OPTIMIZING LACTIC ACID PRODUCTION BY *Lacticaseibacillus rhamnosus* ATCC 7469 IN DIFFERENT GROWTH MEDIA

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ABSTRACT

The study aimed to evaluate the ability of *Lacticaseibacillus rhamnosus* ATCC 7469 to produce lactic acid using standard MRS medium (MRSp) and a formulated MRS medium (MRSF). The investigation involved the analysis of the pH profiles, glucose concentration, biomass, and lactic acid during fermentation, with subsequent calculation of biomass and lactic acid yields, along with maximum productivity. The results revealed a higher final lactic acid concentration in MRSF (21.15 g/L), compared to MRSp (18.21 g/L). However, MRSp showed a higher maximum productivity (0.88 g/L/h) achieved in 24 hours, whereas MRSF required 48 hours. The results indicate a similar variation in the pH profile of MRSf and MRSp media, decreasing from approximately 5.7 to 3.5 and 3.6, respectively. MRSF demonstrated superiority as an ideal medium for *L. rhamnosus* ATCC 7469 growth and lactic acid production, with a production difference of 14%. The microorganism showed efficiency in lactic acid production, highly favorable yield and maximum productivity. The study serves as a basis for future research, necessitating further exploration of the metabolism and physiology details of *L. rhamnosus*.

Keywords: Fermentation. Lactic Acid Bacteria. *Lactobacillaceae*. Organic Acids. Secondary Metabolism.

1 INTRODUCTION

Lactic acid is the most abundant organic acid in nature, and as forms, it can exist as D (-) and L (+)[1]. This acid is essential in several industries due to its versatility,[2] being widely used in food, pharmaceuticals, chemicals, cosmetics, agriculture and environmental protection[3–5]. Recently, it has become a crucial raw material in the production of polylactic acid (PLA), a biodegradable polymer that replaces petroleum-derived plastics, contributing to a more sustainable future[6]. Lactic acid is produced mainly by lactic acid bacteria (LAB), with *Lacticaseibacillus rhamnosus* being a key microorganism in the genus *Lacticaseibacillus*, previously classified in the genus *Lactobacillus* [7,8]. Metabolically, this species uses the heterofermentative pathway, that is, in addition to producing lactic acid, these bacteria produce other compounds such as acetate and ethanol, with a maximum yield of 0.5 g of lactic acid per gram of glucose[9]. The production of lactic acid can occur through chemical or biological synthesis, the chemical process being expensive and technically unfeasible[10]. Biological production through bacterial fermentation, responsible for more than 90% of global production, is a promising large-scale option, using renewable sources of carbon and nitrogen[5,11]. This approach offers benefits such as low cost, low temperature, and lower energy consumption, resulting in lactic acid of high optical purity[3,8]. However, challenges in biological production include inhibition due to low pH, temperature, product concentrations and the high cost of the substrate[10]. Therefore, it is essential to investigate the physiological characteristics of lactic acid-producing bacteria in environments with nutrient variation, aiming to face challenges and increase their production through fermentation. Thus, this study aims to evaluate the biotechnological potential of *Lacticaseibacillus rhamnosus* ATCC 7469 in production of lactic acid during fermentation in two culture media.

2 MATERIAL & METHODS

Microorganism: The commercial culture of *L. rhamnosus* ATCC 7469, obtained from Plast Labor (Rio de Janeiro) was preserved in glycerol (10% v/v) at -20 ºC in the freezer (Electrolux FFE24), following the methodology of Chang and Liew (2012). To reactivate the microorganism (inoculum), 2 mL of the preserved bacterial suspension were added to 25 mL of MRS broth (Man, Rogosa, and Sharpe) and incubated in a bacteriological incubator at 37ºC for 24 hours.

Fermentation media: To evaluate the effects of culture media on the growth and metabolism of *L. rhamnosus* ATCC 7469, standard MRS broth (MRSp) and formulated MRS broth (MRSf) were used. The preparation of standard MRS followed the manufacturer's instructions (Kasvi), diluting 52.25 g/L of MRS broth in distilled water. In turn, the broth MRSf was produced following the components present in the standard (commercial) MRS medium (Table 1). Subsequently, were transferred to sealed test tubes and autoclaved at 121°C for 15 minutes.

To evaluate the fermentative capacity (lactic acid production) of *L. rhamnosus* ATCC 7469, standard and formulated MRS were used. The bacterial inoculum was prepared as described in the first item of methodology. Fermentation occurred with the addition of 10% (v/v) bacterial inoculum in sterilized tubes containing 9 mL of MRSp or MRSf medium, resulting in a final volume of 10 mL of medium. The tubes were incubated in a bacteriological incubator (LABOR, SP-101) at 37 ºC for up to 48 hours without shaking. After each pre-defined time interval (0, 6, 12, 24, and 48 hours), the pH, cell concentration (g/L), as well as glucose and lactic acid concentrations (g/L) were assessed. All analysis and assays were conducted in triplicate or duplicate.

Table 1. Chemical composition of the formulated MRS medium (MRSf).

Components	Concentration (g/L)
Glucose	20
Bacteriological peptone	10
Meat extract	8
Sodium acetate	5
Yeast extract	4
Ammonium citrate	2
Dipotassium phosphate	2
Tween 80	1
Magnesium sulfate	0,2
Manganese sulfate	0,05

Determination of Kinetic parameters: Product and biomass yields ($Y_{P/S}$ and $Y_{X/S}$) and maximum volumetric productivity (Q_P) were determined using the concentrations of lactic acid, glucose and biomass obtained during fermentations.

3 RESULTS & DISCUSSION

Figure 1 (A and B) shows the biomass, glucose, lactic acid concentrations, and pH during the fermentations.

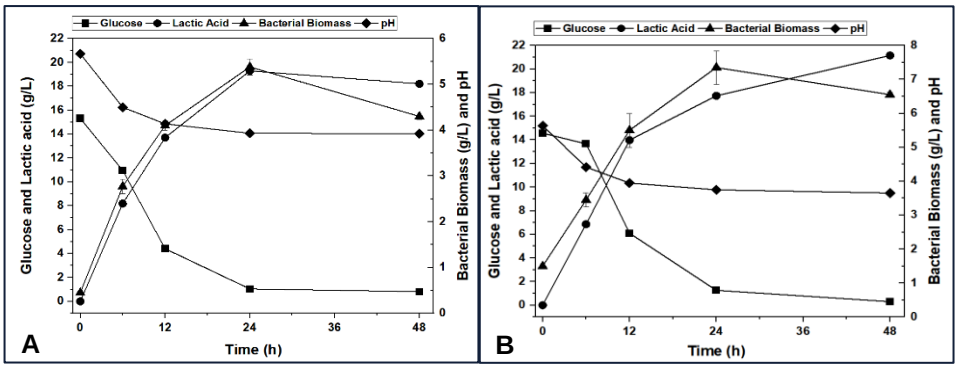

Figure 1. Physiological analysis of *L. rhamnosus* ATCC 7469 bacteria during 48 hours of fermentation in MRSp (a) and MRSf (b) media.

As shown in Figure 1, *L. rhamnosus* exhibited cell growth in both MRSp and MRSf media during the fermentation period, accompanied by high glucose consumption and lactic acid production. The initial biomass in MRSp increased from 0.45 g/L to 5.38 ± 0.18 g/L in 24 hours, while in MRSf it started at 0,65 g/L and reached 7.35 ± 0.49 g/L. The highest values were observed under these conditions throughout the fermentation, with the biomass concentration remaining constant at the end of the period. Glucose consumption in the first 24 hours was significant in both conditions, decreasing from 15.31 g/L to 1.04 ± 0.01 g/L in MRSp and from 14.57 g/L to 1.26 ± 0.01 g/L in MRSf, representing more than 90% consumption. After 48 hours, the glucose concentrations were 0.81 g/L and 0.31 g/L for MRSp and MRSf, respectively, with a consumption rate of 0.30 g/L/h. Glucose consumption and biomass production were accompanied by an increase in lactic acid concentrations and a reduction in pH in both conditions. After 24 hours, the average lactic acid concentrations in MRSp and MRSf were 19.29 ± 0.08 g/L and 17.74 ± 0.12 g/L, respectively, reaching the highest values during the process. At the end of the experiment, there was an increase in MRSf (21 g/L), while the value decreased to 18.21 g/L in MRSp. Regarding the pH profile, a reduction in both conditions was expected during fermentation due to the lactic acid metabolism of the microorganism. In MRSp, the initial and final pH values were 5.67 ± 0.01 and 3.92 ± 0.02, representing a 30% reduction. In MRSf, the pH varied from 5.64 ± 0.02 to 3.65 ± 0.03, a reduction of more than 35% after 48 hours of fermentation (Figure 1). The values for microbial growth and glucose consumption (Figure 1) were higher in the MRSf medium compared to the MRSp medium.

In both conditions, the biomass concentration showed a slight decrease after 48 hours of fermentation in relation to the previous 24 hours, which is directly related to the low glucose concentration in the medium, remaining below 1g/L in all conditions, insufficient to sustain the growth and maintenance of microbial cells. Another parameter that directly influenced the metabolism and physiology of the bacteria was pH. The pH plays a crucial role in the fermentation process, affecting metabolic pathways, enzymatic activity, and fermentation products[12]. The pH varied significantly (~35%) in the MRSf medium compared to the MRSp medium, probably due to increased biomass, cellular activity, and/or lactic acid concentration. The ideal pH values for bacterial growth and metabolite production vary from 3.5 to 9.6 in the literature, depending on the species and strain of the microorganism.[12,13] In this study, initial and final pH values in both conditions were within this range. Bacteria of the genus *Lacticaseibacillus*, such as *L. rhamnosus* ATCC 7469, can grow well in a pH range that varies from acidic to neutral pH[14]. Lactic acid concentrations in both conditions analyzed are in accordance with the patterns observed for *L. rhamnosus*. Despite the similarities between the media, the microorganism produced 3 g/L more lactic acid in the MRSf medium, indicating a significant difference in relation to the MRSp medium, which maintained constant values between 24 and 48 hours of fermentation. *L. rhamnosus* ATCC 7469 is recognized in the literature as a promising producer of lactic acid, particularly the L-lactic acid isomer. Unlike Li et al. (2010), who, using an expensive and complex medium with high concentrations of carbon (>60 g/L) and nitrogen (>30 g/L), achieved a yield of 0.99 g/g with *L. rhamnosus* LA-04, our study demonstrated that *L. rhamnosus* ATCC 7469 presented a yield greater than 1 g/g in a simpler and more economical medium, demonstrating its potential for greater productivity in less complex conditions. Furthermore, *L. rhamnosus* is known to produce lactic acid in unconventional media such as vegetable hydrolysates. In brewery

spent grain hydrolysate (BSG), *L. rhamnosus* ATCC 7469 achieved a maximum lactic acid yield of 0.98 g/g, with a final concentration of approximately 15 g/L after 72 hours of fermentation[16]. In this study, *L. rhamnosus* achieved a lactic acid production of 21 g/L in just 48 hours of fermentation (MRSf), highlighting its promising production capacity in a reduced space of time and in less complex conditions compared to previous studies that used more expensive and complex culture medium.

From the values obtained for glucose consumption, biomass and lactic acid production, kinetic parameters were calculated, including biomass and product yield in relation to the substrate ($Y_{P/S}$ and $Y_{X/S}$), and maximum productivity (Q_P) (Table 2).

Table 2. Kinetic parameters of *L. rhamnosus* ATCC 7469: Biomass and lactic acid yields, and maximum product productivity.

CONDITION	$Y_{X/S}$ (g/g)	$Y_{P/S}$ (g/g)	Q_P (g/L/h)
MRSp	0,27	1,26	0,80
MRSf	0,35	1,48	0,44

In terms of yield and production, MRSf medium showed higher values compared to MRSp medium. However, the maximum lactic acid productivity among the media (Table 2) was achieved in MRSp medium, with maximum production at 24 hours, while in MRSf medium, the highest production was observed after 48 hours of fermentation (Figure 1), influencing the result of maximum lactic acid productivity. The adaptability of the microorganism to the environment positively influences its growth and metabolism, being influenced by biological, physical, chemical or combined factors. As stated by Chang and Liew (2012), although the MRS medium is commonly used for the cultivation of lactic acid bacteria, several strains of *Lacticaseibacillus* have specific nutritional needs, mainly because they are known as fastidious microorganisms, due to their specific nutritional demands. Even with a composition such as MRSp, the MRSf medium proved to be more suitable for the cell growth and lactic acid production by *L. rhamnosus* ATCC 7469, as it presented higher biomass values and product yields throughout the fermentation.

4 CONCLUSION

Given the results presented, the MRSf medium demonstrated greater efficiency in the production of lactic acid by *L. rhamnosus* ATCC 7469, surpassing the results obtained in the MRSp medium. Due to its superior performance, it resulted in a 14% increase (18.21 g/L to 21.15 g/L) in lactic acid production as well as increased biomass in comparison with the MRSp medium. The microorganism showed the ability to produce high concentrations of lactic acid during the fermentation period and demonstrated the ability to grow even at low pH. This initial work is essential for the physiological characterization of this important industrial microorganism and to elucidate further metabolic details of the profile of *L. rhamnosus* ATCC 7469, such as a better understanding and definition of homofermentative or heterofermentative metabolism and present to the industry a strain efficient in the production of lactic acid.

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ACKNOWLEDGEMENTS

The authors express their gratitude to the Federal University of Pernambuco (UFPE) and the Coordination for the Improvement of Higher Education Personnel (CAPES).