10 min) – for enzyme inactivation, and cooled. The polymer was recovered by precipitation in ice-cold ethanol (70%). The generated pellet was resuspended three times in distilled water and converted to powder by lyophilization (Shih et al., 2010; Siqueira et al., 2017).

2.5 Characterization of PHA and levan

The PHA and levan were characterized by Fourier Transform Infrared spectroscopy (FTIR) using a VERTEX 70 instrument (Bruker Optics, USA) under dry air and room temperature (25 °C). The spectra FTIR were obtained in the wavelength range of 4000-400 cm⁻¹ resolution. The levan viscosity was analyzed in a Stabinger Viscometer SVM 3000/G2 equipment (Anton Paar, Graz/Austria), with viscosity measurements in a range between 0.2–20000 mPa·s for dynamic viscosity with automatic temperature control (Djuric et al., 2017).

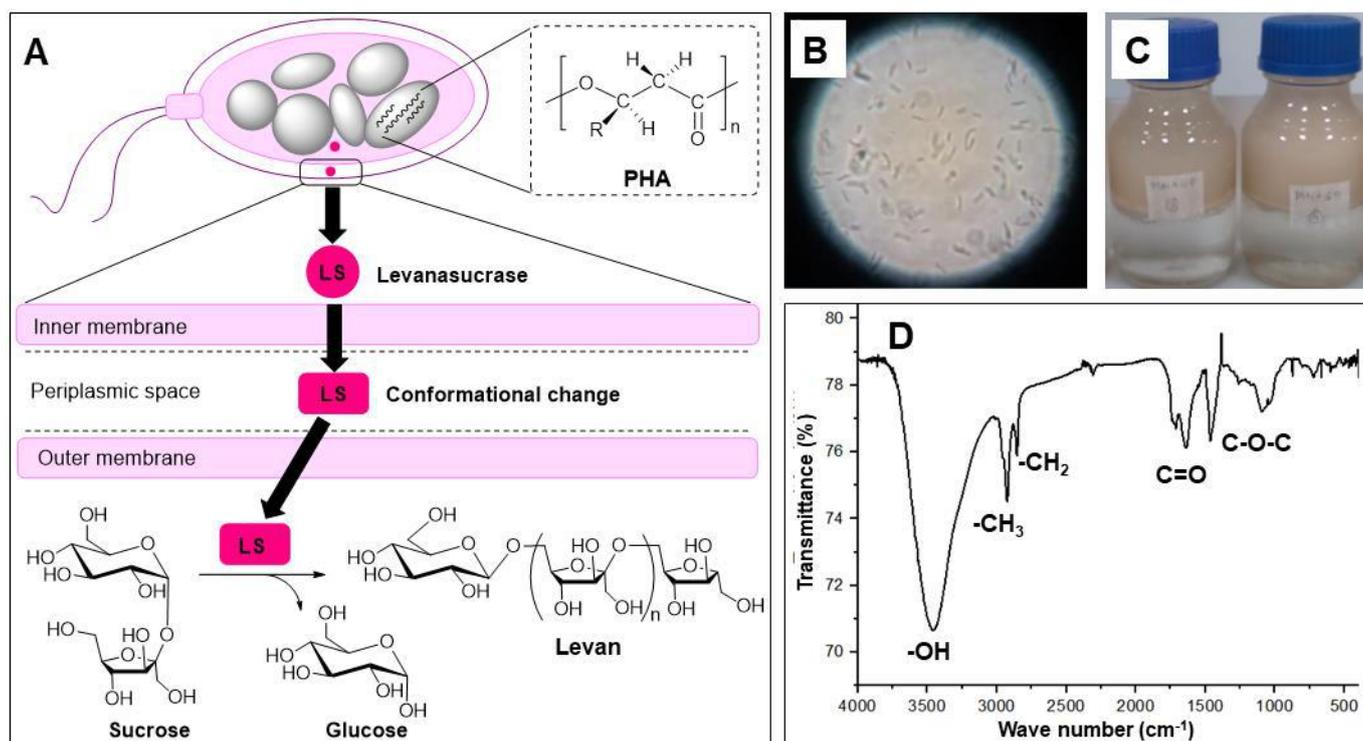
The levan polymer was characterized by TLC and viscosimetric analysis. Characterization by TLC was performed according to Dahech et al. (2013). Briefly, aqueous solutions of levan (1%) were mixed with solutions of oxalic acid (0.5%, 2 mL), heated to 100°C, and neutralized. The elution system consisted of a mixture of chloroform/acetic acid/water (6:7:1, v/v/v) and the developer solution consisted of a solution of sulfuric acid (5%, v/v) in methanol (Dahech et al., 2013).

3. Results

3.1 Analysis of PHA production by *H. smyrnensis* AAD6^T BAE2

The *H. smyrnensis* AAD6^T BAE2 strain is a mutant with a high capacity to produce PHB inside the cell, as well as levan in the extracellular medium (Tohme et al., 2018), (Figure 1).

Figure 1 - Isolation and characterization of PHA produced by *H. smyrnensis* AAD6^T BAE2 strain. In A, an illustration of the *H. smyrnensis* AAD6^T BAE2 strain cell showing the PHA storage granules inside. In addition, it is possible to observe the activation of the enzyme levansucrase in the periplasmic space and its hydrolytic action on sucrose to produce levan in the extracellular medium. In B, light microscopy image of *H. smyrnensis* AAD6^T BAE2 strain. In C, extraction of PHA from the interior of cells with chloroform in basic medium. In D, the characteristic infrared spectrum of PHA (PHB type).



Source: Authors.

The total biomass of *H. smyrnensis* AAD6^T BAE2 was produced approximately 25 g after 168 h in both sucrose-based (2 %) and molasses-containing (2 °Brix) media. The maximum rate of PHA was about 0.015 g in both media. The average PHA productivity was 6.0×10^{-4} g PHA/g biomass.

3.2 Characterization of PHA

The Figure 1.B showed the image of *H. smyrnensis* AAD6^T BAE2 strain seen only by light microscopy. However, the presence of PHA is usually identified using specific dyes such as sudan black B in high-resolution images (Albuquerque et al., 2018), such as those by transmission electron microscopy (TEM) analysis.

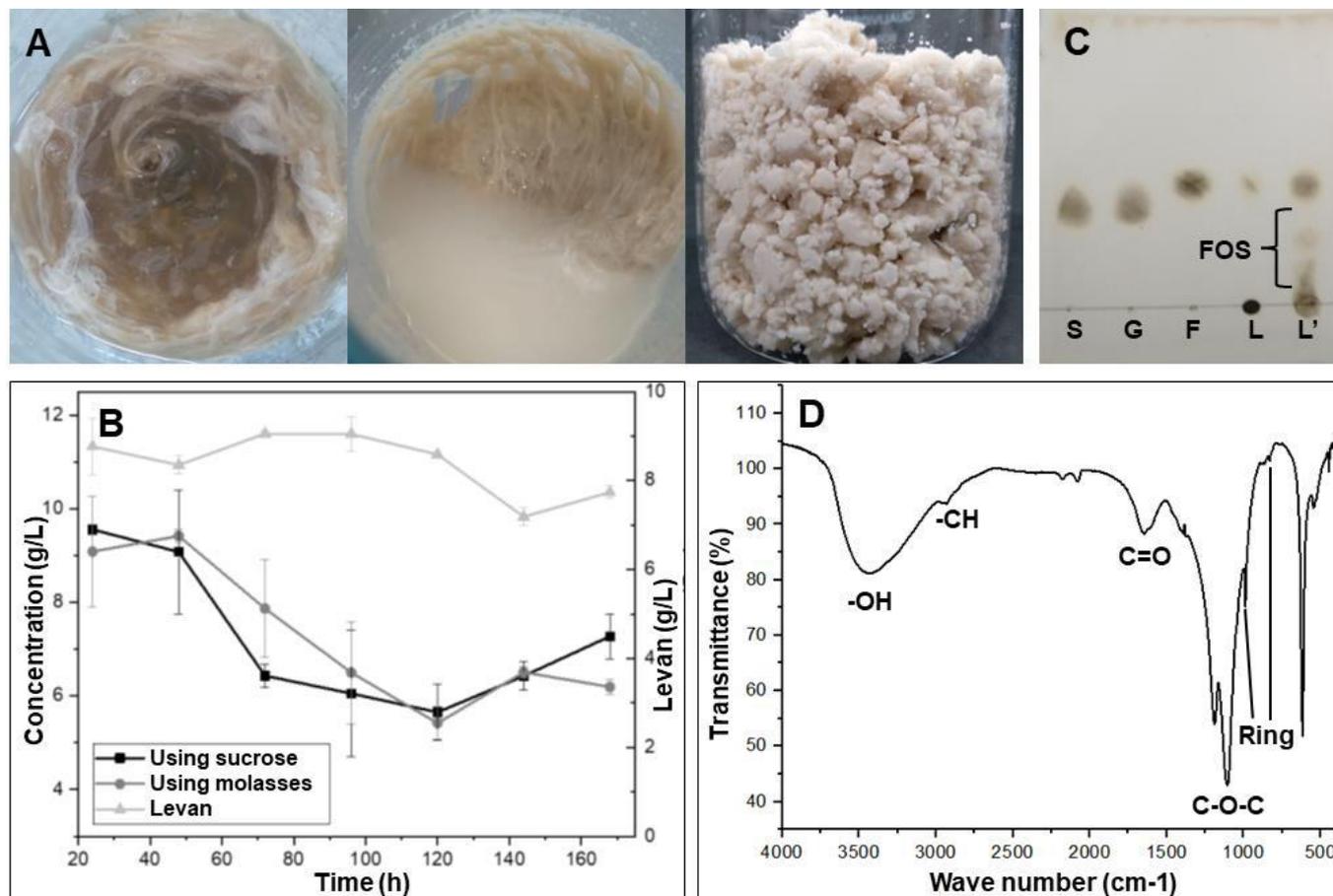
Before its characterization, PHA was extracted with chloroform in a basic medium, forming three distinct phases (Figure 1. C). The lower phase is formed by PHA soluble in chloroform; the intermediate phase (thinner phase) is formed by biomass residues and; the upper phase, is formed by by-products and water-soluble salts. By decanting, the PHA phase in chloroform is isolated, and, by evaporation at room temperature, the PHA is precipitated (Albuquerque et al., 2018).

The PHAs recovered by the digestion method was used for FTIR analyses. PHA analysis by FTIR spectroscopy showed characteristic peaks and signals for PHB (Figure 1.D). There was a strong band between 3460 cm⁻¹, attributed to the -OH stretching vibration. Two signals in the region between 2922 and 2854 cm⁻¹, were attributed to the stretching vibration of -CH₃ and -CH₂, respectively. A signal at 1714 cm⁻¹, corresponding to the C=O stretching group, denoting the presence of a typical carbonyl ester of polyhydroxybutyrate (PHB). Another important signal was found at 1057 cm⁻¹, attributed to C-O-C stretching vibrations, corresponding to the ester bond found in the PHB molecule (Albuquerque et al., 2018; Vega-Vidaurre et al., 2022).

3.3 Evaluation of levan synthesis by *H. smyrnensis* AAD6^T BAE2

Levan was synthesized by *H. smyrnensis* AAD6^T BAE2 in an orbital shaking incubator under controlled conditions of 37 °C, pH 7.0, and 180 rpm, for 168h. The identification of levan was confirmed by observation with the naked eye from the gradual increase in the viscosity of the fermented broth (Shih et al., 2010; Shih et al., 2011). In addition, when the fermented broth was mixed with 70% cold ethanol a fibrous and compact network was observed (Djuric et al., 2017), which is a characteristic associated with this fructan (Figure 2.A).

Figure 2 - Isolation and characterization of levan synthesized by *H. smyrnensis* AAD6^T. In A, sequence of levan extraction using ethanol as a precipitating agent. In B, substrate degradation curves (sucrose and molasses) and levan formation. In C, TLC image of the acid hydrolysis of levan at 1- and 5-min times (where S: sucrose; G: glucose; F: fructose; FOS: fructooligosaccharides; L: levan hydrolyzed for 1 min and L': levan hydrolyzed for 5 min). In D, the characteristic infrared spectrum of levan.



Source: Authors.

As shown in Figure 2. B, there was marked microbial growth in the first 48 h for both 2% sucrose-based and molasses-containing (2 °Brix) media. From this interval, the biomass concentration decreased over time until 120 h of cultivation. The same figure shows the levan production curve, showing an inverse behavior, that is, while the substrates were consumed, there was an increase in the concentration of the biopolymer (Shih et al., 2010; Shih et al., 2011).

In both media (sucrose and molasses) *H. smyrnensis* AAD6^T BAE2 produced only levan at optimal concentrations (Figure 2. B). PHA was not obtained in sufficient concentration to generate each point of the curve, but its concentration at the end of the process was sufficient to characterize it (Albuquerque et al., 2018). The behavior of the biomass was similar for both the sucrose-containing medium and the molasses-based medium (curve not shown). The levan production curve fluctuated somewhat but peaked in the 72–80 h range, where the maximum levan production was approximately 9 g/L (accumulative total: 55 g). These oscillations may be the result of the activation of the levansucrase enzyme, which acts outside the bacterial cell. The levan production curve fluctuated a little, but reached or peaked in the 72-80 h phase, where the maximum levan production was approximately 9 g/L (cumulative total: 55 g). These fluctuations could have been generated by changing the agitation speed, which was altered from 180 to 250 rpm to compensate for bacterial oxygen demand, as verified by Shih et al. (2005).

3.4 Characterization of levan

Crude levan was obtained from the fermented broth, purified and a sample of the white powder was used for further analysis according to Shih et al. (2010). The sugar composition of levan was preliminarily determined by TLC analysis (Figure 2.C). After the acid hydrolysis of levan, the retention time of the acid-hydrolyzed levan sample was the same as the monosaccharide fructose in the TLC analysis (Dahech et al., 2013). Figure 2.C shows a TLC of the partial hydrolysis of levan, where it is possible to obtain intermediates of 3 or 4 fructose units, such as fructooligosaccharides (FOS). Total hydrolysis of levan (not shown) released only fructose as the final product, confirmed by the retention factor value (Rf, the ratio of the distance moved by the compound as compared to the distance moved by the solvent) of acid-hydrolyzed levan which was identical to that of fructose under the same level of ascending solvent condition (Shih et al., 2010; Shih et al., 2010; Shih et al., 2011; Dahech et al., 2013).

The levan FTIR spectrum showed a strong band at 3437 cm^{-1} , which was attributed to the hydroxyl stretching vibration of polyalcohols (Figure 2.D). The bands at 2924 and 2828 cm^{-1} were due to stretching and bending vibrations of $-\text{CH}_2$, respectively. The broadband at 1634 cm^{-1} was attributed to bound water (Dahech et al., 2013). Strong absorption was observed at 1014 cm^{-1} , corresponding to stretching vibrations of the glycosidic bond (C-O-C). Finally, two typical signals were observed at 989 cm^{-1} and 829 cm^{-1} , indicating the presence of the furanoid ring of the levan sugar units (Jathore et al., 2012; Dahech et al., 2013).

Levan solutions produced by *H. smyrnensis* AAD6^T BAE2 (LHS) were characterized as to their viscosity (dynamic viscosity, kinematic viscosity and specific mass) at increasing concentrations (0.05, 0.1, 0.5 and 1.0%, w/v) (Table 1).

Table 1 - Viscosimetric analysis of levan solutions at different concentrations.

Sample	Dynamic Viscosity (mPa.s)	Kinematic viscosity (mm ² /s)	Specific mass (g/cm ³)
LHS - 0,05%	1.0711 ± 0.0033	1.0702 ± 0.0033	0.9999 ± 0.0000
LHS - 0,1%	1.1213 ± 0.0535	1.1215 ± 0.0535	1.0008 ± 0.0000
LHS - 0,5%	1.2260 ± 0.0030	1.2224 ± 0.0030	1.0029 ± 0.0000
LHS - 1,0%	1.4657 ± 0.0043	1.4582 ± 0.0041	1.0052 ± 0.0000

Source: Authors.

The viscosimetric analysis of levan at the different concentrations (Table 1) showed a linear increase in dynamic and kinematic viscosities. In solutions, less than 0.5%, an increase in levan concentrations generates less linear variation, with a less significant slope (Stojković et al., 2015).

The *H. smyrnensis* AAD6^T BAE2 is a strain that combines advantages such as osmoadaptation and halophilicity, allowing the production of biopolymers under non-sterile conditions and under high salinity (Kirtel et al., 2015; Tohme et al., 2018). In this study, the first assays were performed for the co-production of levan and PHA and a copolymer PHB *H. smyrnensis* AAD6^T BAE2 using alternative substrates. The objective of this work was to find the ideal conditions for maximum PHA production in molasses, with levan as a secondary product.

4. Discussion

PHAs are produced by several groups of bacteria, including *Cupriavidus necator* (formerly called *Ralstonia eutropha*), *Alcaligenes eutrophus*, *Protomonas extorquens*, and *Pseudomonas oleovorans* (Albuquerque & Malafaia, 2018; Alves et al., 2022). However, the high total costs for PHA production mainly fall on downstream and upstream processing costs, as well as the use of new and more efficient microorganisms (Tohme et al., 2018; Alves et al., 2022).

In turn, microbial levan is a fructan that has gained considerable scientific and industrial interest recently due to its distinctive properties, such as high solubility in oil and water, strong adhesiveness, good biocompatibility, and film-forming ability (Öner, Hernández & Combie, 2016; Lima et al., 2020; Siqueira et al., 2020). It is composed of fructose residues linked by β -(2,6) fructofuranosidic bonds and is produced from sucrose-based substrates by a variety of bacteria, including the genera *Acetobacter*, *Bacillus*, *Erwinia*, *Gluconobacter*, *Halomonas*, *Microbacterium*, *Pseudomonas*, *Streptococcus* and *Zymomonas* (Öner, Hernández & Combie, 2016).

The co-production of levan and PHB by the *H. smyrnensis* AAD6^T BAE2 strain was first reported by Tohme et al. (2018). In this study, the authors obtained a rate of 0.48 g/L of PHB (26.9%) and 15.3 g/L of levan, in a sucrose-based medium. When glucose was used as a substrate, the rate of PHB was increased almost threefold (1.34 g/L, 45.8%). However, in this same substrate, the production of levan was not reported. According to the authors, this occurred because, during levan biosynthesis, the enzyme levansucrase hydrolyzes sucrose, generating glucose and fructose (Figure 1. A). While fructose is used in the polymerization of levan chains, glucose is used as a source of carbon and energy, with excess glucose remaining in the system. When the rate of glucose accumulation exceeds the rate of utilization, excess glucose negatively influences levansucrase activity and may cause an overall inhibitory effect on levan synthesis (Tohme et al., 2018).

Although the accumulation of glucose is considered a bottleneck in the production of levan, its presence is essential for the production of PHB, the most common type of PHA. PHB biosynthesis begins when glucose is converted to pyruvate via glycolysis. Subsequently, pyruvate is used to form acetyl-CoA. PHB is synthesized from acetyl-CoA through butanoate metabolism. First, acetoacetyl-CoA is formed from acetyl-CoA by 3-ketothiolase (phaA). The product is then reduced by acetoacetyl-CoA reductase (phaB), generating (R)-3-hydroxybutanoyl-CoA. Finally, PHA synthase (phaC) catalyzes the formation of PHB (Tohme et al., 2018; Amaro et al., 2019; Alves et al., 2022).

The FTIR spectroscopic analysis of the PHA sample produced by *H. smyrnensis* AAD6^T BAE2 (Figure 1.D) showed intense characteristic absorption bands for PHB, including C=O typical of esters in 1700 cm⁻¹. The results obtained in this study are in agreement with others reported on FTIR analysis for PHB (Tohme et al., 2018; Vega-Vidaurre et al., 2022). In turn, FTIR characterization of levan showed signs of -OH vibration, C-H vibrational stretching, glycosidic bond elongations (C-O-C), and typical signs of furanosidic rings of sugar units (Dahech et al., 2013). All absorption signals were identical to other levans described in the literature (Jathore et al., 2012; Dahech et al., 2013; Xu et al., 2016).

The acid hydrolysis of levan released as sugar residues only fructose units, which was confirmed by the same retention factor (Rf) in the TLC. The partial hydrolysis of levan assays (Figure 1.C) generated fructose monomers and fructooligosaccharide fragments, as has been described in the literature (Liu et al., 2010). Generally, hydrolysis of levan with organic acids ensures the greater formation of fructose units. In turn, hydrolysis of levan using inorganic acids produces a greater amount of FOS and low molecular weight levan (Dahech et al., 2013; Runyon et al., 2014; Djuric et al., 2017). Different hydrolysis patterns can also result from the molecular weight and degree of branching of levan (Djuric et al., 2017).

One of the most common properties of polysaccharides is their ability to form highly viscous aqueous solutions, even at low concentrations (Stojković et al., 2015). Compared to polysaccharides of similar molecular weight, levan has low intrinsic viscosity ($[\eta]$). The low ($[\eta]$ = 0.07–0.18 dL/g) of levan is attributed to its compact and spherical molecular conformation, where the side branches contribute to this behavior (Arvidson et al., 2006; Kang et al., 2009; Benigar et al., 2015). In this work, levan solutions in the range of 0.05–1.0% showed a linear increase in dynamic and kinematic viscosities. Viscosity is affected by the attractive and repulsive interactions of the polysaccharide chains (Timilsena et al., 2015). Therefore, the linear increase observed in this study may be related to the fact that as the polymer concentration was increased, the interactions between chains prevailed over the repulsive forces, becoming dominant at higher concentration values. This behavior has been reported in uncharged polymers, such as levan (Torres et al., 2015).

5. Conclusion

The integrative approach for the simultaneous biosynthesis of high-value-added microbial products from low-cost substrates represents an important strategy for reducing total production costs. In this work, different compartments of the bacterium *H. smyrnensis* AAD6^T BAE2 were explored for the simultaneous production of PHA (intracellular) and levan (extracellular) using sucrose and molasses as substrates. The biopolymers were characterized by FTIR spectroscopy. Overall, this work reinforces the concept of biorefinery from a holistic zero-waste perspective, so discussed nowadays by the circular economy. These results indicate that *H. smyrnensis* AAD6^T BAE2 strain can be considered a potential cell factory for the dual production of levan and PHA and copolymers as PHB.

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