Biotecnia
Journal of biological and health sciences
http://biotecnia.unison.mx
Universidad de Sonora
ISSN: 1665-1456

*Original Article*

# Evaluation of a minimal prickly pear-based medium for the growth of *Lactobacillus* spp.

Evaluación de un medio mínimo a base de nopal para el crecimiento de *Lactobacillus* spp.

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## ABSTRACT

This study aimed to evaluate the potential of dehydrated wild prickly pear (*Opuntia robusta*) for the growth of *Lactobacillus* spp. A minimal medium based on prickly pear and peptone (NPM) was formulated and separately inoculated with five *Lactobacillus* strains. Growth kinetics were performed to determine growth, total sugar consumption, pH behavior, and lactic acid production compared with a commercial culture medium (MRS). Results show that NPM provides the minimum substrates necessary for the growth of the strains evaluated. *L. fermentum* 634 was the most efficient, reaching an $OD_{630}$ of 0.7667±0.0527 and 1.0639±0.0616 in NPM and MRS, respectively. Notably, no lag phase (λ = 0) was observed in NPM, suggesting rapid bacterial adaptation, while in MRS, *L. fermentum* 634 presented a lag phase of 1.137 h. The generation time in NPM (2.67 h) was comparable to that in MRS (2.31 h), indicating efficient bacterial replication. These results suggest that dehydrated prickly pear could serve as a foundation for formulating culture media. This can be enhanced by incorporating specific substrates necessary for bacteria, optimizing sustainable and economical industrial fermentation processes.

**Keywords:** *Lactobacillus* spp., Wild prickly pear, *O. robusta*, Minimal medium.

## RESUMEN

El objetivo de este estudio fue evaluar el potencial del nopal silvestre deshidratado (*Opuntia robusta*) para el crecimiento de *Lactobacillus* spp. Se formuló un medio mínimo a base de nopal y peptona (NPM) el cual fue inoculado por separado con las cinco cepas de *Lactobacillus.* Se realizaron cinéticas en las cuales se determinaron el crecimiento, el consumo de azúcares totales, el comportamiento del pH y la producción de ácido láctico, comparando con el medio de cultivo comercial (MRS). Los resultados muestran que el medio NPM proporciona los sustratos mínimos necesarios para el crecimiento de las cepas evaluadas, siendo *L. fermentum* 634 la más eficiente, alcanzando una $OD_{630}$ de 0.7667 ± 0.0527 y 1.0639 ± 0.0616 en NPM y MRS respectivamente. Notablemente, no se observó fase de latencia (λ = 0) en NPM, lo que sugiere una rápida adaptación bacteriana, mientras que en MRS *L. fermentum* 634 presentó una fase de latencia de 1.137 h. El tiempo de generación en NPM (2.67 h) fue comparable al de MRS (2.31 h), lo que indica una replicación bacteriana eficiente. Estos resultados sugieren que el nopal puede utilizarse como base para la formulación de medios de cultivo, los cuales pueden mejorarse añadiendo sustratos específicos requeridos por las bacterias, con el fin de optimizar procesos de fermentación industrial sostenibles y económicos.

**Palabras clave:** *Lactobacillus* spp., Nopal silvestre, *O. robusta,* medio mínimo.

## INTRODUCTION

The development of adequate culture media is essential for the growth of lactic acid bacteria such as *Lactobacillus* spp., which is widely used in the food, pharmaceutical, and biotechnology industries. *Lactobacillus* spp plays a key role in food fermentation and produces beneficial compounds, such as organic acids, bacteriocins, and B-complex vitamins, making them essential microorganisms in the formulation of probiotic products (Zheng *et al*., 2020). Likewise, it is necessary for its propagation and maintenance, a culture medium that provides the essential nutrients for its metabolism and growth (Miranda *et al*., 2023).

The MRS is the standard medium for the growth of *Lactobacillus* spp. due to its balanced content of carbohydrates, nitrogen, and minerals, however, its high cost limits its use in large-scale industrial applications, which has driven the search for more accessible and sustainable alternatives (Renschler *et al*., 2020). Among the options evaluated, agro-industrial sources such as whey, cereal extracts, and plant residues have been studied with the purpose of totally or partially replacing the components of the MRS (Ślizewska and Chlebicz-Wójcik, 2020).

*Opuntia robusta* (*O. robusta*) is a wild cactus whose chemical composition makes it an attractive substrate for culture media formulation. It contains 10-15% carbohydrates, including glucose, fructose, and xylose, which can be metabolized by *Lactobacillus* spp. In addition, its dietary fiber, primarily composed of mucilage and pectin, plays a prebiotic role, promoting bacterial growth (Monteiro *et al*., 2023). It is also rich in minerals, such as calcium, magnesium, and potassium, which are essential for bacterial metabolism and the production of organic acids (Reyes Escogido *et al*., 2023).

Unlike other agro-industrial sources such as whey or cereals, *O. robusta* has some advantages that make it a promi-

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**Received: January 7, 2025**
**Accepted: April 8, 2025**
**Published: May 21, 2025**


DOI: 10.18633/biotecnia.v27.2550

sing alternative to develop a new cost-efficient medium based on endemic plants. *Opuntia robusta* is an endemic plant that grows in arid and semi-arid regions and requires little maintenance (Reyes-Escogido *et al*., 2023). Its availability and low production costs allow its use in large-scale industrial processes, unlike MRS media, which includes components that increase its cost (Derabli *et al*., 2022). The nutritional composition and accessibility of *O. robusta* represent a viable and ecologically sustainable option for the growth of *Lactobacillus*.

Bacteria of the genus *Lactobacillus* have been widely used in the food industry to produce yogurt, cheeses, pickles, and other fermented products (Wang *et al*., 2021). Their ability to ferment carbohydrates and produce lactic acid makes them essential in improving food texture, flavor, and preservation (Zheng *et al*., 2020). Additionally, various species of this genus have been recognized as microorganisms with GRAS status (Generally Recognized as Safe), allowing their application in probiotic products with benefits for human health (Yadav *et al*., 2022). Several studies have reported on the ability of *Lactobacillus* to metabolize various sugars as well as complex carbohydrates so that this ability can be an advantage for the formulation of culture media based on economic and sustainable substrates such as *O. robusta* (Derabli *et al*., 2022). The main objective of this study was to evaluate the potential of a minimal medium based on *O. robusta* and peptone (NPM) for the growth of *Lactobacillus* spp., for which parameters such as optical density, carbohydrate consumption, and pH were determined in the *Lactobacillus* spp cultures in NPM comparing them with MRS.

## MATERIAL AND METHODS

### Bacterial strains

The *Lactobacillus* strains used in this work were *Lactobacillus* sp. 319, *Lactobacillus plantarum* 601, *Lactobacillus fermentum* 634, *Lactobacillus paraplantarum* 936, and *Lactobacillus ruminis* 1291. These strains belong to the strain collection of the Metabolism Research Laboratory at the University of Guanajuato and are stored at -80°C. Before use, they were reactivated by inoculating into MRS medium (Merck Millipore, Burlington, MA, USA) through two successive cultures, which were incubated at 37°C for 24-48 hr.

### Preparation and proximate analysis of dehydrated prickly pear

Young cladodes (2 to 3 weeks old) of wild prickly pear *O. robusta* were collected. The collection occurred in Zacatecas, Mexico (22° 13′ 35.76″ N and 101° 44′ 19.85″ W). After removing the spines, the cladodes were washed with water and placed on absorbent paper to remove excess water. The cladodes were cut into 1x1 $cm^2$ cubes and dried at 50°C for 24 hours. Subsequently, the dried cladodes were ground using a coffee grinder; the powder was passed through a 50-mesh sieve to obtain a particle size of 300 µm. The powdered prickly pear was stored in sealed containers at room temperature for later use.

Proximate analysis of the dehydrated prickly pear was performed using AOAC methods (2000). Moisture was determined by weight loss of the sample after drying at 105°C for 4 hours in a drying oven (LabTech, model LDO-080F, Mexico). Total ash was determined by incinerating the sample in a muffle furnace (Felissa FE-360, Mexico) at 550°C. Total fats were determined by gravimetric method using a Soxhlet apparatus with ethyl ether for extraction. For protein determination, a Kjeldahl apparatus (Labconco, USA) was used, and total dietary fiber determination was performed using the TDF kit (Sigma, Aldrich, St. Louis, Missouri, USA) according to the supplier's instructions. For total carbohydrate determination, the sample was prepared according to Monrroy *et al*. (2017), quantification was performed using the anthrone-sulfuric acid method, glucose was used as a standard, and absorbance was read at 630 nm in a microplate reader (Stat Fax ® 4200).

### Culture media

A minimal medium based on prickly pear and peptone (NPM) was prepared without pH adjustment. For these media, 20 grams of dehydrated prickly pear and 10 grams of peptone were weighed per liter of distilled water. MRS medium was also prepared according to the supplier's instructions. All media were sterilized at 121°C for 15 minutes.

It should be noted that tests were previously carried out to assess the ability of *Lactobacillus* to use the *O. robusta* cactus as a substrate. We evaluated a medium consisting only of *O. robusta* (20g/L) (MN), the MRS that replaces glucose with *O. robusta* (20g/L) (MRS-N), and a medium made with *O. robusta* (20g/L) and peptone (10 g/L) (NPM). We decided to use the NPM for this study based on the results.

### Growth of *Lactobacillus* spp. in NPM at different pH levels

*Lactobacillus* spp. strains were inoculated onto solid NPM media and adjusted to different pH levels (6.5, 6.0, 5.5, 5.0, and 4.5). The solid media contained 13 grams of agar-agar per liter and were incubated at 37°C. Bacterial growth was evaluated by observing colonies at 24, 48, and 72-hour intervals. All experiments were performed in triplicate to ensure reproducibility of results.

### Growth kinetics

All strains were reactivated in MRS medium, incubated at 37°C under aerobic conditions, and three successive cultures were performed before the kinetics were conducted.

For the kinetics, Erlenmeyer flasks were prepared with NPM and MRS medium, which was used as a control. Each medium was inoculated with 10% (v/v) of fresh culture. The flasks were incubated at 37°C under aerobic conditions, and strain growth was monitored by reading absorbance at 630 nm in a microplate reader (Stat Fax ® 4200) at 0, 2, 4, 6, 24, 28, and 48 hours.

The cultures were centrifuged at 6,000 rpm for 10 minutes at 8°C (CENTURION Centrifuge FAB U.K.), and the supernatant was recovered for the following assays.

**Determination of pH and lactic acid**

The pH of supernatants obtained at each time point was determined using a potentiometer (Bourns). A graph of pH versus time was plotted. Lactic acid production was quantified by volumetric titration using a standardized 0.1 N NaOH solution (Sigma-Aldrich) and phenolphthalein as an indicator, with the final pH ranging between 8.5 and 8.9. Lactic acid was reported in g/L using the following modified equation from Bouteille *et al*. (2013):

Lactic acid concentration (g/L) = [V × N × PM (lactic acid) / Vm]

Where:
V is the volume of NaOH used in the titration (in L).
N is the concentration of NaOH (0.1 N).
PM (lactic acid) is the molecular weight of lactic acid (90.08 g/mol).
Vm is the sample volume taken for titration (10 mL).

Each experiment was performed in triplicate to ensure the accuracy and reproducibility of the results.

**Determination of total sugars**

To determine the consumption of available sugars in the medium during bacterial growth, total sugars in the supernatant were measured at the previously mentioned time points using a modified anthrone-sulfuric acid method (Hodge and Hofreiter, 1962). Anthrone was prepared at 0.2% in sulfuric acid. For the reaction, the supernatant was diluted 1:100. To 150 µL of the dilution, 600 µL of anthrone-sulfuric acid (Sigma-Aldrich) was carefully added, cooled in a 4°C water bath, mixed, and incubated at 80°C for 10 minutes. After cooling to room temperature, absorbance was read at 630 nm. Glucose was used as a standard for the calibration curve. The assays were performed in triplicate, and average values were obtained.

**Estimation of growth curve using the Gompertz Model**

The growth curve estimation for *Lactobacillus* spp. Strains were performed using the modified Gompertz model (Zwietering *et al*., 1990). The analysis was conducted using Microsoft Excel due to its accessibility and efficient capability for non-linear curve fitting.

The Gompertz model equation was used to fit the experimental data of optical density as a function of time:

$$OD(t) = A \times \exp\left(-\exp\left(\frac{\mu}{A} \times e^{(\lambda - t)} + 1\right)\right)$$

Where:
OD(t): is the optical density at time t.
A: is the upper limit of optical density, representing the maximum achievable optical density.
µ: is the maximum growth rate, indicating the speed of growth during the exponential phase.
λ: is the inflection time, marking the moment when the growth rate is maximum.

Non-linear optimization techniques were applied using Excel's Solver tool to fit the equation to the experimental data. This tool minimized the squared error between observed values and those predicted by the model. As a result, the model parameters (A, µ, λ) were estimated with high precision, and the generation time (GT) was calculated for each strain cultured in each medium, providing a robust and accurate representation of the growth dynamics of the studied strains.

**Statistical analysis**

Statistical analysis was performed using IBM SPSS Statistics software (version 26, IBM Corp., Armonk, NY, USA). Before conducting any tests, data normality was assessed using the Shapiro-Wilk test. Group comparisons were analyzed using one-way analysis of variance (ANOVA) for normally distributed data, with p-values < 0.05 considered statistically significant. The non-parametric Kruskal-Wallis test was employed for data that did not meet normality assumptions. Subsequently, significance values were adjusted using Bonferroni correction for multiple comparisons. In all analyses, p-values < 0.05 were considered statistically significant.

## RESULTS AND DISCUSSION

**Growth kinetics of *Lactobacillus* spp. strains**

Numerous studies describe the physicochemical characteristics of nopal. In this study, proximate analysis of dehydrated nopal revealed a moisture content of 6.67±0.04%, total fiber of 38.74±0.93%, total ash of 19.95±0.05%, total proteins of 4.81±0.10%, total fats of 1.2±0.03%, and total carbohydrates of 36.23±0.12%. These values align with literature reports, which indicate that nopal has a low protein content, generally between 1% and 5% by dry weight, and high dietary fiber and carbohydrate content (Cruz-Rubio *et al*., 2021; Estrada-Sierra *et al*., 2024). Given nopal's low protein content, peptone was added to the culture medium as an additional protein source. Peptone supplements nopal's protein deficiency, providing amino acids and peptides necessary for bacterial growth; it has been shown that peptone supplementation of alternative media is essential for bacterial growth, in this case, because *O. robusta* is deficient in protein (~4.81 ± 0.10%), it was necessary to add peptone as a nitrogen source. Li *et al*. (2022) optimized an economical medium for *Bacillus coagulans* and *Clostridium butyricum* growth, highlighting that the peptone provided essential amino acids and peptides enhancing growth even on unconventional substrates.

Ayivi *et al*. (2022) reported that supplementing alternative plant-based media improves the viability of *Lactobacillus* spp. by increasing the availability of essential nutrients. Other studies have reported that peptone is a determining factor in increasing the cellular performance during the fermentations of *Lactobacillus* spp. on unconventional substrates, highlighting its role in the optimization of alternative media (Kavak *et al*., 2023).

One-way analysis of variance (ANOVA) revealed significant differences in Gompertz-adjusted OD growth kinetics (Table 1) among different strains and culture media at 48

**Table 1.** Growth kinetics and pH of *Lactobacillus* spp. after 48 hours, adjusted using the Gompertz model.
**Tabla 1.** Cinética de crecimiento y pH de *Lactobacillus* spp. después de 48 horas, ajustados mediante el modelo de Gompertz

| Strains | Growth Kinetics (OD 630nm) Media ± SD | | pH Media ± SD | |
|---|---|---|---|---|
| | MRS | NPM | MRS | NPM |
| *L.* sp. 319 | 1.3230 ± 0.0227*** | 0.8649 ± 0.0889*** | 4.21 ± 0.27*** | 4.25 ± 0.33*** |
| *L. plantarum* 601 | 1.4426 ± 0.1027*** | 1.0094 ± 0.0266*** | 4.19 ± 0.12*** | 3.16 ± 0.28*** |
| *L. fermentum* 634 | 1.0639 ± 0.0616*** | 0.7667 ± 0.0527*** | 4.49 ± 0.19*** | 4.02 ± 0.30*** |
| *L. paraplantarum* 936 | 1.4718 ± 0.0885*** | 0.9789 ± 0.0264*** | 4.27 ± 0.33*** | 3.79 ± 0.33*** |
| *L. ruminis* 1291 | 1.0160 ± 0.0498*** | 0.6546 ± 0.0297*** | 4.53 ± 0.17*** | 3.74 ± 0.16*** |

Note: The values are presented as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) was used to compare the means between strains and culture media. All differences were statistically significant (***p < 0.0001). MRS: Man, Rogosa, and Sharpe; NPM: Nopal Peptone Medium.

hours ($p < 0.001$). In MRS medium, *Lactobacillus paraplantarum* 936 showed the highest growth with an OD of 1.4718 ± 0.0885, followed by *Lactobacillus plantarum* 601 with an OD of 1.4426 ± 0.1027. Strains *Lactobacillus* sp. 319, *Lactobacillus fermentum* 634, and *Lactobacillus ruminis* 1291 showed ODs of 1.3230 ± 0.0227, 1.0639 ± 0.0616, and 1.0160 ± 0.0498, respectively.

In NPM medium, *Lactobacillus plantarum* 601 exhibited the best growth with an OD of 1.0094 ± 0.0266, followed by *Lactobacillus paraplantarum* 936 with an OD of 0.9789 ± 0.0264. Strains *Lactobacillus* sp. 319, *Lactobacillus fermentum* 634, and *Lactobacillus ruminis* 1291 showed ODs of 0.8649 ± 0.0889, 0.7667 ± 0.0527, and 0.6546 ± 0.0297, respectively.

As expected, bacterial growth in the MRS medium was superior to that observed in NPM (Figure 1) because glucose is available as simple sugar in the MRS medium, while in the NPM medium, most carbohydrates form complex molecules like polysaccharides and fibers. These molecules require more significant metabolic activity from bacteria to hydrolyze bonds and release sugars, which can then be utilized (Wang *et al*., 2021).

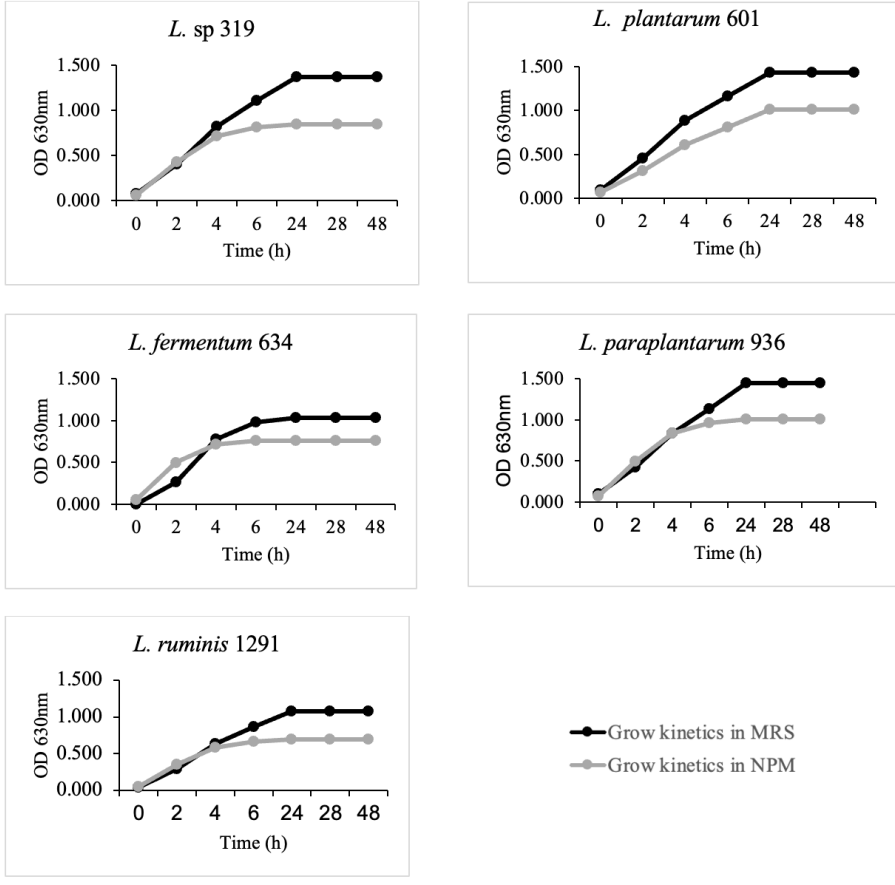


**Figure 1.** Growth kinetics of *Lactobacillus* (*L.*) sp. 319, *L. plantarum* 601, *L. fermentum* 634, *L. paraplantarum* 936, and *L. ruminis* 1291 in MRS (black line) and NPM (gray line) media. Growth was monitored by measuring optical density (OD) at 630 nm over 48 hours.
**Figura 1.** Cinéticas de crecimiento de *Lactobacillus* sp. 319, *L. plantarum* 601, *L. fermentum* 634, *L. paraplantarum* 936 y *L. ruminis* 1291 en MRS (línea negra) y NPM (línea gris). El crecimiento se monitoreó midiendo la densidad óptica (DO) a 630 nm durante 48 horas.

Given this context, it is crucial to evaluate growth parameters such as maximum growth rate (μmax), lag phase (λ), and generation time (GT) to understand better the viability and efficiency of alternative culture media (Table 2). These parameters facilitate an analysis of how different strains perform in terms of growth speed and efficiency, establishing a strong foundation for optimizing medium formulation and enhancing microbial production (Kavak *et al*., 2023).

**Lag phase duration (λ)**

A notable aspect in comparing both media was the duration of the lag phase (λ). Strains cultured in NPM medium, formulated with peptone and nopal, showed no lag phase, initiating growth immediately after inoculation. This suggests that the NPM medium offers conditions for rapid bacterial growth initiation. In contrast, some strains showed a significant lag phase in the MRS medium. *Lactobacillus fermentum* 634 presented a lag phase of 1.137 hours, while *Lactobacillus ruminis* 1291 exhibited a phase of 0.389 hours. These results suggest that MRS medium, while commonly used in lactic acid bacteria fermentation, might need a longer adaptation period for certain strains compared to NPM medium.

This differential behavior may be related to each medium's specific composition. The availability and nature of nutrients in the culture medium are determining factors in microbial growth dynamics. A formulation optimizing nutrient availability has been suggested to reduce or eliminate the lag phase, which appears to be the case for the NPM medium in this study (Khalfallah *et al*., 2023).

The elimination of the lag phase observed in the NPM medium can be attributed to the immediate availability of amino acids necessary for the initial protein synthesis provided by peptone and the carbohydrates provided by *O. robusta*. Although complex, *O. robusta* contains fermentable sugars that bacteria can use after a brief adaptation period. Recent studies have indicated that combining nitrogen and carbohydrate sources can significantly reduce the lag phase in alternative media (Cruz-Rubio *et al*., 2021).

**Maximum growth rate (μmax)**

Maximum growth rates also showed differences between the two media. In the NPM medium, *Lactobacillus fermentum* 634 reached a μmax of 0.260 $h^{-1}$, slightly lower than observed in MRS (0.299 $h^{-1}$). *Lactobacillus plantarum* 601 experienced a more significant reduction in its maximum growth rate in NPM (0.156 $h^{-1}$) compared to MRS (0.227 $h^{-1}$). Although a slight decrease in growth rates was observed in the NPM medium, the absence of a lag phase suggests that, despite this decrease, NPM still provides a favorable environment for rapid initiation of bacterial growth.

Using flour as the primary carbohydrate source in the alternative medium evaluated by Ślizewska and Chlebicz-Wójcik (2020), provides a relevant comparison. In their research, they used a semi-solid medium composed mainly of flours, such as wheat and barley, combined with yeast extracts and peptone as a nitrogen source for culturing *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus fermentum*, and *Lactobacillus acidophilus*. Although some strains showed superior growth in the MRS medium compared to the alternative medium, adequate growth was maintained in the latter, demonstrating that alternative media can be viable and efficient for probiotic production (Ślizewska and Chlebicz-Wójcik, 2020). These results align with those obtained in the present study, where the NPM medium proved highly effective despite a slight reduction in maximum growth rate, especially by not presenting a lag phase.

**Generation time (GT)**

Generation Time (GT) was another evaluated parameter, and strains showed similar generation times in both media. *Lactobacillus* sp. 319 had a GT of 3.16 hours in MRS and 3.22 hours in NPM, indicating similarity in the efficiency of both media to maintain bacterial growth once initiated. This similarity in generation time suggests that once bacteria have grown, both media can effectively sustain their growth. This finding aligns with observations from other studies, which indicate that while generation times may be comparable among different culture media, the initial growth phase and maximum rate can vary based on medium formulation (Ślizewska and Chlebicz-Wójcik, 2020).

The results suggest that the NPM medium has the potential to be a viable alternative to the MRS medium for culturing *Lactobacillus* spp. The absence of a lag phase in NPM suggests that this medium could be particularly useful in applications where rapid growth initiation is critical. These findings are consistent with other works establishing the importance of medium formulation in microbial growth dynamics (Fink, Held, and Manhart, 2023). Medium composition not only affects the lag phase but also the overall efficiency

**Table 2.** Growth parameters of *Lactobacillus* spp. cultures in MRS and NPM.
**Tabla 2.** Parámetros de crecimiento de *Lactobacillus* spp. cultivado en MRS y NPM.

| Strain | μmáx [h−1] | | λ [h] | | TG [h] | |
|---|---|---|---|---|---|---|
| | MRS | NPM | MRS | NPM | MRS | NPM |
| *L.* sp. *319* | 0.219 | 0.215 | 0.163 | 0 | 3.16 | 3.22 |
| *L. plantarum* 601 | 0.227 | 0.156 | 0 | 0 | 3.06 | 4.44 |
| *L. fermentum* 634 | 0.299 | 0.260 | 1.137 | 0 | 2.31 | 2.67 |
| *L. paraplantarum* 936 | 0.212 | 0.246 | 0 | 0 | 3.27 | 2.81 |
| *L. ruminis* 1291 | 0.179 | 0.177 | 0.389 | 0 | 3.88 | 3.92 |

μmax: maximum growth rate; λ: duration of the delay phase; TG - generation time; *MRS:* Man, Rogosa, and Sharpe; NPM: Nopal Peptone Medium.

of bacterial growth, as observed in the results obtained with NPM medium in this study. Furthermore, it has been demonstrated that alternative media, when well-formulated, can offer results comparable to traditional media (Ślizewska and Chlebicz-Wójcik, 2020), reinforcing the validity of NPM medium as a competitive option.

**pH behavior in culture media**

The pH of the medium is a crucial factor in the growth of lactic acid bacteria. In this study, *Lactobacillus* strain growth was evaluated in an NPM medium at native pH (4.7) and at pH 4.5, 5.0, 5.5, 6.0, and 6.5.

Strain growth was observed to be similar across all evaluated pH ranges, including the medium's native pH. However, growth was more consistent and, in some cases, slightly superior in the NPM medium without pH adjustment. These results suggest that *Lactobacillus* strains can adapt well to the NPM medium's natural pH, reducing the need for additional adjustments, as adjusting pH is somewhat time-consuming due to nopal's viscous consistency during culture medium preparation. Therefore, it was decided to use an NPM medium at its native pH of 4.7 for subsequent experiments, as it provided optimal conditions for bacterial growth without the need to modify pH.

At the study's start (0 hours), all strains recorded a pH of 5.70 in the MRS medium and 4.7 in the NPM medium (Figure 2). During the assay, pH level variations were detected among different strains and the two media. At 48 hours, ANOVA analysis showed significant differences in pH, specifically between strains and culture media ($p < 0.001$). In MRS medium, pH values ranged from 4.21 ± 0.27 in *Lactobacillus* sp. 319 to 4.53 ± 0.17 in *Lactobacillus ruminis* 1291. In NPM medium, pH values ranged from 3.16 ± 0.28 in *Lactobacillus plantarum* 601 to 4.25 ± 0.33 in *Lactobacillus* sp. 319 (Table 1).

During strain growth in both media, a progressive pH decrease was observed due to organic acid production, such as acetic acid, propionic acid, and primarily lactic acid. This ability to acidify the medium is crucial for their adaptability and fermentation efficacy. Lactic acid production, typical of *Lactobacillus* spp., is responsible for pH reduction (Szczerbiec *et al*., 2022). Additionally, *Lactobacillus plantarum* and *Lactobacillus crispatus* have been shown to produce

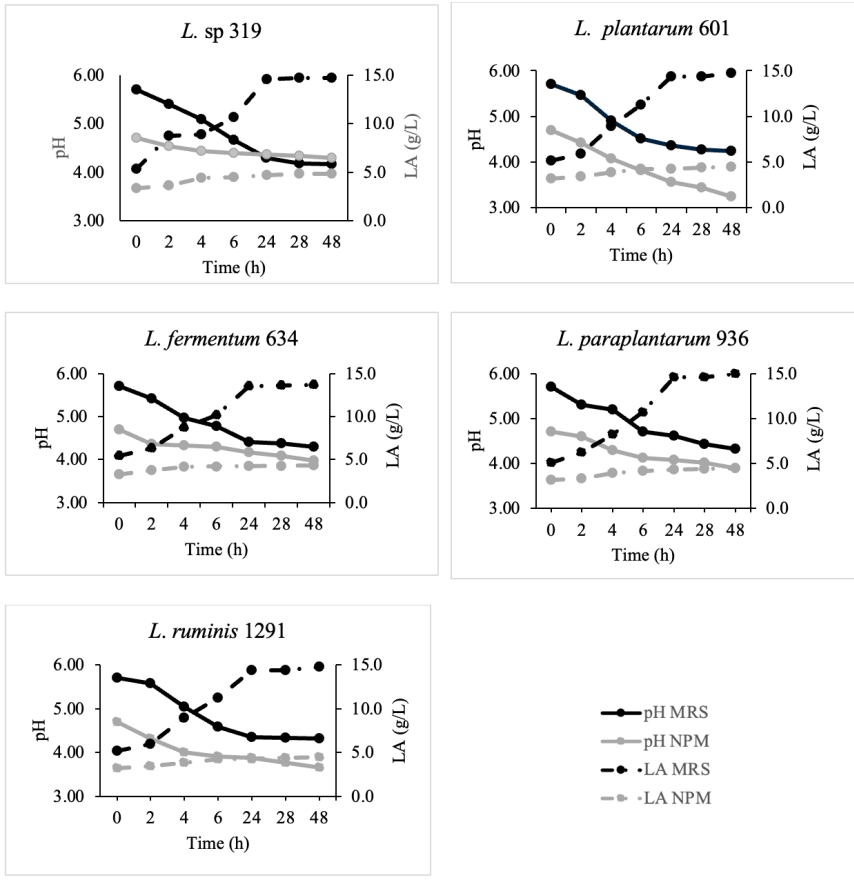


**Figure 2.** Lactic acid (LA) production and pH changes during the growth of *Lactobacillus* (*L.*) sp 319, *L. plantarum* 601, *L. fermentum* 634, *L. paraplantarum* 936, and *L. ruminis* 1291 over 48 hours in MRS (black lines) and NPM (gray lines) media. The solid lines represent pH, the dotted lines represent lactic acid concentration in g/L.

**Figura 2.** Producción de ácido láctico (LA) y cambios de pH durante el crecimiento de *Lactobacillus* sp 319, *L. plantarum* 601, *L. fermentum* 634, *L. paraplantarum* 936 y *L. ruminis* 1291 durante 48 horas en MRS (líneas negras) y NPM (líneas grises). Las líneas continuas representan el pH, las líneas punteadas representan la concentración de ácido láctico en g/L.

these and other organic acids, contributing to pathogenic microorganism inhibition (Zhou *et al*., 2021). This phenomenon is essential in food preservation and fermented product production, as low pH inhibits pathogenic and spoilage microorganism growth.

Ślizewska *et al*. (2020) demonstrated that different *Lactobacillus* strains vary in their ability to acidify the medium, with *Lactobacillus plantarum* standing out for its efficiency in lactic acid production. In their research, this strain reduced medium pH to levels below 4.0 under optimal conditions, underscoring its robustness and adaptability in various substrates, including carrot, beetroot, tomato extracts, whey, and agroindustrial residues. These findings support NPM's viability as an alternative and sustainable culture medium in our study.

**Lactic acid production**

Lactic acid production is a key indicator of *Lactobacillus* strains' fermentative activity. During fermentation, *Lactobacillus* produces various organic acids, including butyric acid, acetic acid, and lactic acid, with the latter being the most relevant due to its widespread use and demand in the food and industries (Wang *et al*., 2021). Therefore, the concentration of lactic acid produced was determined to measure the fermentative activity of the strains used in this study (Figure 2).

According to the results obtained, at 48 hours, a significant variation in lactic acid production was observed between strains and culture media (Table 3). The Kruskal-Wallis test showed significant differences in lactic acid production ($p < 0.005$). In MRS medium, strains *Lactobacillus paraplantarum 936* and *Lactobacillus ruminis* 1291 presented the highest yield with medians [Q1, Q3] of 16.1732 [15.0673, 16.2594] g/L and 15.1668 [15.1090, 15.5403] g/L, respectively. The high lactic acid production in the MRS medium can be attributed to glucose availability, which is the only sugar present and is easily fermented by lactic acid bacteria, favoring growth and metabolic activity (Szczerbiec *et al*., 2022).

In NPM medium, *Lactobacillus ruminis* 1291 and *Lactobacillus paraplantarum* 936 presented the highest yields with medians [Q1, Q3] of 4.6578 [4.5526, 4.6706] g/L and 4.5978 [4.4105, 4.7030] g/L, respectively. The lower production in the NPM medium may be due to the diversity of sugars present, which are not as easily fermentable by lactic acid bacteria compared to the glucose available in the MRS medium (Albergamo *et al*., 2022). The sugar composition in the nopal includes glucose, galactose, arabinose, xylose, and galacturonic acid, among others. This sugar diversity can influence fermentation and lactic acid production by *Lactobacillus* bacteria (Wang *et al*., 2021). Research on the use of plant products fermented with *Lactobacillus* indicates that lactic fermentation of plant matrices increases the nutritional value and sensory quality of final products, thus improving their acceptance and market potential (Le Rouzic *et al*., 2022).

These findings highlight the significant influence of culture medium on the fermentative capacity of *Lactobacillus* spp., which is crucial for optimizing industrial processes that employ these bacteria in the production of lactic acid and other fermentative products. In particular, the NPM medium demonstrates its ability to sustain lactic acid production, which is relevant for exploring more economical and sustainable culture media (Xavier *et al*., 2024).

**Carbohydrate utilization**

The analysis of total carbohydrates is crucial for understanding the metabolic efficiency of *Lactobacillus* strains in different culture media. As carbohydrates are the main energy source for these bacteria during lactic fermentation, evaluating their consumption allows determination of the strains' capacity to metabolize available sugars, which is critical for optimizing the production of lactic acid and other bioactive compounds in alternative media such as nopal (Derabli *et al*., 2022).

Total carbohydrate consumption by lactic acid bacteria was determined at 48 hours in both culture media (Figure 3). Initial total carbohydrate concentrations were 20 g/L in MRS and 5.35 g/L in NPM, with concentrations at 48 hours differing for each strain, as shown in Table 3. The Kruskal-Wallis test indicated significant differences in total carbohydrate content between strains and culture media at 48 hours ($p < 0.005$).

**Table 3.** Lactic acid (LA) production and total carbohydrate (TC) concentration in *Lactobacillus* spp. cultures after 48 hours.
**Tabla 3.** Producción de ácido láctico (AL) y concentración de carbohidratos totales (CT) en los cultivos de *Lactobacillus* spp. después de 48 horas.

| Strains | Lactic acid (g/L) Median [Q1, Q3] | | Total Carbohydrates (g/L) Median [Q1, Q3] | |
|---|---|---|---|---|
| | MRS | NPM | MRS | NPM |
| *L.* sp. 319 | 14.1463 [13.7333, 14.5090] ** | 5.0176 [4.8861, 5.0898] ** | 1.8293 [1.8118, 1.8685]* | 1.4158 [1.3352, 1.4614]* |
| *L. plantarum* 601 | 14.5317 [14.1250, 14.6505] ** | 4.2640 [4.2289, 4.5146]** | 0.6026 [0.5896, 0.6276]* | 2.1122 [2.0587, 2.1130]* |
| *L. fermentum* 634 | 12.5397 [12.4957, 13.4604] ** | 4.2658 [4.2520, 4.3457]** | 2.9922 [2.9161, 3.1182]* | 1.1350 [1.1288, 1.1844]* |
| *L. paraplantarum* 936 | 16.1732 [15.0673, 16.2594] ** | 4.5978 [4.4105, 4.7030]** | 2.1595 [2.0994, 2.2021]* | 1.4610 [1.4503, 1.4916]* |
| *L. ruminis* 1291 | 15.1668 [15.1090, 15.5403] ** | 4.6578 [4.5526, 4.6706]** | 3.7540 [3.7455, 3.9519]* | 1.5250 [1.5218, 1.6660]* |

Note: The values of LA and TC are presented as median [Q1, Q3]. The non-parametric Kruskal-Wallis test was used to compare the medians between strains and culture media. Significance values were adjusted using Bonferroni correction for multiple comparisons. All differences were statistically significant ($p < 0.001$; $p < 0.005$).

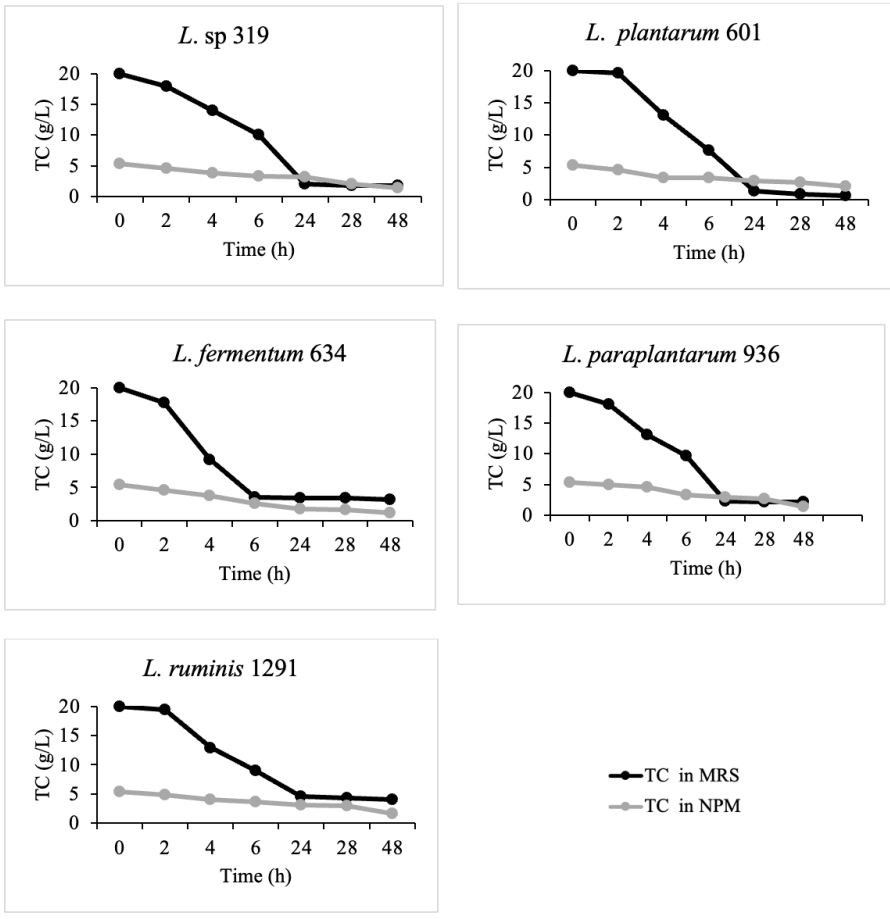


**Figure 3.** Total carbohydrate (TC) consumption by *Lactobacillus* sp. 319, *L. plantarum* 601, *L. fermentum* 634, *L. paraplantarum* 936, and *L. ruminis* 1291, in MRS (black line) and NPM (gray line) media. Carbohydrate consumption was monitored over 48 hours, showing a significant reduction in MRS compared to NPM.

**Figura 3.** Consumo de carbohidratos totales (CT) por *Lactobacillus* sp. 319, *L. plantarum* 601, *L. fermentum* 634, *L. paraplantarum* 936 y *L. ruminis* 1291 en medios MRS (línea negra) y NPM (línea gris). El consumo de carbohidratos se monitoreó durante 48 horas, mostrando una reducción significativa en MRS comparado con NPM.

In the MRS medium, *Lactobacillus ruminis* 1291 showed the highest total carbohydrate concentration with a median [Q1, Q3] of 3.7540 [3.7455, 3.9519] g/L, while *Lactobacillus plantarum* 601 showed the lowest at 0.6026 [0.5896, 0.6276] g/L. In NPM medium, *Lactobacillus plantarum* 601 had the highest total carbohydrate concentration at 2.1122 [2.0587, 2.1130] g/L, while *Lactobacillus fermentum* 634 had the lowest at 1.1350 [1.1288, 1.1844] g/L. This lower total carbohydrate concentration in *Lactobacillus fermentum* suggests high efficiency in breaking down and utilizing complex carbohydrates, possibly due to enzymes like amylases and glucosidases. These enzymes cleave glycosidic bonds, releasing simple sugars rapidly metabolized through glycolysis, indicating a good metabolic capacity for carbohydrate utilization in complex media like NPM (Zheng *et al*., 2020).

This study's results highlight the potential of fermentation in alternative media such as NPM. Despite the complexity introduced by nopal, all strains significantly reduced total carbohydrates, suggesting that with proper optimizations, NPM can be an effective and sustainable medium for lactic acid production. *Lactobacillus plantarum* 601 showed a notable ability to utilize glucose in an MRS medium efficiently, reflected in the lowest carbohydrate concentration. However, the capacity to metabolize carbohydrates in the NPM medium was more limited, as demonstrated by the higher concentration. This could indicate that *Lactobacillus plantarum* 601 lacks the necessary enzymes to hydrolyze glycosidic bonds in nopal's complex carbohydrates (Papadopoulou *et al*., 2023). Nevertheless, *Lactobacillus* can adapt to various nutrient sources, maintaining metabolic activity even under less favorable conditions (Zheng *et al*., 2020).

Papadopolulou *et al*. (2023) used hydrolysates of *Saccharina latissima* (seaweed) and other residues to grow *Lactobacillus*; they reported the production of lactic acid, like our findings with NPM. Ślizewska and Chlebicz-Wójcik (2020) reported that in a medium based on corn, wheat, barley, and rye flour, the maximum growth rate of *Lactobacillus* was higher than in MRS. Durable *et al*. (2022) highlighted the potential of *Opuntia ficus-indica* as a carbon source, supporting its use in sustainable industrial processes. This observation

is in accordance with our findings, where the NPM medium supported the growth of *Lactobacillus* strains, although the final optical density was lower than that in MRS. These similarities suggest that, although alternative media may not match the performance of commercial media, they offer a viable and sustainable option for lactic fermentation, especially in regions where conventional resources are limited or expensive. Although bacterial growth and lactic acid production in the NPM medium is lower than in MRS, an advantage could be the reduction or elimination of the lag phase, which can be an industrial benefit since the bacterial adaptation time is shortened. This effect could be exploited in processes where it is crucial that growth starts quickly and could be optimized through supplementation with specific nutrients or even metabolic engineering of the strains to improve their efficiency in NPM, as Cruz-Rubio *et al*. (2021) suggested.

The differences in the growth of *Lactobacillus* spp. in the NPM compared with MRS can be explained by variations in the metabolic capacities of each strain to use complex carbohydrates. Recent studies reported that *Lactiplantibacillus plantarum* has genetic diversity regarding the presence of genes involved in carbohydrate metabolism, among which can be found those related to the metabolism of cellobiose, galactose, arabinose, and xylose, all of them present in the nopal (Cui *et al*., 2021). Furthermore, enzymes such as amylases and glucosidases are important for hydrolyzing these complex carbohydrates, so their differential expression could influence their growth (Huligere *et al*., 2023). Furthermore, the two-component system (TCS) identified in *Lactobacillus plantarum* acts as a key regulator for its adaptation to different carbohydrate sources, which could explain the differences in adaptability to NPM medium present among *Lactobacillus* strains (Papadopoulou *et al*., 2023). The observed variability in the growth of *Lactobacillus* can be attributed both to the differences in enzymatic profile and to the composition of the medium, where the diversity of sugars in cactus represents a metabolic challenge that only some strains are adapted to overcome efficiently. Our findings are according to this since the NPM medium provided a suitable environment for bacterial growth despite its lower carbohydrate content and complex sugar composition; also, they are consistent with these investigations since the NPM medium provided a suitable environment for bacterial growth despite its lower carbohydrate content and complex sugar composition. This behavior supports the proposal of using NPM in industrial fermentation processes, particularly in regions where the cactus is abundant, contributing to cost reduction and sustainability in the production of metabolites or probiotic propagation.

## CONCLUSIONS

The NPM could be used as an alternative to MRS since it was used by *Lactobacillus* spp as an energy source. In general, all *Lactobacillus* evaluated grew in NPM; *Lactobacillus fermentum* 634 was the strain that showed the best growth. The reduction of total carbohydrates in NPM means that *Lactobacillus* can metabolize the carbohydrates provided by *O. robusta*. The ability of this strain to grow without a lag phase and efficiently use available carbohydrates highlights the potential of *O. robusta* as a substrate in fermentation processes. Although the growth of the strains in NPM was lower than in MRS, there is a rapid adaptation to NPM, which suggests that this medium can maintain the growth of *Lactobacillus* efficiently. It is necessary to continue with trials in which the cactus is evaluated and combined with other substrates to obtain economical and efficient means to produce biomass or metabolites at an industrial level. This presents new opportunities to reduce costs and improve sustainability in food biotechnology using local resources such as the cactus.

## ACKNOWLEDGMENTS

The Consejo Nacional de Humanidades, Ciencia y Tecnología (CONAHCYT) is acknowledged for the grant awarded under the Postdoctoral Fellowship Program for Mexico, which enabled the completion of this research.

## CONFLICTS OF INTEREST

The authors have no financial conflicts of interest to declare.

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