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Preparation of Hydrolyzed Sugarcane Molasses as a Low-Cost Medium for the Mass Production of Probiotic *Lactobacillus paracasei* ssp. *paracasei* F19

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Abstract: In this study, sugarcane molasses (SCM) was pre-treated in a low-cost fermentation medium to produce probiotic biomass of *Lactobacillus paracasei* ssp. *paracasei* F19 (LPPF19) with the combination of dilution, centrifugation, and acid hydrolysis (5 molar sulfuric acid, 60 °C/2 h). Microtox analysis, inductively coupled mass spectrometry (ICP-MS), and high-performance liquid chromatography (HPLC) were used to measure the effects of SCM pretreatment on the fermentation process. The results showed that the hydrolysis of sucrose into glucose and fructose was 98%, which represented an increase of 44.4% in the initial glucose content (fermentation-limiting sugar), and harmful heavy metals, such as arsenic, cadmium, and lead, were reduced by 50.3, 60.0, and 64.3%, respectively. After pretreatment, with the supplementation of only yeast extract and salts (Na, K, Mg, and Mn), a biomass of 9.58 log CFU/mL was achieved, approximately ten times higher than that for the control medium used (MRS/DeMan, Rogosa, and Sharpe). The cost reduction achieved compared to this commercial medium was 68.7% in the laboratory and 78.9% on an industrial scale. This work demonstrated that SCM could be used in a cheaper and more effective alternative fermentation to produce LPPF19.

Keywords: low-cost medium; probiotic; *Lactobacillus paracasei* ssp. *paracasei* F19; hydrolysis; sugarcane molasses

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1. Introduction

Lactobacillus species are one of the most commonly used probiotic cultures belonging to the group of lactic acid bacteria (LAB). According to recent studies [1], the growing probiotic market of *Lactobacillus*, which shows its economic significance, is expected to reach USD 94.48 billion by 2027. Among the most demanded materials in this market are efficient culture media, which can be used for low-cost industrial fermentation.

Recently, a new definition has been proposed for the term "probiotic" to cover all healthy impacts of live probiotic cells and their derivatives in all possible forms, including viable or inviable microbial cells (vegetative or spore, intact or ruptured) that are potentially healthy to the host [2]. Likewise, new technologies to ensure their viability have been reported in multiple studies [3,4].

Among probiotics, *Lactobacillus paracasei* is an important bacterium widely used as a starter or as a bio preservative and has been reported in the production of valuable compounds [5,6], as well as the improvement of industrial wastewater treatment [7].

Considering the complex and costly growth requirements of Lactobacilli (peptones, beef extract, casein hydrolysate, etc.) used for their cultivation at the laboratory scale, the feasibility of replacing such supplements with efficient and economical alternatives has been a chokepoint for the commercial overproduction of probiotic Lactobacillus biomass [8].

In this context, sugarcane molasses (SCM) has been studied as an economical ingredient for Lactobacillus biomass fermentation [9]. SCM is a dark, dense liquid obtained as a byproduct of the sugarcane manufacturing process when more sucrose cannot be obtained by conventional physical methods after the crystallization stage. The process begins with the extraction of the sugarcane juice, followed by sulfitation, clarification, evaporation, and crystallization stages.

Due to its production process, it contains an important mixture of sugars, where sucrose is the main one (30–40%).

Molasses have been reported as materials that could be used to obtain valuable compounds [10,11] and as feedstock in a profitable biorefinery scheme [12]. An adequate pretreatment must convert non-fermentable sugars into assimilable ones by microorganisms and reduce impurities and heavy metals it contains, which can inhibit the growth of bacteria. The Microtox test has been proposed as a standardized toxicity test in aquatic media and is used to analyze the acute toxicity of aqueous samples, such as wastewater from a treatment system [13]; it is also applied in this work as an indicator of toxicity levels in molasses solutions.

Another limitation of molasses is its negligible organic nitrogen content, so adding some nitrogen source is necessary to enhance the growth of microbial strains in molasses-based media [14]. In addition, growth-associated components (glucose, amino acids, etc.) must be supplied in sufficient quantities to achieve the desired cell density and product concentrations.

Previous studies have shown different pretreatments applied to SCM for biotechnological uses, including hydrolysis at a wide range of pH, as an important condition for fermentation [15,16]. Although SCM has been pointed out as a promising substrate for producing some microbial biomass strains, it has not been presented as the sole carbon source in the culture media for Lactobacillus biomass production.

In this work, a feasible SCM pretreatment was developed for *Lactobacillus paracasei* ssp. *paracasei* F19 (LPPF19) biomass production with sucrose fractionation using a combined process of dilution, centrifugation, and acid hydrolysis. In addition, a detailed cost analysis of the ingredients used in the culture broth, both at the laboratory and industrial scale, was included.

2. Materials and Methods

2.1. Raw Material

SCM was obtained from a rum-producing company located in the eastern region of the Dominican Republic (Brugal & Co, San Pedro de Macoris, Dominican Republic, employs it as a secondary raw ingredient). This material was characterized to evaluate the sugar content, metals, and pH level to define the necessary pretreatment to condition it as a growing medium for the probiotic lactic acid bacteria.

After analyzing the results of the SCM composition, we decided to subject it to an acid hydrolysis treatment to increase the sugar content of glucose and fructose. Glucose is a limiting sugar in LPPF19 culture since this carbohydrate can be more efficiently metabolized by lactic acid bacteria [17].

2.2. Microorganism

Lactobacillus paracasei ssp. *paracasei* (F-19) was supplied by Christian Hansen Laboratory of Spain as a freeze-dried culture and was stored at −80 Celsius (°C) until its use. The optimal growth of this microorganism is achieved at a temperature of between 15 and 45 °C [18], where 37 ± 1 °C is the temperature used commercially and applied in the experiments of this work.

2.3. Pretreatment and Hydrolysis Conditions of Molasses

The treatment of molasses includes four main steps—dilution, centrifugation, acid hydrolysis, and neutralization—with two objectives: to reduce impurities and heavy metals in suspension and to transform the sucrose of this raw material into glucose and fructose.

Microtox analysis was performed to determine toxicity with and without centrifugation to evaluate if this process was necessary prior to hydrolysis. Microtox tests are based on the use of biosensors that measure the light emitted by selected bacteria when exposed to a given range of concentrations of the test substances. The light emitted by the bacteria is measured by a light reader, thus establishing a dose–response relationship. Thus, this test helps determine the EC_{50} value (concentration of a contaminant that causes a 50% decrease in the bioluminescence of that microorganism) [19].

The molasses solution was centrifuged at 8000× *g* (relative centrifugal force units) at 15 °C for 15 min (Eppendorf 5810R Centrifuge, Hamburg, Germany). Later, at room temperature, the pH was adjusted with a 20% sodium hydroxide (NaOH) solution (Scharlab, S.L., Barcelona, Spain) at 6.5 and 8.5 pH, selected based on preliminary experiments. Each pH was evaluated with and without centrifugation (8000× *g*) for 15 min. Then, for acid hydrolysis, the reported methodology with modifications was applied [20]. A solution of 5 molars of sulfuric acid (Scharlab, S.L., Barcelona, Spain) (5M H_2SO_4) was added with constant stirring (in the cold) and then heated and maintained at 60 °C for 2 h. Finally, the solid residue was separated. The obtained samples were characterized by the high-performance liquid chromatography (HPLC) method to determine the content of reducing sugars. Experiments were replicated three times, and analysis was carried out in triplicate.

2.4. Media and Culture Conditions for Bacteria

A molasses culture medium (MCM) with an adequate nutrient profile for bacterial growth was prepared from hydrolyzed molasses. The hydrolysates with the greater glucose content were diluted with water to reach 20 g/L of glucose. In addition, MCM contained, per liter, 4 g of yeast extract (Scharlau, Sharlab, Barcelona, Spain) to provide nitrogen source and salts, 2 g of dipotassium hydrogen phosphate (Scharlau), 5 g of sodium acetate trihydrate (Sharlab), 5 g of triammonium citrate (VWR), 2 g of magnesium sulfate heptahydrate (Scharlau), and 0.5 g of manganese chloride tetrahydrate (Alfa Aesar, Haverhill, MA, USA), which together represent 100% of the total salts added to the medium, using the MRS (DeMan, Rogosa, and Shape) as reference. Additionally, 1 mL/L of surfactant agent (Sharlab) was added to MCM to help the biomass growth due to its oleic acid content.

MCM was placed in 250 mL Erlenmeyer's and then autoclaved at 121 °C for 20 min (the working volume was 50 mL). Each flask was inoculated with an average of 0.635 ± 0.019 g of *Lactobacillus paracasei* ssp. *paracasei* (F-19), which has a cell density of 4.9132 log UFC/g. Then, it was incubated at 30 °C for 48 h on a rotary shaker at 120 rpm (revolutions per minute) in aerobic conditions.

Cell growth was assessed using the standard plate count method. After 48 h, samples were collected from the Erlenmeyer flasks and grown on an agar plate with MRS medium (Merck, Madrid, Spain) after decimal dilutions in sterile water until 10^{-5}, 10^{-6}, and 10^{-7}. Plates were incubated at 30 °C for 48 h. The pH was also measured as an indicator of the different stages in the growth cycle of the microorganism.

2.5. Analytical Methods

Sugars present in molasses were quantified using the HPLC technique (Agilent 1100 series HPLC, Agilent Tecnologies, Barcelona, Spain). Each sample was centrifuged at 15,000× *g* and 20 °C for 10 min to remove the suspended solids, and the supernatant was filtered with 0.45 µm nylon filters (Scharlab, S.L., Barcelona, Spain). One gram of supernatant was diluted in 100 mL of distilled water and analyzed with a Bio-Rad AMINEX HPX-87C column, Bio-Rad, Hercules, CA, USA, with the following settings: column temperature: 80 °C, injection volume: 20 µL, refractive index detector: 55 °C, and water as the mobile

phase. Reference compounds of sucrose, glucose, and fructose were used (Teknokroma Analytica, S.A., Barcelona, Spain).

Another critical parameter is the content of metals in the molasses because they can affect probiotic growth. Metal quantification was established using the inductively coupled plasma mass spectrometry (ICP-MS) technique. The operating conditions are shown in Table 1.

Table 1. ICP-MS operating and acquisition parameters.

RF Power (W)	1550
Plasma gas flow (L min^{-1})	15
Carrier gas flow (L min^{-1})	0.85–0.90
Sample flow rate (mL min^{-1})	0.1
He flow rate (mL min^{-1})	4.3
Extraction lens 1 (V)	2
Extraction lens 2 (V)	−140
Omega bias (V)	−30
Omega lens (V)	1
Cell input (V)	−34
QP focus	2
Cell output (V)	−30
Octopole RF (V)	150
Octopole bias	−6
QP bias	−3
Data acquisition	(Dwell time, 300 ms)
Sweeps per replicate	8
Replicates	3
Detection mode	Peak hopping
Isotopes	^{75}As, ^{111}Cd, ^{59}Co, ^{52}Cr, ^{63}Cu, ^{56}Fe, ^{55}Mn, ^{96}Mo, ^{56}Ni $^{208, 207, 206}Pb$, ^{51}V and ^{66}Zn

2.6. Experimental Design

BBD design of response surface methodology was applied to optimize the three selected factors (yeast extract, minerals, and surfactant agent). The factors choice was based on preliminary experiments. The three independent factors used in the current study were investigated at three different levels (−1, 0, +1), as shown in Table 2, and the design consisted of a BBD of 16 executions. The experiments were randomized to minimize the effects of unexplained variables in the observed responses. The statistical analysis was performed with the software package Statgraphics Centurion version XVII.1.12 (StatPoint Technologies Inc., Warrenton, VA, USA).

Table 2. Ingredients and their amounts accompanying molasses for each MCM liter and biomass produced in the assays of Box–Behnken design.

Essay	Yeast Extract, g	Minerals (% Max Level *)	Surfactant Agent, mL	Biomass & (log CFU/mL)
E1	0	50	1	6.77 [a]
E2	4	50	0	9.58 [b]
E3	4	50	1	7.50 [a]
E4	0	50	0	7.86 [a]
E5	2	0	0	7.71 [a]
E6	0	0	0.5	6.51 [a]
E7	2	0	1	6.81 [a]
E8	4	100	0.5	7.41 [a]
E9	0	100	0.5	6.82 [a]
E10	2	100	1	6.99 [a]
E11	4	0	0.5	6.90 [a]

Table 2. *Cont.*

Essay	Yeast Extract, g	Minerals (% Max Level *)	Surfactant Agent, mL	Biomass & (log CFU/mL)
E12	2	100	0	8.58 [c]
E13	2	50	0.5	7.00 [a]
E14	2	50	0.5	6.97 [a]
E15	2	50	0.5	6.94 [a]
E16 #	CF	CF	CF	8.63 [c]

* Maximum amount of added minerals: 14.5 g. # Commercial MRS used as control. CF: Commercial formula. & Different letters ([a], [b] and [c]) mean significant differences in ANOVA at a significance level of 95%.

3. Results and Discussion

3.1. Composition of Raw Material

The cane molasses used in this work had a high density (1.36 g/mL) and were slightly acidic (pH 4.43). The sugar content, measured with HPLC analysis (Figure 1), revealed a sucrose content of 62.0 ± 0.1%, glucose content of 19.1 ± 0.1%, and fructose content of 18.7 ± 0.1% with respect to the total base of sugars, which was around 50–65%. The other major components were water, about 17–25%; nitrogenous, non-nitrogenous, and wax components, 6.9–18%; and ash, 7–15% [21].

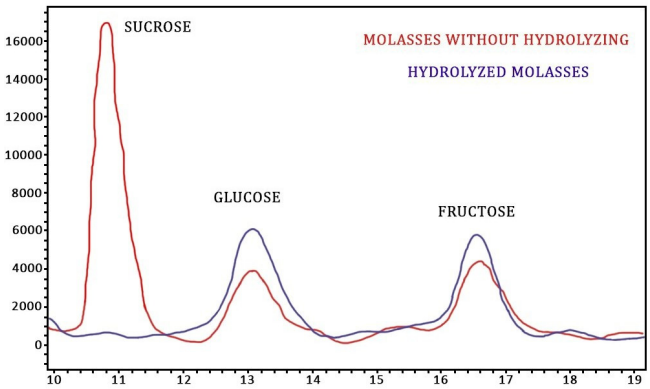

Figure 1. Sugar content in original molasses and after acid hydrolysis, determined by HPLC.

The sugar percentages of the molasses studied were about 3–5% higher than those of other molasses used in previously reported studies [22]. These results could be due to the natural hydrolysis that occurs in molasses stored in warm and slightly acidic environments, such as the geographical area where molasses were obtained for this research. In addition, the chemical composition of molasses is highly variable because it depends on agricultural factors (plant variety, maturity, climate, and soil), the industrial efficiency of sugar production, and the storage time [23].

3.2. Molasses Pretreatment and Hydrolysis Conditions

As a first step to reducing impurities and the metal content of the molasses, centrifugation prior to hydrolysis was carried out. The centrifugation procedure alone appeared unsatisfactory for removing impurities in molasses and avoiding the toxic effect on the cells because the bacteria could not grow in the molasses.

The high viscosity of the molasses used in these experiments made it very difficult to carry out the centrifugation and hydrolysis tests in the required conditions. Subsequently, different dilutions in water (100, 140, 200, and 400 g molasses/L) were studied.

Figure 2 presents the results of the hydrolysis of molasses at pH 8.5, with centrifugation, which was the most favorable for sucrose hydrolyzation.

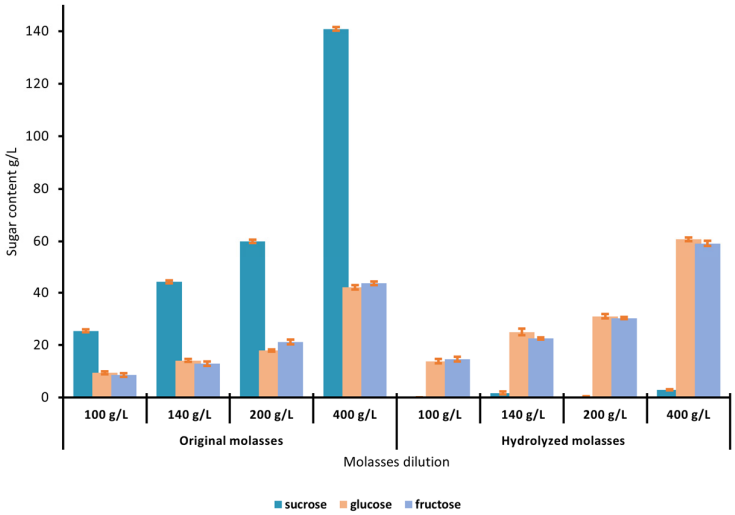

Figure 2. Sugar content g/L (gram/liter) in original and hydrolyzed molasses with previous centrifugation, adjustment to pH 8.5, and subsequent hydrolyzation.

According to these results, it was observed that a dilution of 400 g of molasses/L of water was the most suitable condition for achieving the best use of sugars. This dilution was enough to reduce viscosity to a value that enabled the sample processing to achieve sufficiently high amounts of sucrose so that after its fractionation, it could obtain optimum quantities of glucose in the growth medium.

In the case of pH 8.5 (400 g/L molasses dilution), sucrose presented a decrease from 140.8 g/L to 2.9 g/L (98%). Subsequent sucrose fractionation gave rise to a glucose increase from 42.1 g/L to 60.8 g/L, 44.4%, while at pH 6.5, the glucose increase was only 37.5%. One-way analysis of variance (ANOVA) at a 95% confidence level indicated statistically significant differences between the means. Likewise, the fructose increase was 35.8% at pH 8.5, while at pH 6.5, it was only 32.4%. These results showed that pH 8.5 was the most favorable for sucrose hydrolyzation.

3.3. Microtox Test

The toxicity of molasses was determined with the Microtox test in toxicity units (TUs). There is an international agreement for the classification of ecotoxicity: $0 < TUs \leq 0.07$ for low toxicity, $0.07 < TUs \leq 2.67$ for medium toxicity, $2.67 < TUs \leq 5.86$ for high toxicity, and $TUs > 5.86$ for very high toxicity.

The results presented in Table 3 show high toxicity for all samples, with a $TU_{50} > 3$. However, it is observed that the lowest toxicity was obtained when combining the hydrolysis plus centrifugation processes at pH 8.5 (with significant differences at a 95% confidence level with respect to pH 6.5).

Table 3. Toxicity of molasses samples analyzed (Microtox test).

Sample	TU	EC_{50}, %
Molasses without hydrolysis	21	4.8
Molasses with hydrolysis, pH = 6.5	18	5.6
Molasses with hydrolysis and centrifugation, pH = 6.5	19	5.3
Molasses with hydrolysis, pH = 8.5	15	6.7
Molasses with hydrolysis and centrifugation, pH = 8.5	7.1	14

TU: Toxitiy units, $100/EC_{50}$, EC_{50}: Percent of tested sample causing a 50% letal effect.

The high concentration of heavy metals in molasses has been pointed out by some authors, as well as the requirement of acid hydrolysis to achieve heavy metals' precipitation [24]. Figure 1 presents a chromatogram of molasses with and without hydrolysis. In

addition, with the combination of centrifugation and acid hydrolysis, it was possible to significantly reduce toxic metals present in the molasses, such as (50.3%), Cd (64.3%), Cu (63.0%), and Pb (55.6%), which are presented in Table 4. The levels of heavy metal reduction achieved with the molasses treatment favored the creation of a suitable environment for the growth of LPPF19 bacteria.

Table 4. Metal reduction in molasses after pretreatment.

Metal *	Concentration (µg /L)	Reduction (%) after Pre-Treatment
V	72.5	61.2
Cr	73.2	31.7
Mn	6835.0	57.5
Fe	24,963.0	72.9
Co	135.0	55.0
Ni	446.0	33.9
Cu	2027.0	63.0
Zn	1160.0	51.6
As	15.3	50.3
Mo	30.1	40.2
Cd	1.4	64.3
Pb	23.9	60.0

* V Vanadium, Cr Chrome, Mn Manganese, Fe Iron, Co Cobalt, Ni Nickel, Cu Copper, Zn Zinc, As Arsenic, Mo molybdenum, Cd Cadmium, Pb Lead.

Other authors have reported lower amounts of heavy metals in the molasses studied than in this investigation, noting that the absence of the potentially toxic metals Cd, Pb, and Ni was beneficial for microbial growth [25]. The impact of metals on microbial activity could be due to a reduction in the number of viable cells as a consequence of the death of less-tolerant species due to toxicity; moreover, as a second reason, metals could reduce the metabolic activity of population survivors. Thus, it was reported that cadmium could cause damage to nucleic acid and denature cell proteins; chromium could cause the inhibition of oxygen uptake; and zinc could cause a decrease in biomass and inhibition of the growth of microorganisms. [26].

3.4. Optimization of the Culture Medium

Using the best molasses treatment conditions, 16 fermentation experiments were carried out in BBD design, as pointed out in Section 2. The results of bacterial growth in these experiments are presented in Figure 3. Trials with different colors in this figure mean that they present significant differences in the one-way analysis of variance, (ANOVA) with a confidence level of 95%.

Regarding the detailed analysis of the differences in the Tukey test, it was observed that the means had a notable difference in treatment 2 with respect to all the means of the others, including the control treatment, Assay 16.

When comparing the control treatment E16 with the others, it was observed that there were statistically significant differences, with a confidence level of 95%, in the means of almost all of the treatments, except Treatment 12. In turn, Treatment E12 presented differences from all the treatments except the control, E16.

The maximum growth rate was obtained in the trial of Assay E2, where the exponential growth phase was reached, with 9.58 log CFU/mL at 48 h, 10% higher than the control pathway. Assay E12 had the next highest growth of the 16 trials performed. According to these results, adding yeast extract and the selected mineral salts supported a successful fermentation process.

The most important nutritional requirements for LAB are glucose, amino acids, and minerals [27]. On this basis, a nutrient balance was designed for culture media with SCM. Additionally, a direct relationship between the protein/carbohydrate concentration and the growth of different Lactobacillus strains has been reported [28,29]. Likewise, increased

growth of LPPF16 has been reported when sodium ascorbate, sodium pyruvate, manganese sulfate, and cysteine were used as growth enhancers in the culture broth [30]. In summary, the growth of LAB, influenced by the nutrients and pH of the medium, was established, as well as the removal of toxic components present in the medium.

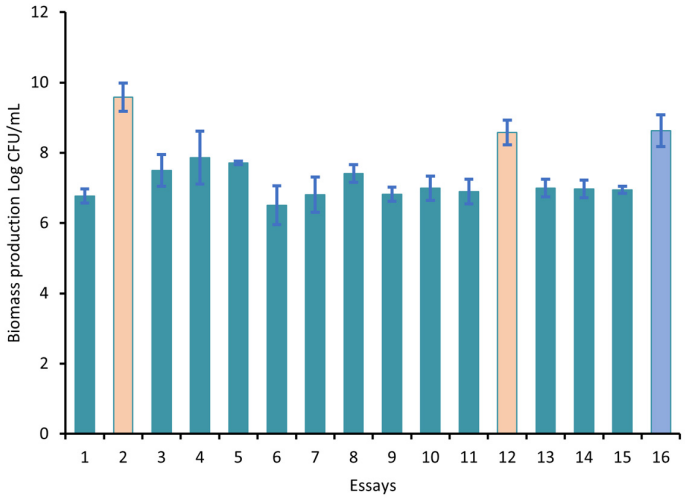

Figure 3. Growth of biomass of *Lactobacillus paracasei* ssp. *paracasei* (F-19) in 16 Box–Behnken design (BBD) combination nutrients for optimized culture media, Log CFU/mL: logarithm colony forming units/milliliter.

In relation to minerals salts, some authors reported that magnesium (Mg) is an essential element for the growth of Lactobacilli such as *Lactobacillus plantarum* [31], and manganese (Mn) is essential for the growth and metabolic activity of LAB organisms due to its biological effects on the structure and activation of numerous enzymes [32,33]. Furthermore, potassium (K) and sodium (Na), among others, have been reported to be essential for the enzymatic activity of LAB [34]. Likewise, the enrichment of culture medium with selected nutrients is used to maximize the productivity of LAB in a cost–benefit scheme [35]. Thus, in this work, we decided to add selected mineral salts, including phosphates, manganese, and magnesium, to the engineered culture to increase biomass production, according to Section 2.3 of the manuscript.

Some authors have studied the use of SCM as an ingredient for the production of targeted Lactobacillus strains, reporting up to 5.3×10^9 CFU/mL of *Lactobacillus paracasei* NRRL B-4564 [36] and 1.6×10^{10} CFU/mL of *Lactobacillus plantarum* [37].

In this work, it was found that the E2 treatment with the highest probiotic biomass production corresponded to the combination of 4 g/L of yeast extract with 50% minerals (Tukey's test, 95% significance). Treatment E12 reached the second position in biomass growth production, similar to the control (see Table 2 and Figure 3).

Yeast extract has been evaluated in other studies, as the main supplement, along with SCMes in the production of Lactobacilli, reporting that 2.5 g/L of yeast extract was sufficient to obtain high productivity of *Lactobacillus delbrueckii* mutant Uc-3 in batch fermentation, observing a buffering capacity of molasses [9]. Figures 4 and 5 show the bacterial growth and pH trajectory of E2 and control assays, respectively.

Additionally, in this study, it was observed that the pH dropped drastically from 6.09 to 4.0 during the fermentation of the molasses-prepared culture medium in Assay E2, indicating a good fermentation pathway, similar to that shown by the control, in which the pH dropped from 6.01 to 3.73 at the same time. Comparable results to those found in this investigation have been reported by authors who observed a drastic decrease in pH from 6.8 to 4.5 in fermented broths designed for lactic acid production with a Lactobacillus strain [38].

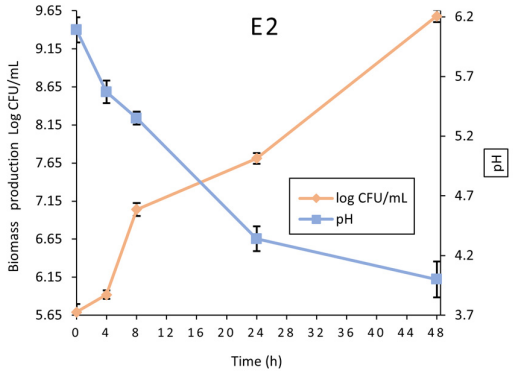

Figure 4. Growth of biomass and pH in fermentation of *Lactobacillus paracasei* ssp. *paracasei* (F-19) in Assay E2.

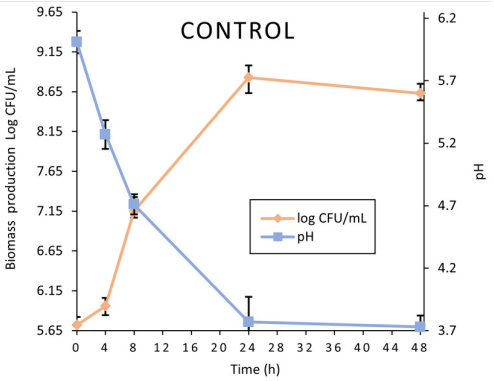

Figure 5. Growth of biomass and pH in fermentation of *Lactobacillus paracasei* ssp. *paracasei* (F-19) in Control assay.

The utilization of sugars by LPPF19 during fermentation is presented in Figure 6, corresponding to E2 and control trials.

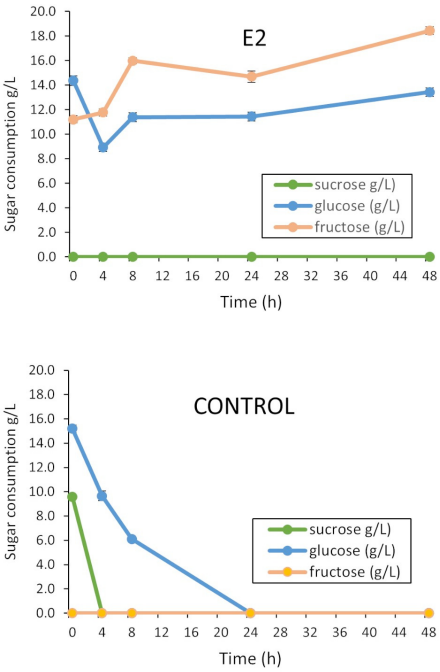

Figure 6. Sugar consumption by *Lactobacillus paracasei* ssp. *paracasei* (F-19) in E2 and control assays.

The glucose (limiting sugar) consumption in Assay E2 ranged from 14.4 g/L to 8.9 g/L (38.2%) in the first 4 h, similar to the control pathway, where glucose consumption decreases sharply from 15.2 g/L to 9.7 g/L (36.1%) at the same time. In addition, fructose showed a consumption range of 16.0 g/L to 14.7 g/L (8.1%) during the first 24 h of fermentation. Some authors have reported a similar fermentation pathway using media with molasses as substrate, in which the Lactobacillus strains also utilized glucose slightly faster than fructose during their growth [39].

These results demonstrate that sugarcane molasses, with the sole supplementation of selected minerals and yeast extract, after the proposed pretreatment, is a medium with the right nutrient balance to achieve significant probiotic biomass production when using LPPF19 as a reference.

3.5. Cost of Molasses Culture Medium (MCM)

The cost of raw materials used in the preparation of MCM is presented in Table 5. Data refer to the market price in the Dominican Republic for molasses [40] and Spain for the rest of the ingredients in 2020. The total cost of raw materials in preparing molasses culture media at the laboratory level is 1.23 EUR/L, while at the industrial level, it is 0.83 EUR/L. Even if the manufacturing cost of one product in ordinary chemical processing plants varies depending on many factors, such as operating labor and maintenance costs, raw materials are one of the principal components; it is estimated that they represent 33% of manufacturing costs [41]. In this case, the manufacturing cost of the molasses culture medium at laboratory levels could be estimated at 3.73 EUR/L and industrial levels at 2.51 EUR/L. Both costs are dramatically lesser than the cost of the control medium used in this research (11.93 EUR/L, 2020, Spain, price market data).

Table 5. Cost of preparing molasses culture medium (MCM) for Laboratory and Industry.

Raw Material	Quantity/L of MCM	Laboratory		Industry	
		Market Price *	Cost of MCM (EUR/L)	Market Price *	Cost of MCM (EUR/L)
Molasses	400 g	0.669 **	0.0706	0.669 **	0.0706
Yeast extract	4.00 g	42.14/500 g	0.3371	125/25 kg	0.0200
Dipotassium hydrogen phosphate	2.00 g	40.32/kg	0.0806	592.50/25 kg	0.0474
Sodium acetate trihydrate	5.00 g	19.21/kg	0.0961	197.50/25 kg	0.0395
Triammonium citrate	2.00 g	38.70/250 g	0.3096	38.70/250 g	0.3096
Magnesium sulfate heptahydrate	0.20 g	15.30/100 g	0.0306	142.50/25 kg	0.0011
Manganese sulfate tetrahydrate	0.05 g	26.60/100 g	0.0133	24.70/kg	0.0012
Surfactant agent	1.00 mL	23.50/25 mL	0.9400	138.6/L	0.1386
Molasses Pre-treatment					
Sulfuric acid 98%	8.80 mL	20.26/L	0.1783	20.26/L	0.1783
Sodium hydroxide	0.67 g	155/5 kg	0.0208	155/5 kg	0.0208
Total			1.2310		0.8271

* 2020, Spain, market prices according presentation (EUR/g, EUR/L, etc.) ** Dominican Republic price market, in EUR/gallon (INAZUCAR, 2019). EUR: EU currency, euros.

The reduction cost at laboratory levels, which respect to the control medium, comprises 68.7%, while at industrial levels, it comprises 78.9%. This cost evaluation reveals that cane molasses is an attractive material that can be used as a principal carbon source ingredient in a low-cost culture medium for producing *Lactobacillus paracasei* ssp. *paracasei* F19.

4. Conclusions

The results showed that conditioning sugarcane molasses with the pretreatment of subsequent dilutions, centrifugations, and hydrolysis made it possible to transform it into a hydrolysate with high glucose content (60.8 g/L), comprising 44% of the initial content, and obtain a technically viable and low-cost culture medium for growing probiotic *Lactobacillus paracasei* ssp. *paracasei* F-19 after enrichment with nitrogen and mineral sources.

Biomass production reached 9.58 log CFU/mL (ten times higher than the control). Cost analysis showed percent reductions, with respect to the control, of nearly 70% in the laboratory and close to 80% in the industry.

To improve the fermentation process, since not all of the glucose was consumed, future studies are needed to consider a greater utilization of sugars by Lactobacilli in media with sugarcane molasses.

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