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Furan Inhibitor Effects on Native Lactic Acid Bacteria from Maule Region: Growth Kinetics and Lactic Acid Production from Agroindustrial Waste

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Abstract

Native Lactic Acid Bacteria (LAB) isolated from fruits and vegetables in Chile's Maule Region were identified as *Lactiplantibacillus plantarum* and *L. pentosus*. Their growth and lactic acid production were evaluated under furan inhibitor stress (furfural and 5-methylfurfural (5-MF)) relevant to agroindustrial waste valorization. In fruit hydrolysates containing natural furans (11–57 mg/L), LAB grew similarly to inhibitor-free controls and produced 17–25 g/L lactic acid. Under controlled conditions, furfural at 12 g/L reduced bacterial growth by over 80% and decreased lactic acid production to only 2 g/L. In contrast, 5-MF at the same concentration allowed substantially higher tolerance, with up to 7 g/L lactic acid. Benchmarked directly against the commercial reference strain *Lactiplantibacillus plantarum* PL8014 under identical conditions, the native isolate B1 matched or exceeded PL8014's lactic acid output in most furfural and 5-MF concentrations tested, including the two most severe 5-MF conditions. Logistic growth and Luedeking–Piret models accurately described both bacterial growth dynamics and lactic acid production kinetics under all inhibitory conditions ($R^2 > 0.96$). Inhibitor concentration proved more critical than inhibitor type, although furfural consistently showed stronger potency. These findings confirm the biotechnological potential of native Maule LAB and provide quantitative kinetic parameters for optimizing lactic acid production from lignocellulosic waste, supporting circular economy strategies.

Keywords: lactic acid bacteria; furan inhibitors; agroindustrial waste; growth kinetics

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1. Introduction

The rapid progress of industrialization has generated significant benefits for population quality of life. However, this advancement, particularly in the agribusiness sector, has been accompanied by the generation of huge quantities of waste, causing environmental pollution [1]. Today's consumer society model has generated highly complex manufacturing processes that produce increasing amounts of waste [2].

Implementing a circular economy is imperative to valorize the massively generated organic waste. Currently, companies lack valorization pathways that would allow them to transform this waste into valuable byproducts. The traditional disposal methods for agroindustrial wastes are landfill storage and outdoor incineration, which cause further contamination and do not generate valuable products [3], among other environmental impacts. Agroindustrial waste represents a rich source of organic compounds, composed of structural biopolymers such as cellulose, hemicelluloses, pectin, and lignin. In addition, these residues contain waxes, salts, water, starch, tannins, and proteins [4]. These components can be transformed through chemical or biotechnological processes, offering an alternative for developing technologies oriented toward more sustainable conversion, such as biorefineries that can efficiently transform a broad range of biomass feedstocks into commercially feasible biofuels and bioproducts [5]. Specifically, these materials can serve as important substrates for producing several enzymes, such as glycohydrolases and oxidoreductases [6], as well as fermentable substrates rich in carbon and nitrogen, which are essential nutrients for microbial growth.

Among microorganisms of industrial interest, lactic acid bacteria (LAB) stand out for their fundamental role in fermentation processes and food production. Although there is wide diversity of LAB strains, exploring understudied niches, such as organic fruits and vegetables, could lead to the isolation of unique strains with interesting properties. The characterization of local microorganisms through biochemical and molecular approaches is essential to preserve and understand regional microbial diversity. Agroindustrial lignocellulosic waste is particularly interesting due to its high content of polysaccharides, proteins, and starch [7]. However, its rigid structure [8] requires pretreatments such as acid hydrolysis to release fermentable sugars. Unfortunately, these processes generate inhibitory compounds such as furans (furfural, 5-MF, and hydroxymethylfurfural), which cause cellular stress [9] and negatively affect microbial metabolism, protein structure, and in some cases lead to apoptosis [10].

There is a differential impact on homofermentative and heterofermentative LAB. Homofermentative LAB are more susceptible to inhibition by furan derivatives, showing reduced growth rates and metabolic activity, leading to decreased lactic acid production. This is due to their less effective detoxification mechanisms compared to heterofermentative strains. In contrast, heterofermentative LAB exhibit enhanced growth kinetics and sugar consumption in the presence of furan derivatives. This occurs because furfural and HMF promote the reoxidation of NAD and $NADP^+$, generating higher conversion rates, growth, and sugar consumption [11]. They possess more effective detoxification pathways, allowing them to mitigate the inhibitory effects of these compounds and maintain lactic acid production [12]. Furan derivatives can inhibit specific enzymes, cause DNA and cell membrane damage, and elicit cellular redox imbalance [13]. For instance, in *Sporolactobacillus inulinus*, phenolic compounds such as syringaldehyde were found to be more inhibitory than furans, causing membrane damage and affecting key enzymes involved in D-lactic acid fermentation [14]. The presence of furan derivatives can disrupt normal metabolic pathways, leading to reduced efficiency in lactic acid production. This is particularly evident in strains that lack robust detoxification mechanisms [15].

The impact of furans on LAB metabolism is particularly relevant, since these compounds not only inhibit bacterial growth but also affect lactic acid production. Understanding the metabolic response of LAB to these inhibitors is crucial for optimizing the reuse of agroindustrial byproducts, considering that furans can originate from both pretreatments and natural decomposition of waste. The advantages in producing metabolites of interest, combined with the use of low-cost energy sources, represent a significant commercial opportunity. Scaling up these processes requires understanding the optimal conditions

to maximize both bacterial growth and lactic acid production. In this context, the present study aims to taxonomically identify native LAB isolated from fruits and vegetables of the Maule Region, Chile, and evaluate their performance against furan inhibitors. We hypothesized that furfural and 5-MF exert differential inhibitory effects on LAB growth and lactic acid production at equivalent concentrations, with furfural being more potent due to differences in their chemical structure and toxicity mechanisms. To test this, we quantified kinetic parameters using logistic and Luedeking–Piret models across a range of inhibitor concentrations, enabling a structured comparison of both compounds.

2. Materials and Methods

2.1. Bacterial Strains and Culture Conditions

Commercial strains were obtained from stocks stored in the laboratory at −80 °C. Strains derived from the Maule Region were collected by swabbing the surface of local, organically grown fruits and vegetables (beetroot, radish, and strawberry) using sterile peptone water. Serial dilutions of these washings were plated on de Man, Rogosa, and Sharpe (MRS) agar (Merck KGaA, Darmstadt, Germany) and incubated at 37 °C for 48 h under anaerobic conditions. Morphologically distinct colonies were selected, purified by successive streaking on MRS agar, and stored in MRS broth supplemented with 20% (*v*/*v*) glycerol at −80 °C for further characterization.

2.2. Biochemical Characterization of Lactic Acid Bacteria

The biochemical characterization of LAB was performed using a series of standardized tests. Initially, Gram staining was conducted according to the methodology described by [NO_PRINTED_FORM] [16]. Oxygen requirement was evaluated by incubating cultures under simulated anaerobic conditions using the AnaeroCult system (Merck KGaA, Darmstadt, Germany). The catalase test was performed by applying hydrogen peroxide directly onto colonies and observing the absence of bubble formation. Fermentative capacity was analyzed in phenol red broth supplemented with glucose, fructose, and sucrose, where acid production was evidenced by a color change in the indicator from red to yellow. Finally, lactic acid activity was qualitatively confirmed on MRS agar plates supplemented with calcium carbonate, where the formation of dissolution halos around colonies after anaerobic incubation indicated acid production.

2.3. Bacterial Taxonomic Identification

The samples used for DNA extraction were isolated from beetroot, radish, and strawberry, with only beetroot in triplicate. DNA extraction was performed from fresh cultures, obtaining biomass from 1 mL of each sample. Following extraction, an electrophoretic run was performed on a 2% agarose gel. The genomic DNA obtained was amplified by PCR using the DNA sample, distilled water, Enhancer, Taq polymerase, mix, and primers FD1 (27f) (5′-AGAGTTTGATCCTGGCTCAG-3′) and RP2 (1492r) (5′-ACGGCTACCTTGTTACGACTT-3′) [17] with an annealing temperature of 60 °C. The thermocycling conditions were: initial denaturation at 95 °C for 1 min; denaturation at 95 °C for 30 s; annealing at 60 °C for 45 s; extension at 72 °C for 2 min; and final extension at 72 °C for 5 min. The steps from denaturation to extension were repeated for 32 cycles. After amplification, the PCR product was quantified using a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and visualized by 2% agarose gel electrophoresis using GelRed. The DNA was sent for sequencing to the sequencing platform using an ABI PRISM 3500 xl (Applied Biosystems, Foster City, CA, USA) at the Pontifical Catholic University of Chile. Quality control for the 16S sequences was first performed using DNA Baser version 5.21.0 (Heracle BioSoft, Pitești, Romania), removing low-quality nucleotides at both the

beginning and end of the sequence. The second software was BioEdit version 5.0.9 (Ibis Biosciences, Carlsbad, CA, USA), used to assemble the forward and reverse sequences to generate a consensus sequence. The consensus sequence was entered into BLASTn (nr) for analysis and similarity searching against the database. The 16S rRNA gene sequences of strains B1, B2, B3 and F1 have been deposited in the NCBI GenBank database under accession numbers PZ868143, PZ868144, PZ868145 and PZ868146, respectively.

2.4. Cultures with Hydrolysate as Carbon Source

Cultures were performed using hydrolysates from apple and pear waste previously obtained by acid-free thermal hydrolysis: dried fruit residues (peel and endocarp) were ground in an IKA A11 basic analytical mill (IKA-Werke GmbH & Co. KG, Staufen, Germany) and incubated in distilled water at 80 °C for 2 h, following the acid-free thermohydrolysis approach for agricultural residues described by [18], at concentrations of 50 and 85 g/L, respectively. Modified MRS medium (without casein, meat extract, and Tween 80) was used, with the following final composition: 6 g yeast extract, 1.2 g K_2HPO_4, 3 g sodium acetate, 0.12 g $MgSO_4 \cdot 7H_2O$, 1.2 g $(NH_4)_3$, 0.03 g $MnSO_4 \cdot H_2O$. All cultures were developed in flasks (50 mL of MRS medium in 100 mL Erlenmeyer flasks at 37 °C with an initial pH of 6.4). All hydrolysate cultures were performed in three independent biological replicates (n = 3) using the same standardized inoculum (OD_{600} = 0.05) and incubation conditions described in Section 2.5. A control culture using commercial glucose (8 g/L) instead of hydrolysate was included as a reference for non-inhibited growth. Total reducing sugar content of the apple and pear hydrolysates was quantified using the 3,5-dinitrosalicylic acid (DNS) colorimetric method [19], and hydrolysate volumes were adjusted so that all cultures received an equivalent total sugar concentration, thereby isolating the effect of furan content from differences in available carbon source.

2.5. Culture Under Inhibitory Furan Conditions

All cultures were performed in modified MRS medium (composition: 6 g/L yeast extract, 8 g/L glucose, 1.2 g/L K_2HPO_4, 3 g/L sodium acetate, 0.12 g/L $MgSO_4 \cdot 7H_2O$, 1.2 g/L $(NH_4)_3$, 0.03 g/L $MnSO_4 \cdot H_2O$, initial pH 6.4). As a standardized initial inoculum, an $OD_{600nm} = 0.05 \pm 0.01$ ($\approx 1.5 \times 10^7$ CFU/mL) was used from a washed overnight culture. Four concentrations of each inhibitor were evaluated: 3, 6, 9, and 12 g/L for furfural (density 1.29 g/mL) and 5-methylfurfural (5-MF, density 1.1 g/mL). Although natural fruit hydrolysates contain low furan concentrations (under 100 mg/L), the concentrations selected for these assays (3 to 12 g/L) were strategically chosen to simulate severe industrial conditions, such as harsh acid pretreatment of highly recalcitrant lignocellulosic biomass. Testing these extreme ranges allows for the determination of absolute tolerance thresholds of the native strains and ensures a robust mathematical modeling of relative inhibitory potency under worst-case biorefinery scenarios [10]. The following controls were included: (i) Positive control (no inhibitor): medium with 8 g/L glucose without furans; (ii) Sterility control: uninoculated medium; (iii) Basal production control: medium without added glucose. All experiments were performed as three independent biological replicates (n = 3) in 100 mL Erlenmeyer flasks containing 50 mL of medium, incubated at 37 °C for 72 h under orbital agitation (150 rpm). Growth was monitored by spectrophotometry (OD_{600}) twice daily. To ensure measurements remained strictly within the linear range of the Lambert-Beer law (OD_{600} between 0.1 and 0.8), aliquots with higher turbidities were serially diluted with fresh sterile MRS medium prior to reading, and the recorded values were multiplied by the corresponding dilution factor. Lactic acid concentration was quantified every 24 h by HPLC. The concentrations of furfural and 5-methylfurfural in the

apple and pear hydrolysates were quantified prior to inoculation using the GC/MS/MS method described in Section 2.7.

2.6. High-Performance Liquid Chromatography (HPLC)

Lactic acid samples were diluted and filtered (0.45 µm pore size). The equipment used to identify lactic acid concentrations was a YOUNG CHROMPASS HPLC ELSD/UV (Young In Chromass, Anyang, Republic of Korea) with a flow rate of 0.8 mL/min, a Kromasil C18w column (4.6 × 250 mm), a column temperature of 30 °C, an injection volume of 20 µL, and a mobile phase of 100% 10 mM K_2HPO_4. Lactic acid detection was performed at 210 nm.

2.7. SPME-GC/MS/MS Chromatographic Conditions

For the extraction of volatile compounds, 3.5 mL of hydrolyzed sample was taken prior to the extraction procedure. The extraction of volatile compounds from the headspace of the samples was performed using a polydimethylsiloxane/carboxen/divinylbenzene (PDMS/CAR/DVB) fiber at a temperature of 50 °C. The fiber exposure time was 15 min.

The analysis was performed on a Thermo TSQ DUO instrument (Thermo Fischer Scientific, Waltham, MA, USA) operated in Selected Reaction Monitoring (SRM) mode. Helium was used as the mobile phase at a flow rate of 1 mL/min, and the injector temperature was maintained at 230 °C. An RTX-5 ms column was used under the following temperature program: initial temperature of 50 °C held for 1 min, increased to 110 °C at 10 °C/min and held for 10 min, then increased to 166 °C at 3 °C/min and held for 2 min, and finally increased to 270 °C at 30 °C/min. The total run time was 42 min. The ion source and transfer line temperatures were both set to 280 °C.

For the quantification of furfural and 5-methylfurfural, the SRM method was optimized to select appropriate collision energies for the fragmentation of parent ions and the monitoring of daughter ions. Specifically, for furfural, the transition 95 → 39 m/z was used for quantification, and 39 → 37 m/z for qualification. For 5-methylfurfural, the transition 110 → 109 m/z was used for quantification, and 81 → 53 m/z for qualification (Table 1). Qualitative and quantitative data were processed using Chromeleon software version 7.2.10 (Thermo Fisher Scientific, Waltham, MA, USA).

Table 1. Optimized SRM conditions for furfural and 5-methylfurfural.

Analyte	Precursor (*m*/*z*)	Product (*m*/*z*)	Collision Energy (eV)	Type
Furfural	95	39	15	Quantitative
Furfural	39	37	15	Qualitative
5-Methylfurfural	110	109	5	Quantitative
5-Methylfurfural	81	53	5	Qualitative

2.8. Mathematical Modeling

The kinetic mathematical models were applied specifically to evaluate the growth and lactic acid production dynamics of the selected native strain, *L. pentosus* B1. Kinetic mathematical models of bacterial growth have been widely used in predictive microbiology to parametrically model and thus obtain logical models, reliable estimates, and replicability of numerical results. In this sense, the Luedeking–Piret model is quite efficient, adequately fitting and capable of extrapolating various culture scenarios.

$$dX/dt = r(1 - X/K)X$$

$$dC/dt = \beta_1 X + \beta_2 dX/dt$$

where the maximum growth rate r and the maximum medium capacity K are parameters specific to logistic bacterial growth, while β_1 and β_2 represent the rates of lactic acid production excluding and including growth velocity, respectively (Table 2). The inhibitory effect of furfural and 5-MF is presented as a variation in the parameters, including both growth parameters r and K, as well as the Luedeking–Piret coefficients β_1 and β_2 for lactic acid production.

Table 2. Variable/Parameter for modeling.

Variable/Parameter	Definition	Unit of Measurement
X	Bacterial from beets biomass concentration (B1)	Optical Density (OD) at 600 nm
C	Lactic acid concentration	$g{\cdot}L^{-1}$
r	Maximum specific growth rate	h^{-1}
K	Carrying capacity	Optical Density (OD) at 600 nm
β_1	Non-growth-associated lactic acid production coefficient	$g{\cdot}L^{-1}{\cdot}h^{-1}{\cdot}OD^{-1}$
β_2	Growth-associated lactic acid production coefficient	$g{\cdot}L^{-1}{\cdot}OD^{-1}$

Parameters were calculated from the mean of replicates. The fit was performed using nonlinear least squares minimization, employing the Levenberg–Marquardt algorithm from the scipy.optimize package of SciPy 1.16.3 (Python 3.11). The dynamics of bacterial growth and lactic acid production were solved numerically using the scipy.integrate package. Additionally, the NumPy 2.1.3 package was used for matrix arrays and vector operations, and Matplotlib 3.10.0 for visualization of both data and theoretical models. To evaluate the fit of the implemented models, the coefficient of determination (R^2) was used, which measures the proportion of variability explained by the theoretical model versus observed data; the root mean square error (RMSE), which considers the average magnitude of prediction errors in the measurement units of each variable; and the mean absolute error (MAE), which measures the absolute difference between observed and theoretical values. Subsequently, the estimated parameter values were graphically compared at different concentrations of each inhibitor, thus analyzing the compound effect on bacterial growth and lactic acid production.

2.9. Statistical Analysis

Statistical analysis of the data was performed using RStudio, version 4.4.1. Analysis of variance (ANOVA) tests, both simple and two-way, were performed, followed by Tukey's tests to establish comparisons between variables. A confidence level of 95% ($p < 0.05$) was established.

3. Results

3.1. Molecular Characterization of Lactic Acid Bacteria

Initial biochemical screening confirmed the basic characteristics of the LAB isolates. All selected strains (B1, B2, B3, F1, R1) were Gram-positive, catalase-negative, and exhibited robust growth under microaerophilic and anaerobic conditions. Furthermore, they demonstrated active fermentative capacity in phenol red broth supplemented with glucose, fructose, and sucrose, and produced distinct dissolution halos on MRS agar plates supplemented with calcium carbonate, qualitatively confirming strong lactic acid production prior to molecular identification. The sequences obtained were curated and assembled using DNA Baser v5.21.0 and BioEdit v5.0.9. Taxonomic identification was performed using BLASTn against the NCBI database, considering a percentage of identity $\geq$99%. While the 16S rRNA gene sequence analysis revealed that several closely related species

within the *Lactiplantibacillus plantarum* group share >99% sequence identity, *L. pentosus* and *L. plantarum* were reported as the top distinct hits with the highest alignment scores and coverage in the NCBI database. Two predominant species were identified: *Lactiplantibacillus pentosus* and *Lactiplantibacillus plantarum* (Table 3). Of the five isolates characterized, all five showed BLASTn identity $\geq$ 99% to their respective top-hit reference sequences (range 99.25–100%; Table 3), supporting reliable taxonomic assignment at the species level, or at the species-group level for isolates within the closely related *L. plantarum* group. Although *Enterococcus durans* was also isolated from radish (strain R1, 99.82% identity), this strain was excluded from subsequent experiments. *Enterococcus durans* belongs to the family Enterococcaceae and differs substantially from *Lactiplantibacillus* spp. in its fermentative profile and lactic acid yield. Furthermore, certain *Enterococcus* species are classified as opportunistic pathogens, which limits their suitability for biorefinery applications within a circular economy framework. Therefore, subsequent analyses focused exclusively on *Lactiplantibacillus pentosus* and *L. plantarum* strains, which are well-characterized producers of lactic acid with established industrial relevance.

Table 3. Molecular identification of LAB strains isolated from fruits and vegetables in the Maule Region. GenBank accession numbers for the 16S rRNA sequences of strains B1, B2, B3 and F1 are provided in Section 2.3.

Origin	Strain	Identification	% Identity
Beetroot	B1	*Lactiplantibacillus pentosus* SKL-18	100%
Beetroot	B2	*Lactiplantibacillus plantarum* 1873	99.25%
Beetroot	B3	*Lactiplantibacillus pentosus* CUC110	99.90%
Strawberry	F1	*Lactiplantibacillus plantarum* SCZH-7	99.91%
Radish	R1	*Enterococcus durans* AcCk25	99.82%

3.2. Furan Concentrations in Fruit Hydrolysates

The concentrations of furfural and 5-methylfurfural were determined in apple and pear hydrolysates prior to bacterial inoculation using the SPME-GC/MS/MS method. The apple hydrolysate presented furfural and 5-methylfurfural concentrations of 45.12 mg/L and 32.14 mg/L, respectively. In contrast, the pear hydrolysate showed higher levels of both furanic compounds, with furfural reaching 94.25 mg/L and 5-methylfurfural 51.76 mg/L. These values are consistent with the natural formation of furans during mild acid-free hydrolysis of pectin-rich fruit residues and are within the range of concentrations previously reported for similar agroindustrial wastes.

3.3. Growth in Fruit Hydrolysates and Selection of the Working Strain

In cultures utilizing apple and pear hydrolysates containing low organic furan concentrations (11–57 mg/L), the isolated LAB strains demonstrated robust growth and efficient carbohydrate consumption. Lactic acid production reached concentrations between 17 and 25 g/L (Figure 1), confirming that the native microbiota of the Maule Region possesses an implicit tolerance to agricultural degradation byproducts. Specifically, isolate B1 exhibited the highest biomass accumulation and the most rapid initial lactic acid productivity during the first 24 h of the fruit hydrolysate screening. Its performance was directly benchmarked, under identical conditions, against a commercial reference strain (*Lactiplantibacillus plantarum* PL8014; see Section 3.6), positioning it as the most robust candidate for severe stress testing among the strains directly compared. Native isolates B2, B3, and F1 were taxonomically characterized (Table 3) but were not carried forward into the hydrolysate and

furan-inhibition kinetic assays, which focused exclusively on the highest-performing strain (B1) to preserve experimental parsimony.

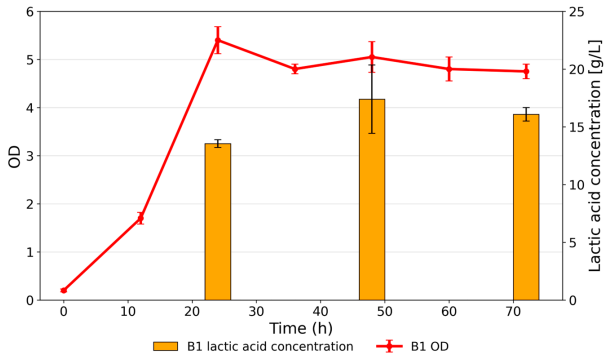


Figure 1. Growth kinetics (OD) and lactic acid production of *Lactiplantibacillus pentosus* B1 in fruit hydrolysates.

Based on these preliminary screening results, the native strain isolated from beetroot (Replicate B1, molecularly identified as *Lactiplantibacillus pentosus*) was selected as the sole design model for the subsequent controlled furan inhibition assays. This selection was justified by its superior kinetic performance, high reproducibility, and rapid adaptation during the hydrolysate fermentation phase, as illustrated in the growth profiles of Figure 1. Consequently, to maintain mathematical parsimony and focus the modeling efforts on the comparative toxicity threshold of the inhibitors, this specific high-yielding native strain (B1) was exclusively used as the biological model for all subsequent high-concentration trials.

3.4. Inhibition of Growth and Production by Furans

In the absence of inhibitors (positive control), strain B1 exhibited unhindered growth reaching a maximum OD_{600} of 6.2 ± 0.15 and a maximum lactic acid production of 14.5 ± 0.3 g/L at 72 h. These values served as the baseline carrying capacity and maximum production reference for the subsequent inhibition models.

3.4.1. Effect of Furfural

Furfural proved to be a potent inhibitor of bacterial growth. At a concentration of 6 g/L, a reduction of over 50% in the growth rate was observed, while at 12 g/L the inhibition exceeded 80% (Figure 2). In parallel, lactic acid production decreased drastically, reaching only 2 g/L at the highest inhibitor concentration.

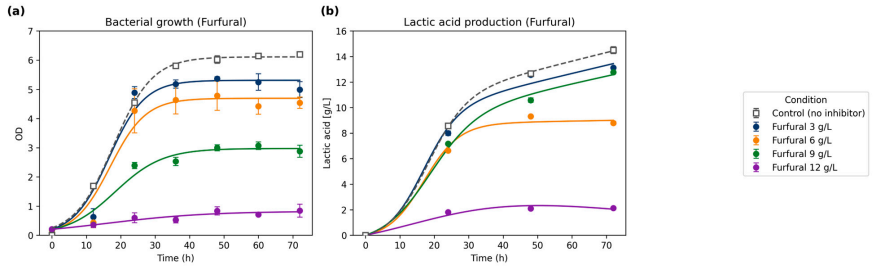


Figure 2. Bacterial growth (**a**) and lactic acid production (**b**) kinetics of *Lactiplantibacillus pentosus* B1 under furfural stress at 3, 6, 9 and 12 g/L. Symbols show the mean ± SD of three biological replicates (error bars shown, some smaller than the symbol); solid lines are the logistic (**a**) and Luedeking–Piret (**b**) model fits. The open gray squares and dashed line show a real triplicate control series (no inhibitor; mean ± SD, n = 3) collected for this response and fitted with the same logistic (**a**) and Luedeking–Piret (**b**) models; the 72 h values (OD 6.2 ± 0.15; 14.5 ± 0.3 g/L lactic acid) match those reported in Section 3.4.

3.4.2. Effect of 5-Methylfurfural (5-MF)

In contrast to furfural, 5-MF exhibited a significantly lower inhibitory effect. Even at the highest concentration tested (12 g/L), LAB maintained a lactic acid production of up to 7 g/L, demonstrating greater tolerance to this compound (Figure 3).

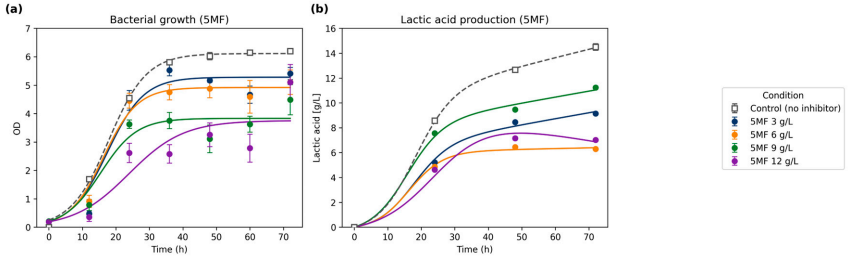


Figure 3. Bacterial growth (**a**) and lactic acid production (**b**) kinetics of *Lactiplantibacillus pentosus* B1 under 5-MF stress at 3, 6, 9 and 12 g/L. Symbols show the mean ± SD of three biological replicates (error bars shown, some smaller than the symbol); solid lines are the logistic (**a**) and Luedeking–Piret (**b**) model fits. The open gray squares and dashed line show a real triplicate control series (no inhibitor; mean ± SD, n = 3) collected for this response and fitted with the same logistic (**a**) and Luedeking–Piret (**b**) models; the 72 h values (OD 6.2 ± 0.15; 14.5 ± 0.3 g/L lactic acid) match those reported in Section 3.4.

3.5. Mathematical Modeling of the Kinetics

The fit of the logistic and Luedeking–Piret models to the experimental data showed coefficients of determination (R^2) greater than 0.96 under most evaluated conditions (Table 4), indicating a high goodness-of-fit. Furthermore, the low values obtained for the Root Mean Square Error (RMSE) and Mean Absolute Error (MAE) robustly validate the predictive capability of the proposed logistic and Luedeking–Piret models across the different tested stress regimes, minimizing the error in estimating actual biomass and product concentrations. The kinetic parameters of growth (r) and carrying capacity (K) decreased monotonically with increasing furfural concentration, whereas for 5-MF they followed a non-linear pattern, with optimal growth observed at intermediate inhibitor concentrations (Figure 4), suggesting differential inhibition mechanisms between the two furanic compounds. Lactic acid production is predominantly influenced by the growth-associated production coefficient (β_2), showing opposite relationships depending on the inhibitor type. The error analysis (RMSE and MAE) revealed that the model has limitations in predicting the inhibitory effect of 5-MF at high concentrations, particularly for bacterial biomass. Statistical analysis (ANOVA, $p < 0.05$) confirmed that the reductions in the kinetic parameters were highly significant for furfural at all tested concentrations compared to the inhibitor-free control.

Table 4. Adjustment measures for the mathematical model.

Inhibitor	Concentration (g/L)		R^2	RMSE	MAE
Furfural	3	Biomass	0.9610	0.4184	0.3064
		Lactic acid	0.9957	0.3443	0.2850
	6	Biomass	0.9734	0.3090	0.2295
		Lactic acid	0.9951	0.2597	0.2150
	9	Biomass	0.9980	0.0503	0.0414
		Lactic acid	1.0000	0.0130	0.0107
	12	Biomass	0.9626	0.0500	0.0393
		Lactic acid	0.9991	0.0267	0.0220

Table 4. *Cont.*

Inhibitor	Concentration (g/L)		R^2	RMSE	MAE
5-MF	3	Biomass	0.9911	0.1386	0.1041
		Lactic acid	0.9998	0.0692	0.0571
	6	Biomass	0.9821	0.2695	0.2014
		Lactic acid	0.9958	0.2409	0.1995
	9	Biomass	0.9048	0.4962	0.3867
		Lactic acid	0.9996	0.0965	0.0795
	12	Biomass	0.8388	0.7792	0.5610
		Lactic acid	0.9379	0.2185	0.1779

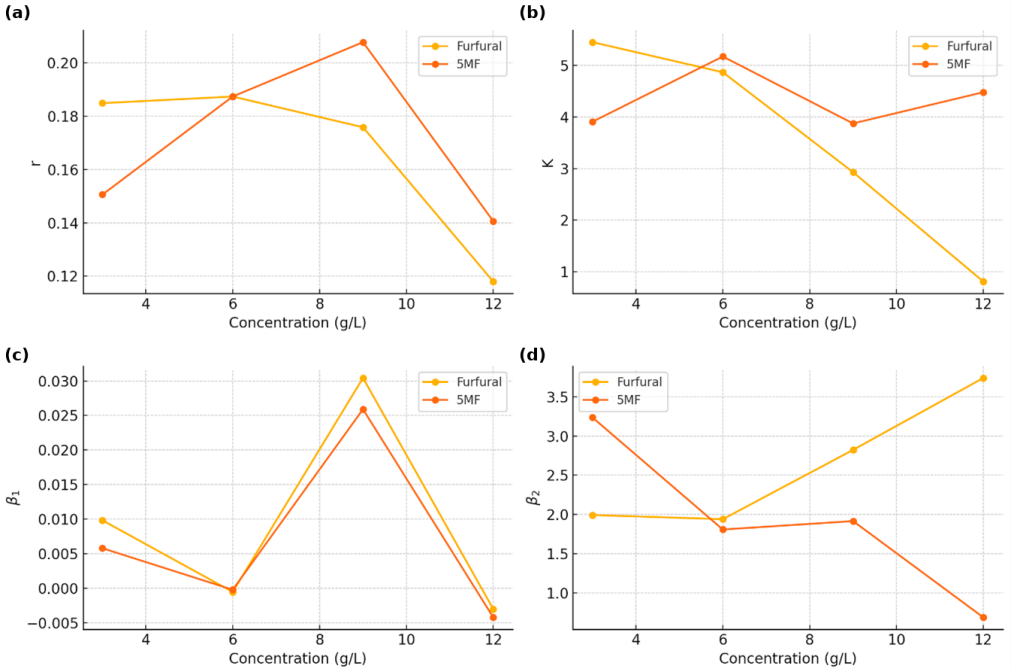


Figure 4. Variation in estimated kinetic parameters for *Lactiplantibacillus pentosus* B1 according to differences in inhibitor type and concentration: (**a**) growth rate *r*; (**b**) carrying capacity *K*; (**c**) non-growth-associated production coefficient β_1; and (**d**) growth-associated production coefficient β_2. In all panels, furfural and 5-MF are identified in the legend of each panel.

3.6. Comparative Performance Against a Commercial Reference Strain

To directly address the comparison with the commercial strain included in the experimental design (Section 2.1), the growth of B1 was benchmarked against the commercial reference strain *Lactiplantibacillus plantarum* PL8014 under identical culture conditions, spanning both fruit hydrolysates and all eight furan-inhibitory conditions (Figures 5 and 6). B1 reached a higher biomass yield than PL8014 at low-to-intermediate furfural concentrations (3, 6 and 9 g/L) and at most 5-MF concentrations, consistent with an adaptive advantage of the native isolate under moderate stress. At the highest furfural concentration tested (12 g/L), this trend reversed: PL8014 maintained a comparatively higher final OD than B1 (relative reduction in final biomass from 3 to 12 g/L of 63.7% for PL8014 versus 83.1% for B1), indicating that the two strains differ not only in overall tolerance but also in how that tolerance scales with inhibitor dose. Growth (OD600) of PL8014 was measured in triplicate under both furfural and 5-MF stress (mean ± SD, $n = 3$), matching B1's replication for these conditions; in the fruit hydrolysates (Figure 5a,b), PL8014 remains a single reference culture.

In addition to biomass, lactic acid production was quantified by HPLC for both strains under both furfural and 5-MF stress, each in triplicate (mean ± SD, n = 3). These results are presented in Figure 7 and Table 5 below.

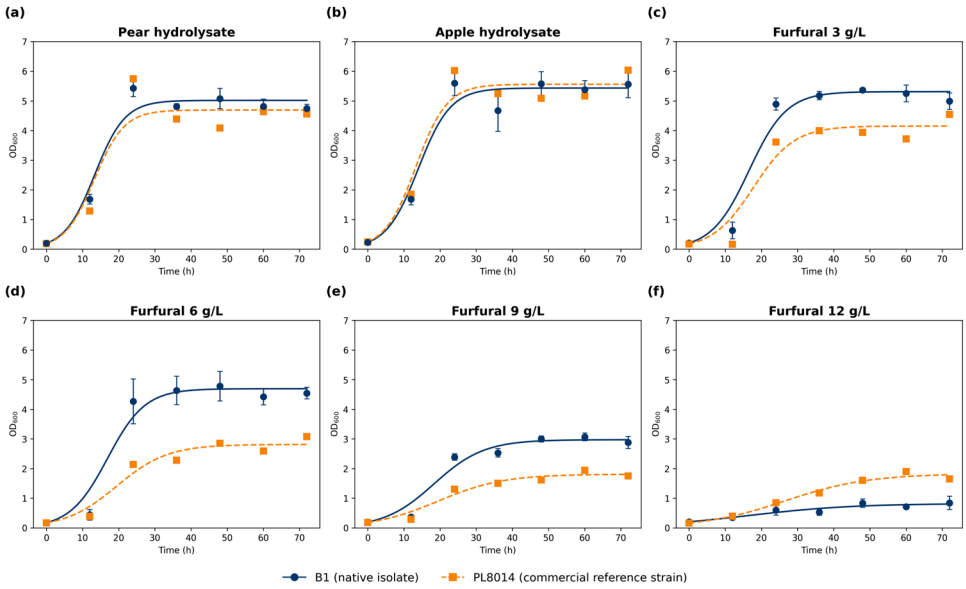


Figure 5. Comparative growth (OD600) of the native isolate *Lactiplantibacillus pentosus* B1 (solid navy circles, mean ± SD, n = 3) and the commercial reference strain *Lactiplantibacillus plantarum* PL8014 (orange squares) in pear (**a**) and apple (**b**) hydrolysates (PL8014: single reference culture) and under furfural stress at 3 (**c**), 6 (**d**), 9 (**e**) and 12 (**f**) g/L (PL8014: mean ± SD, n = 3, matching B1's replication). Lines show logistic growth model fits.

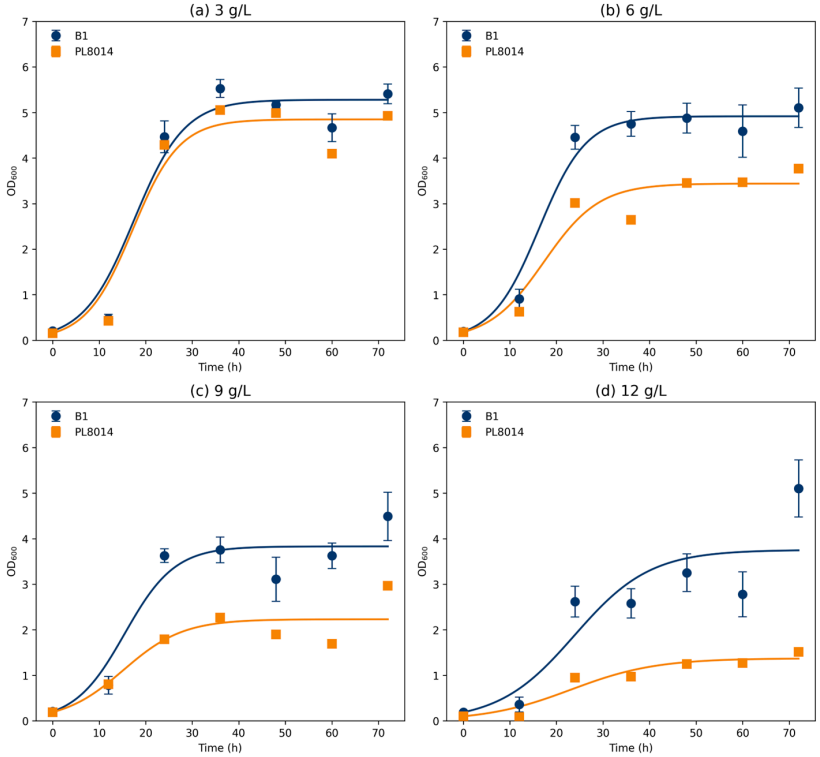


Figure 6. Comparative growth (OD600) of the native isolate *Lactiplantibacillus pentosus* B1 (solid navy circles, mean ± SD, n = 3) and the commercial reference strain *Lactiplantibacillus plantarum* PL8014 (orange squares, mean ± SD, n = 3) under 5-MF stress at 3 (**a**), 6 (**b**), 9 (**c**) and 12 (**d**) g/L. Both strains were assayed in triplicate under this condition. Lines show logistic growth model fits.

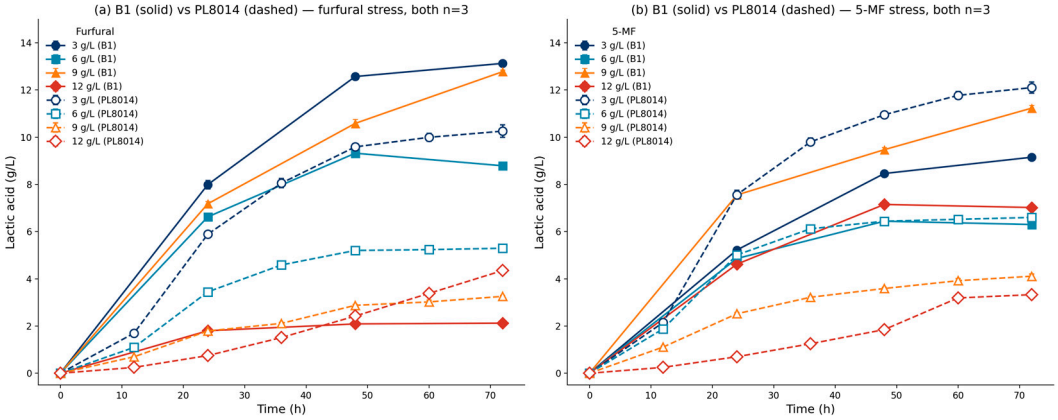


Figure 7. Measured lactic acid production over time by the native isolate *Lactiplantibacillus pentosus* B1 (filled symbols, solid lines) and the commercial reference strain *Lactiplantibacillus plantarum* PL8014 (open symbols, dashed lines) under furfural stress (**a**) and 5-MF stress (**b**), at 3, 6, 9 and 12 g/L. B1 and PL8014 values (both panels) are the mean ± SD of three biological replicates (error bars shown, some smaller than the symbol). B1 was sampled at 24, 48 and 72 h in both conditions; PL8014 was sampled every 12 h. Lines connect measured points and do not represent a fitted model.

Table 5. Lactic acid production (g/L) at 72 h for B1 and PL8014 under furfural and 5-MF stress (mean ± SD, n = 3 for all).

Concentration (g/L)	B1—Furfural (Mean ± SD)	PL8014—Furfural (Mean ± SD)	B1—5-MF (Mean ± SD)	PL8014—5-MF (Mean ± SD)
3	13.12 ± 0.10	10.26 ± 0.27	9.15 ± 0.08	12.10 ± 0.23
6	8.79 ± 0.14	5.29 ± 0.11	6.30 ± 0.06	6.60 ± 0.13
9	12.77 ± 0.08	3.26 ± 0.03	11.23 ± 0.12	4.11 ± 0.09
12	2.12 ± 0.01	4.35 ± 0.09	7.02 ± 0.05	3.33 ± 0.03

Lactic acid production followed a dose-dependent decline consistent with the growth results. Under furfural stress, B1 produced 13.12 ± 0.10, 8.79 ± 0.14, 12.77 ± 0.08 and 2.12 ± 0.01 g/L of lactic acid at 72 h at 3, 6, 9 and 12 g/L, respectively (mean ± SD, n = 3; Figure 7a, Table 5). Under 5-MF stress, B1 produced 9.15 ± 0.08, 6.30 ± 0.06, 11.23 ± 0.12 and 7.02 ± 0.05 g/L (mean ± SD, n = 3) at the same concentrations, while PL8014 produced 12.10 ± 0.23, 6.60 ± 0.13, 4.11 ± 0.09 and 3.33 ± 0.03 g/L (mean ± SD, n = 3; Figure 7b, Table 5). Because both strains were assayed under 5-MF, this condition allows a direct, inhibitor-matched comparison: at low concentrations (3 and 6 g/L), PL8014 modestly outproduced B1, whereas at 9 and 12 g/L this pattern reversed, with B1 producing 2.7- and 2.1-fold more lactic acid than PL8014, respectively. This crossover mirrors the growth-based pattern described above, in which B1's tolerance advantage becomes more pronounced at higher inhibitor concentrations, and reinforces that B1's robustness under severe furanic stress extends from biomass accumulation to product formation. Under furfural stress, PL8014 produced 10.26 ± 0.27, 5.29 ± 0.11, 3.26 ± 0.03 and 4.35 ± 0.09 g/L of lactic acid at 72 h at 3, 6, 9 and 12 g/L, respectively (mean ± SD, n = 3; Figure 7a, Table 5). This comparison shows a crossover consistent with the growth data: B1 outproduced PL8014 at 3, 6 and 9 g/L, but PL8014 produced more lactic acid than B1 at 12 g/L (4.35 vs. 2.12 g/L), mirroring PL8014's comparatively greater tolerance to severe furfural stress already observed for biomass accumulation.

Across the eight furfural and 5-MF concentrations tested, B1 produced more lactic acid than PL8014 in five of eight conditions (furfural at 3, 6 and 9 g/L; 5-MF at 9 and 12 g/L), while PL8014 produced more only at furfural 12 g/L and at the two lowest 5-MF concentrations (3 and 6 g/L).

4. Discussion

4.1. Biotechnological Potential of Native Strains

The identification of *L. pentosus* and *L. plantarum* in fruits and vegetables of the Maule Region corroborates the presence of native LAB with biotechnological potential. The ability of these strains to develop in fruit hydrolysates with low furan concentrations (11–57 mg/L) suggests their adaptation to environments with natural inhibitors, representing an advantage for applications in biorefineries. While native *Lactiplantibacillus* strains are often valued for food-safety and probiotic applications, our experimental data instead highlight a distinct, industrially relevant property: robust tolerance to severe furanic stress. Compared with other furan-tolerant LABs recently reported, our native *L. pentosus* B1 strain demonstrates exceptional resilience. For instance, some robust *L. plantarum* isolates have been characterized for producing lactic acid at maximum thresholds of 8.0 g/L of furfural or 6.0 g/L of hydroxymethylfurfural (HMF) [20], and extensive metabolic engineering or adaptive evolution is often required to push tolerance limits beyond 10 g/L in other lactic acid producers [21]. By comparison, our kinetic models show that the native strain B1 can sustain lactic acid production (up to 7 g/L) even under extreme stress conditions of 12 g/L of 5-MF. This substantial natural tolerance is highly competitive against the baseline resistance of other heterofermentative and homofermentative strains documented in lignocellulosic detoxification studies [11,12]. The ability to maintain production decoupled from severe biomass inhibition highlights unique metabolic pathways in these regional strains, making them exceptional candidates for the direct valorization of highly recalcitrant agroindustrial waste without prior detoxification. It is important to acknowledge that distinguishing between *L. pentosus* and *L. plantarum* species using solely 16S rRNA sequencing presents classical taxonomic limitations due to their high genetic similarity. Consistent with this, BLASTn analysis of the deposited 16S rRNA sequences against the NCBI 16S ribosomal RNA (Bacteria and Archaea) type-strain database showed nearly identical percent identities between the top-ranked *L. plantarum* and *L. pentosus* hits (differences $\leq$ 0.07%, corresponding to a single nucleotide across the ~1440 bp alignment), underscoring that species-level resolution within the *L. plantarum* group requires additional markers such as recA. However, the exceptional functional performance of strain B1 validates its selection for the proposed biotechnological applications, regardless of definitive species-level resolution.

Beyond this literature comparison, the direct, side-by-side benchmarking against the commercial reference strain *Lactiplantibacillus plantarum* PL8014 (Section 3.6) provides more definitive evidence of B1's industrial competitiveness. Under identical culture conditions, B1 produced more lactic acid than PL8014 in five of the eight furfural and 5-MF concentrations tested, including the two highest-severity 5-MF conditions (9 and 12 g/L), where B1 outproduced PL8014 by 2.7- and 2.1-fold, respectively. PL8014 matched or exceeded B1 only at the two lowest 5-MF concentrations (3 and 6 g/L) and at the most severe furfural stress tested (12 g/L)—the latter consistent with PL8014's comparatively greater growth tolerance under that specific condition (relative reduction in final biomass from 3 to 12 g/L furfural of 63.7% for PL8014 versus 83.1% for B1; Section 3.6). Taken together, these results indicate that the native isolate B1 performs at least as well as, and under several of the more industrially relevant high-stress conditions considerably better than, an established commercial production strain, supporting its viability as a native alternative for lactic acid production from furan-containing lignocellulosic hydrolysates.

4.2. Differential Inhibition Mechanisms by Furans

While the specific intracellular mechanisms of inhibition were not directly measured in this study, the severe depression of the kinetic growth parameters (r and K) mathematically

modeled in our experiments strongly aligns with hypothesized mechanisms in the literature, such as cell membrane disruption and energy diversion toward detoxification pathways. The marked difference in inhibition exerted by furfural and 5-MF can be attributed to their distinct chemical properties and toxicity mechanisms. [NO_PRINTED_FORM] [15] attribute the greater toxicity of furfural to its capacity to disrupt cell membrane integrity and alter key enzyme systems. Our results, showing an 80% reduction in growth at 12 g/L of furfural, agree with [NO_PRINTED_FORM] [22], who reported a significant effect on the adaptive phase of microbial cultures from 2 g/L of furans [22]. The non-linear relationship observed between inhibitor concentration and lactic acid production suggests the existence of critical thresholds and possible microbial detoxification mechanisms. Particularly interesting is the behavior of 5-MF, which, despite reducing bacterial growth, allowed substantial lactic acid production, indicating a possible uncoupling between growth and metabolic production under stress conditions. The stark contrast in the decay profiles of the kinetic parameters exhibiting a monotonic collapse for furfural versus a non-linear adjustment for 5-MF strongly implies divergent stress response routes. Nevertheless, future transcriptomic and metabolomic assays are required to definitively confirm these distinct intracellular detoxification mechanisms.

Some LAB, such as *Levilactobacillus brevis* (formerly *Lactobacillus brevis*), have shown strong resistance to furan derivatives, utilizing detoxification pathways to maintain lactic acid production without the need for external detoxification processes [23]. The addition of certain nutrients, such as glutamic acid, has been shown to reverse the inhibitory effects of compounds like maltol on *Lacticaseibacillus casei* (formerly *Lactobacillus casei*), suggesting a potential strategy to mitigate furan inhibition [24]. While furan derivatives pose a significant challenge to lactic acid production, the ability of certain LAB to detoxify these compounds offers promising avenues for industrial applications. For example, in *Limosilactobacillus reuteri*, in the presence of 1 g/L furfural, the growth rate was stimulated by about 25% and biomass yield by 15%, with furfural also being rapidly assimilated from the culture as an electron acceptor [25]. The development of robust strains through metabolic engineering or the use of nutrient supplementation could enhance the efficiency of lactic acid fermentation processes. Additionally, understanding the specific mechanisms of inhibition and detoxification can guide the selection and optimization of bacterial strains for biofuel and biochemical production from lignocellulosic biomass. However, the variability in response among different strains highlights the need for tailored approaches to address the specific challenges posed by furan derivatives in microbial fermentation.

4.3. Implications of Mathematical Modeling

The excellent fit of the kinetic models ($R^2 > 0.96$) validates their usefulness for predicting LAB behavior under inhibitory conditions. The kinetic parameters r and K exhibited a monotonically decreasing trend with increasing furfural concentrations. These decreases reflect a progressive inhibitory effect, which may be due to membrane damage, increased energy costs for cellular stress responses and detoxification pathways, or enzyme inactivation [26–28]. The non-linear behavior observed for 5-MF suggests more complex inhibition mechanisms, possibly related to adaptation or detoxification processes. This may indicate a partial adjustment of metabolism that allows LAB to maintain metabolic activity and lactic acid synthesis despite the slowdown in biomass growth, which is common under inhibitor-induced stress [26]. The model's limitations in accurately predicting biomass under high 5-MF concentrations (RMSE = 0.7792 at 12 g/L) indicate the need to incorporate additional parameters that capture the specific tolerance mechanisms developed by LAB. From a modeling perspective, robust and well-fitted kinetic models are a valuable tool for evaluating sustainable and competitive bioprocesses, particularly when responses to in-

hibitor stress are non-linear and dispersed, as observed here for 5-MF. This fit was achieved without overfitting, preserving the principle of parsimony while still representing bacterial dynamics accurately [29,30].

4.4. Perspectives and Future Studies

Greater sensitivity to furfural than to 5-MF has important implications for the design of biorefinery processes. Pretreatment strategies that minimize furfural formation or allow its selective removal could significantly improve yields in lactic acid production from lignocellulosic waste. The ability of native LAB to produce lactic acid in the presence of 5-MF suggests their potential for fermentation processes using hydrolysates with specific inhibitor profiles. Future studies should explore the capacity of these strains to detoxify inhibitory media and evaluate their performance under conditions closer to industrial ones. To fully elucidate the molecular mechanisms responsible for the observed differences in tolerance, additional research encompassing transcriptomic and proteomic analyses is required. Furthermore, evaluating the effect of furan mixtures and their interaction with other common lignocellulosic inhibitors, alongside exploring microbial conditioning strategies, will be critical to enhance strain robustness for large-scale applications.

5. Conclusions

This study demonstrates the presence of native LAB (*Lactiplantibacillus pentosus* and *L. plantarum*) in fruits and vegetables of the Maule Region, with notable biotechnological potential for lactic acid production from agroindustrial waste. The strains showed the capacity to develop efficiently in fruit hydrolysates with low natural furan concentrations (11–57 mg/L), producing 17–25 g/L of lactic acid. The evaluation of furanic inhibitors revealed that furfural exerts a significantly greater inhibitory effect than 5-MF, reducing bacterial growth by more than 80% and lactic acid production to just 2 g/L at a concentration of 12 g/L. Inhibitor concentration proved to be a more determining factor than its type, although furfural showed greater inhibitory potency from concentrations as low as 3 g/L. The applied mathematical models adequately described the microbial kinetics under inhibitory stress ($R^2 > 0.96$), validating their use for the optimization of fermentative processes. Direct benchmarking against the commercial reference strain *Lactiplantibacillus plantarum* PL8014 under identical conditions further showed that B1 matched or outperformed this established production strain in most furfural and 5-MF concentrations tested, particularly under the more severe stress conditions, supporting its potential as a native alternative for industrial lactic acid production. Future research should explore pretreatment strategies that minimize furfural formation, as well as microbial adaptation mechanisms that allow improving tolerance to these inhibitors, thus optimizing the sustainable production of lactic acid. While these findings highlight the biotechnological potential of native LAB from the Maule Region, it is important to note that these assays were conducted under controlled laboratory conditions. Further research, including scale-up studies in bioreactors and the evaluation of complex inhibitor mixtures, is necessary to fully assess their viability for industrial biorefineries. Nevertheless, the quantitative kinetic parameters obtained provide a valuable baseline that contributes to the ongoing development of agroindustrial waste valorization and circular economy strategies.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The 16S rRNA gene sequences generated in this study for strains B1, B2, B3 and F1 have been deposited in the NCBI GenBank database under accession numbers PZ868143, PZ868144, PZ868145 and PZ868146, respectively (see Table 3). Other data that support the findings of this study, including the raw growth curves and lactic acid production values, are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

LAB	Lactic Acid Bacteria
5-MF	5-Methylfurfural
MRS	de Man, Rogosa and Sharpe
HPLC	High-Performance Liquid Chromatography
SPME	Solid-Phase Microextraction
GC/MS/MS	Gas Chromatography/Tandem Mass Spectrometry
PDMS/CAR/DVB	Polydimethylsiloxane/carboxen/divinylbenzene
SRM	Selected Reaction Monitoring
OD	Optical Density
CFU	Colony Forming Units
DNA	Deoxyribonucleic Acid
PCR	Polymerase Chain Reaction
RMSE	Root Mean Square Error
MAE	Mean Absolute Error
ANOVA	Analysis of Variance

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