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RESEARCH PAPER

Blackberry juice as a vehicle of potential probiotic bacteria isolated from aguamiel, blackberry, and pulque: consortium analysis and kinetic growth

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Abstract

Lactic acid bacteria (LAB) are widely recognized as probiotics, offering various health benefits. The hypothesis that the formation of bacterial consortia enhances these health benefits is based on the premise that synergistic interactions between strains increase viability and metabolic activity. The aim of this study was to examine the interactions between LAB strains isolated from aguamiel, blackberry, and pulque to assess their probiotic potential and incorporation into blackberry juice. Previously isolated LAB strains from three samples of aguamiel, blackberry, and pulque were used. The identification of these strains was facilitated by using an API kit. As a result, 16 distinct co-cultures were established, each composed of three unique strains. A total of four co-cultures exhibited growth of all strains through mutualistic interactions. These co-cultures (Co-1, Co-6, Co-11 and Co-13), were considered bacterial consortia and were selected for the evaluation of growth kinetics in MRS medium and blackberry juice fermentation. These strains showed a reduced exponential phase, allowing them to reach the stationary phase more quickly. This accelerated adaptation suggests that LAB consortia can adjust more effectively to new environmental conditions, suggesting their potential use as probiotics for functional beverage applications.

Keywords

Aguamiel, Blackberry juice, Pulque, Lactic acid bacteria, Mutualism, Probiotics

Introduction

In recent years, there has been an increased interest in the use of probiotics to promote health. The regular consumption of these substances has been demonstrated to be both safe and beneficial (Ayivi et al. 2020). Lactic acid bacteria (LAB) have emerged as a particularly promising solution in this regard, as they are considered safe for human consumption (Bujna et al. 2018; Latif et al. 2023). Similarly, LAB plays a vital role in food preservation by preventing the proliferation of harmful microorganisms. In addition to this function, LAB has also been shown to improve the sensory attributes of fermented foods, including taste and aroma (Costa et al. 2013; Chwastek et al. 2015; Bintsis 2018).

Furthermore, it has been shown that LAB ingestion modulates the proliferation of intestinal pathogens, pre-

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vents the onset of various gastrointestinal diseases resulting from intestinal dysbiosis, and contributes to the restoration of an optimal gut microbiota. This process has also been shown to improve intestinal barrier function and enhance host immunomodulation (Arqués et al. 2015; Bujna et al. 2018). Studies have indicated that the minimum therapeutic dose (MTD) of LAB, which has demonstrated positive results in the treatment of conditions such as diarrhea, colitis, *Helicobacter pylori* infections, and cancer, is typically between 7 and 9 Log CFU/mL (Reid et al. 2003; Neffe-Skocińska et al. 2018).

LAB are found in the intestinal mucosa of animals, plants, and fermented foods (Teneva-Angelova et al. 2018). Different strains of LAB have been isolated from diverse sources, including maguey sap (aguamiel), where species such as *L. plantarum*, *P. acidilactici*, *L. casei*, *L. lactis*, *Leuconostoc*, and *Pediococcus* have been identified. In pulque, a fermented beverage derived from aguamiel, strains like *L. kefiri*, *L. acidophilus*, *L. hilgardii*, *L. sanfranciscensis*, *L. lactis*, *L. piscium*, *L. plantarum*, *L. citreum*, *L. gelidum*, and *L. brevis* have been isolated (Cervantes-Elizarrarás et al. 2019; Chacón-Vargas et al. 2020; Alcántara-Zavala et al. 2021; Noriega-Juárez et al. 2025). Additionally, *L. plantarum* (Barache et al. 2020) and 10 strains of *W. cibaria* (Di Cagno et al. 2009) have been discovered on blackberry fruits.

Commercial preparations containing lactic acid bacteria (LAB) are widely available, whether in monocultures or mixed cultures (Havenaar and Huis In't Veld 1992; Bongaerts and Severijnen 2016). However, a comprehensive understanding of the complex interactions between these microorganisms is essential for optimizing the biotransformation of substrates into essential, health-promoting products (Canon et al. 2020). A fundamental concept in evaluating these interactions is mutualism, where symbiosis fosters positive and reciprocal relationships among the species within the consortium (García et al. 2019). A notable example of this phenomenon is the simultaneous presence of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* in yogurt (Bronstein 2009).

The development of healthy beverages incorporating LAB has generated considerable interest. LAB consortia have demonstrated increased growth in different matrices such as papaya, apple, pineapple, and orange juice (Wanger 2021). However, the development and survival of probiotics in fruit juices depend on the strain used, the pH level, the dissolved oxygen content, and the sugar concentration (Maia et al. 2023). Cranberry, blueberry, blackcurrant, and strawberry juices contain high levels of phenolic antioxidants. Consequently, there has been an increase in the industrial production of health beverages containing these fruits (Huang et al. 2012). Blackberries are also an important source of polyphenols, potent antioxidants with anti-inflammatory and anticancer properties (Tarrah et al. 2018; Van de Velde et al. 2019; Gil-Martínez et al. 2023; Memete et al. 2023) also contain short-chain fatty acids, vitamins, exopolysaccharides and organic acids (Bintsis 2018; Bujna et al. 2018). Furthermore, the polysaccharides and polyphenols in blackberries also have prebiotic effects (Dou et al. 2021; Xi et al. 2024), thus classifying them as functional foods that offer significant health benefits to consumers. Additionally, the low pH of blackberries, which typically ranges from 3.3 to 3.4, inhibits the proliferation of pathogenic bacteria while promoting the growth of acid-tolerant lactic acid bacteria (LAB) strains (Moraes et al. 2020; Siddiqui et al. 2023).

Therefore, the objective of this study was to investigate the interactions of LAB strains isolated from aguamiel, blackberry, and pulque, identified as probiotic candidates for use in blackberry juice.

Materials and methods

Biological material, characterization, and identification

The LAB strains utilized in this study were obtained from the microbial collection of the Food Techno-functionality Laboratory at the Institute of Health Sciences, Autonomous University of the State of Hidalgo. These were previously isolated from fermented beverages, including aguamiel, blackberry fruit (*Rubus fruticosus* cv. Tupy) and pulque. Isolated colonies were characterized by their morphology (small, beige-white colonies with a creamy consistency), Gram-positive staining, negative motility, and negative catalase activity, following standard procedures (Yelnetty et al. 2014; Ismail et al. 2018). The strains were preserved in a solution of glycerol at a temperature of -70 °C for the purpose of long-term storage. For the present study, two strains from each source were selected based on their enhanced resistance to acidic pH and antimicrobial activity (see Table 1), and two strains was used as positive control, *L. rhamnosus* (C1), and *L. casei* (C2). To reactivate the bacteria, the LAB strains were subcultured twice on MRS agar using the microdrop technique and incubated at 37 °C (LabTech, LSI-3016A, Korea) for 24 hours (Naghili et al. 2013). Following the incubation period, a single isolated colony from each strain was selected and cultivated in MRS broth. The cultures were then subjected to incubation at 37 °C for a duration of 24 hours.

Table 1. Selected Lactic Acid Bacteria strains.

Origin	Resistant to pH 2 to 3	Antimicrobial activity
Aguamiel (A)	A2	A1
Blackberry (B)	B1	B2
Pulque (P)	P1	P2
Control+ (C+)	*L. rhammosus*	
	L. casei	

Biochemical identification

The genus (A1, A2, B1, B2, P1 and P2) was identified using the API Kit (VWR, USA), following the manufacturer's instructions. A cloudy suspension of the LAB colonies was prepared in sterile distilled water and then adjusted to McFarland standard. The requisite amount of suspension was added to achieve McFarland Standard 0.4, and the mixture was thoroughly homogenized.

In order to establish a humid environment, 10 milliliters of sterile distilled water were added to the bottom of each gallery well. Subsequently, the gallery strips were meticulously positioned within the designated wells, and the inoculum was distributed with caution to avert the formation of bubbles. The galleries were sealed with sterile paraffin oil, the lid was closed, and the setup was incubated at 37 °C for 24 and 48 hours in an incubator (LabTech, LSI-3016A, Korea). The observed color change from purple to yellow indicated the onset of carbohydrate fermentation. The results were recorded as positive for carbohydrate fermentation and identified using the values provided by the API Kit.

Microbial interactions analysis

A total of 16 distinct co-cultures were established by employing LAB strains, with each co-culture comprising three distinct strains. These strains were cultivated on MRS medium plates. The plates were then subjected to an incubation period at 37 °C for a duration of 24 hours, with all parameters of the incubation process being held constant. The selection of co-cultures was based on visual observation. Positive mutualism (+/+) was defined as uniform growth, with *Lacticaseibacillus rhamnosus* and *Lacticaseibacillus casei* (Zheng et al. 2020) serving as positive controls. The strains that exhibited inhibitory effects on one another were excluded from further consideration. The presence of three strains in each co-culture was verified through Gram staining. The co-cultures combinations demonstrating optimal uniform growth were selected for subsequent assays.

Evaluation of growth kinetics in MRS medium

The growth kinetics of the LAB strains were evaluated using the microdrop technique, according to Strahsburger et al. (2016), with slight modifications. A colony from each strain was inoculated into 10 mL of sterile MRS broth and incubated at 37 °C for 24 hours and at predetermined time points (0, 2, 4, 6, 8, 10, 12, and 24 hours). At these time points, 100 µL aliquots were taken, and viable counts were determined by serially diluted 10 times in peptone water and subsequently plated onto MRS agar plates in triplicate. The results were expressed as the logarithm of colony-forming units per milliliter (Log CFU/mL).

Juice blackberry elaboration and inoculum

Blackberries (*Rubus fruticosus* cv. Tupi) were harvested from Atotonilco el Grande, Hidalgo, Mexico, in April 2020. The fruit was thoroughly washed under running water to remove any remaining leaves and soil. Subsequently, were submerged in drinking water with bactericide (Microdyn) following the instructions strictly, placed in a strainer to facilitate the removal of excess water, emptied into resealable bags and frozen at -32 °C for later use.

The fruits were homogenized using a conventional blender, followed by filtration through a strainer and centrifugation (Allegra 25, Beckman Coulter, USA) at 10,000 rpm for 20 minutes at 4 °C. This procedure was performed to achieve the desired juice clarification. The clarified juice (supernatant) was subjected to thermoultrasonication (Sonics and Materials Inc., VCX 1500, Newtown, CT, USA) in a sterile environment under the following conditions: a temperature of 50 °C was maintained for 17 minutes, and the microbiological quality of the sample was assessed by the no presence of aerobic mesophiles and enterobacteria (Cervantes-Elizarrarás et al. 2017). The juice was then frozen at -35 °C in sterile containers for subsequent inoculation with LAB cultures.

To the inoculum, each LAB strain was individually cultured in MRS liquid medium (BD, Sparks, MD, USA) for 24 hours at 37 °C in an incubator (LabTech, LSI-3016A, Korea). Two milliliters aliquots of each culture were collected at 2 hours intervals over a 24 hours period to determine the concentration of colony-forming units per milliliter (CFU/mL) (Chen et al. 2019). The calculations were performed to determine the appropriate inoculum concentration of each strain in the consortium to achieve the MTD of 9 log CFU/mL in 10 mL at the end of fermentation (Ayivi and Ibrahim 2022). Subsequently, the required aliquots (mL) of each strain were obtained and resuspended in a 1:10 dilution in thermoultrasonicated blackberry juice.

Kinetic parameters of the microbial growth

The kinetic parameters of microbial growth were calculated to assess metabolic differences among the LAB. The growth rate (µ) was determined using Equation (1). The generation time (g) and growth constant (K) were calculated using Equations (2) and (3), respectively. The initial (N0) and final (Nx) biomass concentrations corresponded to the selected time points in the logarithmic phase, which were denoted as t0 and tx, respectively. These calculations yielded insights into the growth dynamics and metabolic behavior of the LAB strains during fermentation.

$$\mu = [\ln(Nx) - \ln(N0)]/(tx - t0) \quad (1)$$

$$g = \ln(2)/\mu \quad (2)$$

$$k = 1/g \quad (3)$$

Statistical analysis

The analysis was performed in triplicate on three batches. Microbiological data from the samples were compared and analyzed using a one-way analysis of variance (ANOVA) with SPSS version 12.0.1 for Windows (SPSS Inc., Chicago, Illinois). Tukey's test was used, with a significance level of $p < 0.05$, to identify significant differences between the variables. In addition, a Student's t-test was performed to compare the initial and final stages of fermentation for each sample.

Results and discussion

LAB identification

The identification of LAB strains was conducted in accordance with the technical data sheet. Strains A1 and B1, isolated from aguamiel and blackberry, respectively, were identified as *Lactiplantibacillus plantarum*. Strains A2 and B2 were identified as *Pediococcus acidilactici*, while strain P1 was identified as *Secundilactobacillus collinoides*, and strain P2 as *Levilactobacillus brevis*. Furthermore, strain C1 was identified as *Lacticaseibacillus rhamnosus*, while strain C2 was identified as *Lacticaseibacillus casei* (Table 2).

Analysis of mutualistic interactions of lactic acid bacteria

Sixteen bacterial mixtures (M1–M16) were analyzed (Table 3), which only four (M1, M6, M11 and M13) which designate as consortia (Co), because exhibited growth of all strains through mutualistic interactions: Co-1 (*L. plantarum* [B1], *L. plantarum* [A1], and *P. acidilactici* [A2]); Co-6 (*L. brevis* [P2], *P. acidilactici* [A2], and *L. plantarum* [A1]); Co-11 (*L. plantarum* [B1], *P. acidilactici* [A2], and *L. brevis* [P2]) and Co-13 (*L. brevis* [P2], *L. plantarum* [A1], and *L. plantarum* [B1]) in the absence of an inhibition zone, similar to positive control (C+; *L rhamnosus* and *L. casei*). While other bacterial mixtures e.g. the M16 (*L. plantarum*, *P. acidilactici*, *S. collinoides*), showed no bacterial interaction (Fig. 1). The consortia were selected for the following determinations. The results were noteworthy due to the scarcity of studies using wild-type strains in the investigation of mutualistic interactions (Xu et al. 2018; Bancalari et al. 2020; Zhang et al. 2025). A study was conducted using a mixed culture of three strains to generate secondary metabolites, with the aim of improving the sensory and functional quality of fermented foods. The study revealed a substantial increase in compounds associated with fruity aromas (Zhao et al. 2025). On the other hand, isolating wild-type strains to facilitate the development of

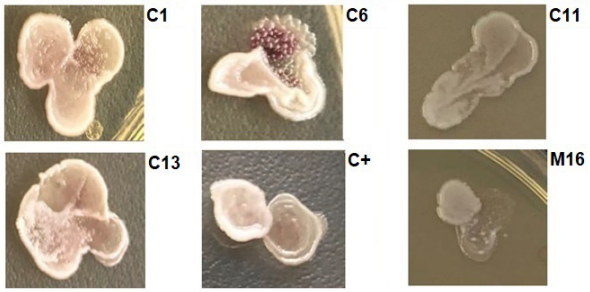

Figure 1. Mutualistic interaction of combination of three strain obtained of vegetal sources. Co-1 (mix *L. plantarum*, *L. plantarum* and *P. acidilactici*), Co-6 (*L. brevis*, *P. acidilactici* and *L. plantarum*), Co-11 (*L. plantarum*, *P. acidilactici* and *L. brevis*) and Co13 (*L. brevis*, *L. plantarum* and *L. plantarum*), M17 (*L. plantarum*, *P. acidilactici* and *S. collinoides*).

Table 2. Bacterial identification by API Test.

Carbohydrate	Aguamiel		Blackberry		Pulque		Controls +	
	A1	A2	B1	B2	P1	P2	C1	C2
Witness	-	-	-	-	-	-	-	-
Glycerol	+	-	-	-	-	-	-	-
Erhytrol	-	-	-	-	-	-	-	-
D-arabinose	-	-	-	-	-	-	+	-
L-arabinose	-	+	+	+	-	+	-	-
D-ribose	+	+	+	+	+	+	-	+
D-xilose	-	+	-	+	+	+	-	-
L-xilose	-	-	-	-	-	-	-	-
D-adanitol	-	-	-	-	-	-	-	+
Methyl-βd-xilopyranoside	-	-	-	-	-	-	-	-
D-galactose	+	+	+	+	-	-	+	+
D-glucose	+	+	+	+	+	+	+	+
D-fructose	+	+	+	+	+	+	+	+
D-mamnose	+	+	+	+	-	-	+	+
L-sorbose	+	-	-	-	-	-	-	+
L-rhamnose	+	-	-	-	-	-	-	-
Dulcitol	-	-	-	-	-	-	+	-
Inositol	-	-	-	-	-	-	+	-
D-mannitol	+	-	+	-	-	-	+	+
D-sorbitol	+	-	-	-	-	-	+	+
Methyl-αD-manopyranoside	+	-	-	-	-	-	-	-
Methyl-αD-glucopyranoside	+	-	-	-	-	-	-	-
N-acetylglucosamine	+	+	+	+	+	+	+	+
Amygdalin	+	-	-	-	-	-	+	
Arbutin	+	-	+	-	-	-	+	+
Esculin ferric citrate	+	+	+	+	+	+	+	+
Salicin	+	-	+	-	-	-	+	+
D-cellobiose	+	+	+	+	-	-	+	+
D-maltose	+	+	+	+	+	+	-	+
D-lactose (bovine origen)	+	-	+	-	-	-	-	+
D-melibiose	+	-	+	-	-	+	-	-
D-sucrose	+	-	+	-	-	-	-	-
D-trehalose	+	-	+	-	-	-	+	+
Inulin	-	-	-	-	-	-	-	-
D-melezitose	+	-	-	-	-	-	+	-
D-rafinose	+	-	+	-	-	-	-	-
Strach	-	-	-	-	-	-	-	-
Glycogen	-	-	-	-	-	-	-	-
Xylitol	-	-	-	-	-	-	-	-
Gentiobiose	+	+	+	+	-	-	+	+
D-turanose	+	-	+	-	-	-	-	+
D-lyxose	-	-	-	-	-	-	-	-
D-tagatose	-	+	-	+	-	-	+	+
D-fucose	-	-	-	-	-	-	-	-
L-fucosae	-	-	-	-	-	-	+	-
D-arabitol	-	-	-	-	-	-	-	-
L-arabitol	-	-	-	-	-	-	-	-
Potassium gluconate	+	+	-	+	+	+	+	+
2-Potassium ketogluconate	-	-	-	-	-	-	-	-
5-Potassium ketogluconate	-	-	-	-	+	+	-	-
Identificación	Lp	Pa	Lp	Pa	Sc	Lb	Lr	Lc

Lp = *Lactiplantibacillus plantarum;* **Pa** = *Pedicoccus acidilactici;* **Sc** = *Secundilactobacillus collinoides;* **Lb** = *Levilactobacillus brevis;* **Lr** = *Lacticaseibacillus rhamnosus;* **Lc** = *Lacticaseibacillus casei;* **+** = Sugar fermemtation; **-** = No sugar fermentation.

Table 3. Lactic Acid Bacteria co-cultures.

Bacterial Mixture	LAB 1	LAB 2	LAB 3
M1	*L. plantarum* (*B1*)	*L. plantarum* (*A1*)	*P. acidilactici* (*A2*)
M2	*L. plantarum* (*B1*)	*L. plantarum* (*A1*)	*S. collinoides* (*P1*)
M3	*P. acidilactici* (*B2*)	*P. acidilactici* (*A2*)	*L. brevis* (*P2*)
M4	*L. plantarum* (*B1*)	*P. acidilactici* (*A2*)	*S. collinoides* (*P1*)
M5	*L. plantarum* (*A1*)	*L. brevis* (*P2*)	*P. acidilactici* (*B2*)
M6	*L. brevis* (*P2*)	*P. acidilactici* (*A2*)	*L. plantarum* (*A1*)
M7	*S. collinoides* (*P1*)	*L. plantarum* (*A1*)	*L. brevis* (*P2*)
M8	*S. collinoides* (*P1*)	*P. acidilactici* (*A2*)	*L. brevis* (*P2*)
M9	*S. collinoides* (*P1*)	*L. plantarum* (*A1*)	*P. acidilactici* (*A2*)
M10	*L. plantarum* (*B1*)	*P. acidilactici* (*B2*)	*L. brevis* (*P2*)
M11	*L. plantarum* (*B1*)	*P. acidilactici* (*A2*)	*L. brevis* (*P2*)
M12	*S. collinoides* (*P1*)	*P. acidilactici* (*B2*)	*P. acidilactici* (*A2*)
M13	*L. brevis* (*P2*)	*L. plantarum* (*A1*)	*L. plantarum* (*B1*)
M14	*L. brevis* (*P2*)	*L. plantarum* (*B1*)	*S. collinoides* (*P1*)
M15	*P. acidilactici* (*B2*)	*L. plantarum* (*A1*)	*S. collinoides* (*P1*)
M16	*L. plantarum* (*B1*)	*P. acidilactici* (*B2*)	*S. collinoides* (*P1*)
C+	*L. rhamnosus* (*C1*)	*L. casei* (*C2*)	-

L: *Lactobacillus*; **P**: *Pediococcus*; **C+**: Positive control. **A1** and **A2**: strains from aguamiel, **B1** y **B2**: strains from blackberry; **P1** y **P2**: strains from pulque.

mutualistic interactions could be an alternative solution, since most domesticated strains experience a decrease in the expression of genes essential for establishing interactions between them (Liu et al. 2019).

This finding aligns with the observations reported by Lee et al. (2014), who demonstrated that while *L. salivarius* can only utilize the limited free amino acids present in MRS medium, its growth and lactate production are enhanced in a mixed culture with *P. acidilactici*. This enhancement is attributable to the capacity of *L. salivarius* to utilize the amino acids released through the proteolytic activity of *P. acidilactici*. In a similar vein, studies by Toe et al. (2019) and Zotta et al. (2017) have reported that LAB strains possess a range of proteolytic activities, enabling the release of distinct amino acids. Furthermore, Wang et al. (2020) have observed that LAB fermentation also liberates other molecules, including sugars, short-chain fatty acids, and vitamins. Consequently, in heterogeneous cultures comprising various *Lactobacillus acidophilus* strains, the metabolic activity of each strain is indispensable for facilitating efficient substrate conversion, enhancing biotransformation, and improving the utilization of fermentation by-products through nutrient exchange an interaction frequently termed cross-feeding (Canon et al. 2020). In the case of the Co-1, Co-6, and Co-11 consortia, the release of amino acids, carbohydrates, and vitamins may have increased their availability for uptake and use by the different *Lactobacillus* species comprising the consortia.

In contrast, the results indicated that the presence of *P. acidilactici* [B2] or *S. collinoides* [P1] in the co-cultures resulted in inhibitory effects on bacterial interactions. As demonstrated in the research by Li et al. (2021), various strains of *P. acidilactici* have been documented to manifest distinct genetic characteristics, encompassing chromosomal genetic material, plasmids, and prophages. These genetic traits have the potential to yield disparate bacteriocins (Li et al. 2021). These bacteriocins, in conjunction with other proteins, have been demonstrated to modulate symbiotic interactions within bacterial consortia by impacting motility and chemotaxis (Raina et al. 2019; Wiesmann et al. 2023).

However, the bacteriostatic or bactericidal effects of these bacteriocins can be counteracted by resistance mechanisms, such as neutralizing the negative charge on the bacterial cell wall through teichoic acid (Debabov et al. 2000). In Gram-positive bacteria, this process depends on four proteins from the Dlt operon (Dlt A-D). Inactivating these proteins has been shown to enhance sensitivity to bacteriocins (Nakao et al. 2000). This behavior has been observed in *L. rhamnosus*, which is part of the positive control (C+). Another resistance mechanism involves modifying anionic phospholipids with L-lysine to create lysylphosphatidylglycerol (a basic phospholipid), which adds a positive charge to the bacterial membrane, aiding in protection against bacteriocin effects (Khatib et al. 2016; Kumariya et al. 2019). These mechanisms may provide a theoretical foundation for the observed mutualistic interactions in consortia Co-1, Co-6, Co-11, Co-13, and C+.

The results of the present study underscore the intricacy of microbial interactions in LAB co-cultures, and the significance of comprehending how these interactions can impact the collective performance of bacterial consortia in fruit juice fermentation (Liao et al. 2023). The positive mutualism observed in Co-1, Co-6, Co-11, and Co-13 highlights the potential benefits of cross-feeding mechanisms, wherein different strains contribute to each other's growth by exchanging essential nutrients. This mutualistic behavior has been demonstrated to enhance biotransformation processes and ensure more efficient substrate conversion, which could have significant applications in the development of functional foods or probiotics (Naseem et al. 2023). Furthermore, the inhibitory effects observed in co-cultures containing specific strains, such as *P. acidilactici* [B2] and *S. collinoides* [P1], lays stress on the competitive nature of LAB strains which may be influenced by genetic factors such as bacteriocin production (Mousanejadi et al. 2023). These findings emphasizes the need for a deeper understanding of strain-specific interactions, as the balance between mutualistic and antagonistic effects is crucial for optimizing microbial consortia for biotechnological applications. By exploring these dynamics, future research could contribute to enhancing the design of microbial communities for targeted fermentation processes, with the potential to improve product quality and microbial stability.

Growth kinetics in MRS medium

Microbial growth kinetics are critical for advancing microbial analysis and enhancing biotechnological processes (Üçok and Sert 2020). The growth of four consortia and control (Co-1, Co-6, Co-11, Co-13, and C+) in MRS broth was tracked over a 24 hours period (Fig. 2). The maxi-

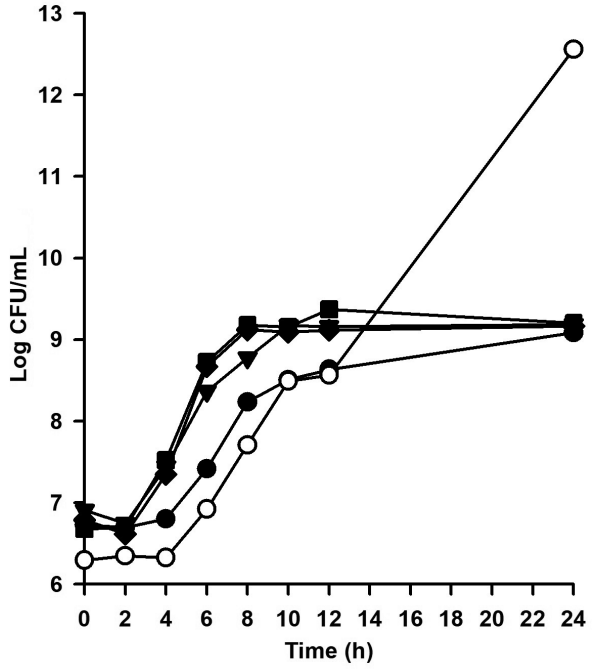

Figure 2. Growth Kinetic of LAB consortia, on broth MRS. ● = Co-1 (Consortium formed by *L. plantarum* [B1], *L. plantarum* [A1] and *P. acidilactici* [A2]), ■ = Co-6 (Consortium formed by *L. brevis* [P2], *P. acidilactici* [A2] and *L. plantarum*[A1]), ▼ = Co-11 (Consortium formed by *L. plantarum* [B1], *P. acidilactici* [A2] and *L. brevis* [P2]), ◆ = Co-13 (Consortium formed by *L. brevis* [P2], *L. plantarum* [A1] and *L. plantarum* [B1]) and O = C+ (Positive control, co-culture o Consortium formed by f *L. rhamnosus* and *L. casei*); CFU Colony Forming Units: n = 3.

mum specific growth rate of the control consortium was observed during the exponential phase, which lasted until 24 hours, showing a maximum count of 12.56 ± 0.02 Log CFU/mL, the highest bacterial count, despite starting with the lowest initial count of 6.28 ± 0.07 Log CFU/mL. The initial bacterial count for the selected LAB consortia averaged 6.64 Log CFU/mL, and they remained in the exponential phase for approximately two hours, with the exception of Co-1, which had an adaptation phase lasting 4 hours. The exponential phase for the other consortia lasted between 4 and 6 hours, followed by a stationary phase that continued until the end of the 24 hours incubation period.

The maximum bacterial count recorded was 9.16 ± 0.15 and 9.12 ± 0.01 Log CFU/mL for Co-6 and Co-13, respectively, both presented the highest counts, while Co-1 showed the lowest rate at 10 hours. The observed outcomes can be ascribed to the occurrence of *L. brevis* within the Co-6 and Co-13 consortia. It has been demonstrated that the presence of diverse microbial species can exert a substantial impact on bacterial growth kinetics (Üçok and Sert 2020). It has been established that a variety of factors, including pH level, the availability of nutrients, substrate concentration, the duration of the incubation period, and the temperature, all exert an influence on the logarithmic growth rate (Najafpour 2007; Śliżewska and Chlebicz-Wójcik 2020).

The varying growth rates observed in this study suggest a strain-dependent effect. In order to understand the behavior of mutualistic interactions, the quantification (Log CFU/mL) of each strain that makes up the consortia was analyzed (Table 4). *L. plantarum* isolated from blackberry exhibited a significantly lower count ($p < 0.05$) at 7.24 Log CFU/mL, while *L. plantarum* from aguamiel, *L. rhamnosus*, and *L. casei* (used as positive controls) reached a value of 9.5 Log CFU/mL, which was significantly higher than the other LAB strains.

Similar findings were observed with *P. pentosaceus* and *L. lactis*, which attained a maximum count of 9.4 Log CFU/mL, as well as with *W. cibaria*, which achieved 9.8 Log CFU/mL (Pellegrino et al. 2019). Conversely, lower counts were reported for *L. plantarum*, *L. brevis*, *L. paracasei*, *L. fermentum*, and *L. pentosus*, with values ranging around 8.4 Log CFU/mL (Garcia et al. 2016). A maximum count of 9 Log CFU/mL in *P. pentosaceus* isolated from date palm was achieved after 29 hours of incubation, while *Leuconostoc citreum*, *Leuconostoc mesenteroides*, and *Leuconostoc pseudo-mesenteroides*, isolated from beef sausages, turkey, and cow's milk, respectively, reached comparable counts after 34 hours (Abid et al. 2018).

The growth of *L. plantarum*, *L. brevis*, *L. paracasei*, *L. fermentum*, and *L. pentosus* isolated from fruit pulp was studied. It was found that after 24 hours of incubation, the growth rate ranged from 8.1 to 8.4 Log CFU/mL, *L. brevis* and *L. pentosus* showed the highest growth, though no significant differences were observed between them (Garcia et al. 2016).

The results obtained in this investigation reveal that both Co-1 and Co-13, containing *L. plantarum* isolated from blackberry, demonstrated reduced growth in monoculture (Table 4). However, the presence of *L. brevis* in the consortia has been observed to enhance the growth rate.

Inoculum calculation for blackberry juice fermentation

The inoculation of the LAB consortia into blackberry juice was conducted according to the maximum value of the MTD, which was 9 Log CFU/mL. Equal concentrations of each LAB strain were utilized, and the bacterial count was documented after 24 hours in accordance with Table 4.

The necessary inoculum (mL) for each LAB strain was calculated (Table 5), producing a value of 3 Log CFU/mL.

Table 4. LAB count after 24 hours of incubation in MRS broth.

LAB	Aguamiel		Blackberry	Pulque	C+	
	L. plantarum	*P. acidilactici*	*L. plantarum*	*L. brevis*	*L. rhamnosus*	*L. casei*
Log CFU/mL	9.55±0.09[a]	9.17±0.08[b]	7.24±0.09[c]	9.31±0.03[b]	9.56±0.02[a]	9.55±0.12[a]

C+: Positive control; **CFU**: Colony Forming Units; **LAB**; Lactic Acid Bacteria; [a-c] Different letters indicate significant differences ($p < 0.05$) between samples.

Table 5. Inoculum required (mL) from each LAB to cover the minimum therapeutic unit for inoculation into the thermoultasonicated juice (10mL).

Consortia/LAB	Aguamiel		Blackberry	Pulque	C+	
	L. plantarum	*P. acidilactici*	*L. plantarum*	*L. brevis*	*L. rhamnosus*	*L. casei*
Co-1	0.314	0.327	0.414	-	-	-
Co-6	0.314	0.327	-	0.326	-	-
Co-11	-	0.327	0.414	0.326	-	-
Co-13	0.314	-	0.414	0.326	-	-
C+	-	-	-	-	0.471	0.471

Co-1 (Consortium formed by *L. plantarum* [B1], *L. plantarum* [A1] and *P. acidilactici* [A2]), Co-6 (Consortium formed by *L. brevis* [P2], *P. acidilactici* [A2] and *L. plantarum* [A1]), Co-11 (Consortium formed by *L. plantarum* [B1], *P. acidilactici* [A2] and *L. brevis* [P2]), Co-13 (Consortium formed by *L. brevis* [P2], *L. plantarum* [A1] and *L.plantarum* [B1]) and C+ (Positive control, co-culture o Consortium formed by f *L. rhamnosus* and *L. casei*); -: absence of inoculum; each inoculum in mL of each LAB corresponds to 3 Log CFU.

The inoculum level was selected to guarantee that the final consortium of the three strains attained the target value of 9 Log CFU/mL. In a related study, *L. acidophilus*, *L. plantarum*, *L. rhamnosus*, and *L. casei* were utilized to ferment apple juice, starting with an initial inoculum concentration of 9 Log CFU/mL. Comparable inoculum levels were utilized in juices derived from apple varieties, inoculated with *L. acidophilus*, *L. plantarum*, and *L. fermentum* (Peng et al. 2021), as well as in blackberry juice fermented with *L. plantarum* (Kwaw et al. 2017).

LAB strains are known for their safe interactions with the human body, aiding in the restoration of gut microbiota and metabolic regulation, along with various health benefits (Mays and Nair 2018). In addition, inoculation at the MTD of 9 Log CFU/mL has exhibited therapeutic effects for a variety of health conditions, including diarrhea, colitis, and cancer (Neffe-Skocińska et al. 2018). For instance, the supplementation of *L. rhamnosus* in diabetic and obese mice has shown hypoglycemic effects and the regulation of blood lipid levels, which can be attributed to the production of short-chain fatty acids by LAB (Wang et al. 2020).

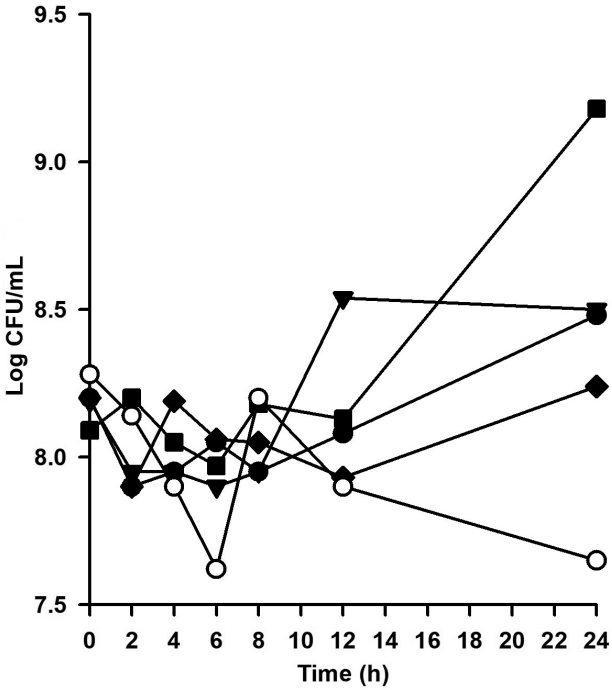

Figure 3. Growth Kinetic of LAB consortia on blackberry juice, on thermoultrasonicated blackberry juice. ● = Co-1 (Consortium formed by *L. plantarum* [B1], *L. plantarum* [A1] and *P. acidilactici* [A2]), ■ = Co-6 (Consortium formed by *L. brevis* [P2], *P. acidilactici* [A2] and *L. plantarum* [A1]), ▼ = Co-11 (Consortium formed by *L. plantarum* [B1], *P. acidilactici* [A2] and *L. brevis* [P2]), ◆ = Co-13 (Consortium formed by *L. brevis* [P2], *L. plantarum* [A1] and *L. plantarum* [B1]) and ○ = C+ (Positive control, co-culture o Consortium formed by *L. rhamnosus* and *L. casei*); CFU Colony Forming Units: n = 3.

Blackberry juice fermentation kinetics

Following the determination of the requisite inoculum (3 Log CFU/mL) for the fermentation process, the growth kinetics of the LAB consortia in thermo-ultrasonicated blackberry juice were measured. The thermo-ultrasonicated juice was free from aerobic mesophiles and enterobacteria. Growth kinetics were monitored over 24 hours, and the growth pattern varied. The mean LAB count at the commencement and conclusion of fermentation was 8.17 and 8.34 Log CFU/mL, respectively (Fig. 3).

When comparing the growth kinetics of the LAB consortia over time, no significant differences ($p > 0.05$) were observed among the Co-1, Co-11, and Co-13. However, the Co-6 consortia demonstrated a significant increase ($p < 0.05$) in LAB count at 24 hours (1 Log CFU/mL), reaching MTD (9 Log CFU/mL), while the positive control exhibited a significant decrease in counts of less than 8 Log CFU/mL at 24 hours.

A comparable trend was identified in a study in which a co-culture of *L. plantarum*, *L. spicheri*, and *L. reuteri* was inoculated into cherry and broccoli juices, resulting in increases of 1 and 1.5 Log CFU/mL, respectively, compared to the initial count (Filannino et al. 2015). In contrast, the final LAB count in apple juice fermented with *L. acidophilus*, *L. plantarum*, *L. rhamnosus*, and *L. casei* was reported to be less than 6.5–7.8 Log CFU/mL (Chen et al. 2019).

The growth of LAB during fermentation is influenced by several factors, with the initial inoculum size being one of the most critical determinants (Chen et al. 2022). Typically, LAB grows to a maximum concentration of 8 to 9 Log CFU/g, depending on the medium and fermentation conditions (George et al. 2018). The initial concentration of the inoculum directly affects how quickly and efficient-

ly the LAB can establish themselves in the substrate. A higher initial inoculum generally leads to faster colonization and growth, but it must be balanced to avoid excessive competition for limited nutrients, which could hinder overall bacterial development (Zhong et al. 2023). Additionally, the quality and availability of nutrients in the fermentation medium, including carbohydrates, amino acids, and vitamins, are essential for the optimal growth of LAB. A recent report has highlighted the benefits of using fermented juices as carriers for bacteria with probiotic potential. Besides, the presence of phenolic compounds and the high concentration of simple carbohydrates in fruit juices renders them an ideal medium for these microorganisms, despite their minimal nitrogen sources (Yuan et al. 2024).

Another significant factor influencing LAB growth is the production of lactic acid during fermentation. During the process of LAB metabolizing sugars, the production of lactic acid ensues, which has the capacity to reduce the pH of the medium. This, in turn, engenders an environment that may either promote or impede bacterial growth, contingent upon the specific strain in question (Tian et al. 2021). The excessive accumulation of lactic acid can result in a phenomenon known as acid stress, in which the bacteria's growth rate slows and their metabolic activity diminishes (Othman et al. 2017). However, certain LAB strains exhibit elevated acid tolerance, enabling them to persist and thrive under acidic conditions. Furthermore, the accumulation of other fermentation by-products, such as ethanol and short-chain fatty acids (SCFAs), has been demonstrated to impact bacterial growth. These by-products may function as signals for microbial adaptation, thereby enhancing resistance mechanisms, or they may directly suppress the growth of competing microbes, providing a selective advantage for LAB (Tian et al. 2021).

Fermented products, whether from plant or animal sources, often contain native LAB, which can profoundly impact human health (Miranda et al. 2021). LAB play a key role in modulating the intestinal microbiome by promoting balanced gut flora, enhancing immune function, and contributing to the production of beneficial metabolites such as SCFAs (Pasolli et al. 2020). These bacteria have shown antimicrobial properties, outcompeting pathogenic bacteria and reducing gastrointestinal disorder incidence. Additionally, consuming LAB-enriched fermented foods is associated with various health benefits, including improved digestion, regulation of blood lipids, and potential anti-inflammatory effects. The capacity of LAB to endure and adjust to the gastrointestinal tract's acidic milieu renders them efficacious probiotics, imparting therapeutic benefits to the host while cultivating a more salubrious intestinal ecosystem. Therefore, a comprehensive understanding of the factors influencing LAB growth during fermentation is essential for optimizing the production of these beneficial microorganisms and their utilization in functional foods (Abedin et al. 2024).

Kinetic parameters of the microbial growth

In addition to the observed growth patterns, it is important to examine the kinetic parameters that govern microbial growth during fermentation, including duplication time (g), generation rate, and the specific growth constant (k). These parameters are critical for comprehending the metabolic activity of LAB in blackberry juice, insights into their efficiency and adaptability during the fermentation process. The duration required for the bacterial population to double (g), serves as a critical metric for assessing the efficiency of the fermentation process. This parameter directly reflects the efficiency with which the bacterial population increases, making it a valuable tool for understanding and optimizing the fermentation process. In this study, while the growth trajectory remained predominantly linear, the calculation of duplication time revealed that certain consortiums, such as Co-6, exhibited a faster growth rate compared to others. This observation was substantiated by a significant increase in LAB count at the 24-hour mark.

Furthermore, the generation rate, defined as the number of generations the bacterial population undergoes per hour, can be utilized to ascertain the rate of bacterial proliferation under specific conditions. The specific k, offers a more comprehensive measure of the overall growth efficiency of the bacterial cultures. The generation rate and growth constant are influenced by factors such as nutrient availability, pH, and fermentation by-products. These parameters were calculated and compared across the different LAB consortia in order to assess their relative performance during fermentation. A deeper understanding of these kinetic parameters could facilitate the optimization of fermentation processes, enhance product yields, and applications of LAB in food and probiotic production.

The kinetic parameters describing microbial growth (specific growth rate (μ), g, and k) were determined for each LAB consortia inoculated into thermoultrasonicated

Table 6. Kinetic parameters calculated from the growth curve of each LAB consortium.

Kinetic parameter	LAB consortia on thermoultrasonicated blackberry juice				
	Co-1	Co-6	Co-11	Co-13	C+
m	0.052±0.001^{a}	0.0250±0.006^{b}	0.023±0.001^{b}	0.025±0.006^{b}	0.053±0.002^{a}
g	13.21±0.91^{a}	27.62±0.23^{b}	27.62±0.23^{b}	27.35±0.34^{b}	12.87±0.93^{a}
k	0.075±0.00^{a}	0.036±0.002^{b}	0.034±0.003^{b}	0.036±0.007^{b}	0.077±0.008^{c}

Co-1 (Consortium formed by *L. plantarum* [B1], *L. plantarum* [A1] and *P. acidilactici* [A2]), Co-6 (Consortium formed by *L. brevis* [P2], *P. acidilactici* [A2] and *L. plantarum* [A1]), Co-11 (Consortium formed by *L. plantarum* [B1], *P. acidilactici* [A2] and *L. brevis* [P2]), Co-13 (Consortium formed by *L. brevis* [P2], *L. plantarum* [A1] and *L.plantarum* [B1]) and C+ (Positive control, co-culture o Consortium formed by f *L. rhamnosus* and *L. casei*); CFU Colony Forming Units: n= 3. Different letters indicate significant differences ($p < 0.05$) between LAB mixtures.

blackberry juice (Table 6). The findings indicated substantial disparities ($p < 0.05$) among the tested formulations. The Co-1, composed of *L. plantarum* strains B1 and A1, along with *P. acidilactici* A2, exhibited the highest specific growth rate ($\mu = 0.052 \pm 0.001$ h^{-1}) and a correspondingly low generation time ($g = 13.21 \pm 0.91$ h), closely comparable to the control consortium (C+), which consisted of *L. rhamnosus* and *L. casei* ($\mu = 0.053 \pm 0.002$ h^{-1}; $g = 12.87 \pm 0.93$ h). These two treatments also exhibited the highest growth rate constants ($k = 0.075 \pm 0.000$ for Co-1, and 0.077 ± 0.008 for C+), indicating efficient microbial adaptation and proliferation under the tested conditions.

In contrast, the remaining LAB consortia (Co-6, Co-11, and Co-13) showed significantly lower growth kinetics. Specific growth rates ranged from 0.023 to 0.025 h^{-1}, with generation times nearly doubling that of Co-1 and C+ (ranging from 27.35 to 29.14 h). Similarly, their growth rate constants were markedly lower ($k \approx 0.034$–0.036), suggesting a reduced ability to grow rapidly in the thermoultrasonicated blackberry matrix.

These findings are in line with previous studies reporting that *L. plantarum* strains often exhibit superior growth and acidification rates in fruit-based substrates due to their metabolic flexibility and robustness under mildly acidic conditions (Yuan et al. 2024). Furthermore, the incorporation of *P. acidilactici* in Co-1 may have contributed to synergistic growth dynamics, consistent with reports indicating enhanced fermentation kinetics in mixed LAB cultures (Yang et al. 2025). In contrast, combinations involving *L. brevis* exhibited a tendency towards diminished growth rates, a phenomenon that may be attributed to competitive interactions or diminished adaptability to the polyphenol-rich milieu characteristic of blackberry juice (Maia et al. 2023). Taken together, these results underscore the significance of microbial composition in optimizing probiotic fermentation in fruit-based matrices.

Conclusion

The termoultrasonicated blackberry juice is an optimum medium to fermentation of consortia of wild strains from vegetable sources. The results suggest that certain strain combinations, such as Co-6, promote enhanced growth during fermentation, potentially due to synergistic interactions between them. Juice fermented inoculated with Co-6 could be an alternative as functional because it complies with minimum therapeutic dose. However, the results of the kinetic parameter results suggest that also Co-1 is a promising candidate for the development of functional blackberry-based fermented beverages.

This study underscores the significance of strain selection in optimizing fermentation outcomes and the potential health benefits of LAB-fermented products. The proliferation of beneficial LAB during fermentation has the potential to exert a favorable influence on the human microbiome, thereby promoting benefits such as enhanced gut health and metabolic regulation. Further research is necessary to refine the strain combinations for various fermentation applications and to maximize the functional properties of fermented beverages.

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Additional information

Conflict of interest
The authors have declared that no competing interests exist.

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Data availability
All of the data that support the findings of this study are available in the main text.