Growth Kinetics Of *Lactobacillus acidophilus*, *Lactiplantibacillus plantarum* And *Lacticaseibacillus rhamnosus* Under Different Cultivation Conditions

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Abstract: Probiotics are live microorganisms that benefit the health of the host when administered in adequate amounts. Among the various microorganisms with probiotic activity, lactic acid bacteria (LAB) are commonly used for this purpose. In this perspective, we sought to evaluate the growth kinetics of strains of the species *Lactobacillus acidophilus*, *Lactiplantibacillus plantarum*, and *Lacticaseibacillus rhamnosus*, identifying suitable parameters for the cultivation process. The experiment was conducted with the addition of 1% inoculum in MRS broth. Microbial growth and specific rate ($\mu_{specific}$) were determined by measuring optical density (OD_{600}) and cell viability on plates. Three treatments were tested (no agitation, 100, and 150 RPM) at a constant temperature of 37°C. The results obtained demonstrate that *L. plantarum* presented the highest specific growth rate ($\mu_{specific}$=0.34/h), followed by *L. rhamnosus* ($\mu_{specific}$=0.32/h) and *L. acidophilus* (0.19/h). It was also demonstrated that all strains showed a decline in viability in the agitation treatments after 24 hours of cultivation. The kinetic values evaluated suggest that the three strains can be cultivated under static conditions for a period of 24 hours without compromising the productivity of the bioprocess in terms of cell viability.

Keywords: Probiotics; Lactic Acid Bacteria; Bioprocess.

1. Introduction

Probiotics are live microorganisms that, when consumed in adequate amounts, provide health benefits to the host [1]. They primarily act in the gastrointestinal tract, where their main mechanisms include inhibiting pathogenic microbes, restoring the gut microbiota, strengthening the intestinal barrier, modulating the immune system, and influencing both the endocrine and nervous systems [2].

Lactic Acid Bacteria (LAB) are commonly used as commercial probiotics due to their classification as Generally Recognized As Safe (GRAS) [3]. The commercial production of probiotics involves complex processes that demand strict quality control to ensure efficacy. Key requirements include a minimum concentration of viable microorganisms (10^6 CFU/g), along with stability, reproducibility, and cost-effectiveness [4].

This study aimed to evaluate the microbial growth kinetics of three LAB strains used as probiotics and to assess the impact of agitation on biomass production.

2. Methodology

The specific growth rate (μspecific) of the strains was determined using a high-throughput experimental design approach, which allows for the collection of a larger number of data points in less time and with minimal material usage [5]. This was accomplished through optical density (OD) measurements via spectrophotometry and

viability assessments based on colony-forming unit (CFU) counts.

2.1. Activation of Bacterial Strains

To activate the strains, the lyophilized samples were thawed and resuspended in sterile MRS broth, then incubated at 37 °C for 24 hours. An aliquot equivalent to 1% (v/v) of the medium volume was subsequently transferred to a fresh tube containing MRS broth and incubated again at 37 °C for approximately 12 hours (overnight) to allow for bacterial adaptation to the culture medium.

2.2. Static Condition

Following activation of the lyophilized strain, an aliquot corresponding to 1% (v/v) of the cultured cells was inoculated into test tubes containing 5 mL of MRS broth. The tubes were incubated at 37 °C in a microbiological incubator to allow for the growth of new cultures. Microbial growth was monitored by measuring optical density at 600 nm (OD600) using a UV/VIS spectrophotometer at time intervals of 2, 4, 6, 8, 12, 24, 36, and 48 hours. Cell viability was assessed at 0, 6, 12, 24, and 48 hours using the Drop Plate method [6], with samples plated on MRS agar and incubated at 37 °C for 48 hours in an anaerobic jar.

2.3. Agitation Condition at 100 and 150 RPM

For this assay, Erlenmeyer flasks containing 20 mL of MRS broth were inoculated with 1% (v/v) of the activated strains and incubated at 37 °C in an orbital shaker at 100 and 150 rpm. Microbial growth was monitored following the same protocol as the static assay, with evaluations conducted at 2, 6, 12, 24, and 48 hours of cultivation.

2.4. Data Collection

The experiment was performed in triplicate using sacrifice samples. Results, expressed as Absorbance Units (AU) and CFU/mL, were plotted over time (t) to generate growth curves. The specific growth rate (μspecific) was determined by fitting a straight line to the logarithmic phase of growth and applying the following equation:

$$\mu = \frac{(In\ X2 - In\ X1)}{(t2 - t1)} \quad (1)$$

X_1 = OD_{600} at time t_1

X_2 = OD_{600}at time t_2

ln = natural logarithm

3. Results and discussion

Under the tested conditions, all three strains reached maximum growth after 24 hours of cultivation. The highest cell concentration was observed for *L. plantarum* (2.15×10^9 CFU/mL), followed by *L. rhamnosus* (1.8×10^9 CFU/mL) and *L. acidophilus* (9.7×10^7 CFU/mL) (Figures 3, 4, and 5). *L. plantarum* also exhibited the highest specific growth rate (μ

= 0.34 h^{-1}), followed by *L. rhamnosus* (μ = 0.32 h^{-1}) and *L. acidophilus* (μ = 0.19 h^{-1}) (Table 1).

Table 1. Specific growth rates (μ) and residual standard deviations calculated by linear regression during the exponential growth phase (4–10 hours) for various LAB strains.

Microorganism	$\mu_{specifics}(h^{-1})$	Standard Deviation
L. acidophilus	0.3434	0.174
L. plantarum	0.3434	0.3634
L. rhamnosus	0,3214	0,2828

Exposure of the strains to agitation at 100 rpm resulted in a marked decrease in cell viability, with counts falling to 1.40 × 10^4 CFU/mL after 48 hours (Graph 1). A comparable growth pattern followed by a decline was also observed under agitation at 150 rpm (Graphs 2 and 3)

Figure 1. Cell growth of *L. plantarum* over time under static cultivation.

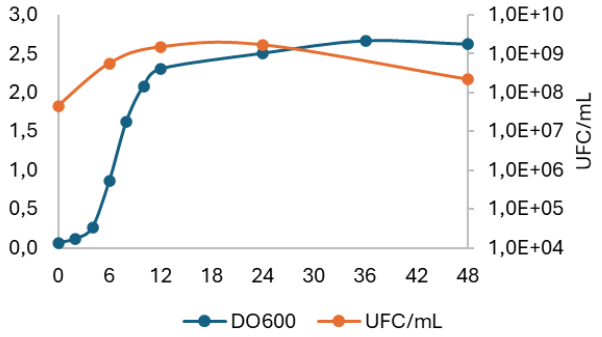


Figure 2. Cell growth of *L. plantarum* over time under cultivation with agitation at 100 rpm.

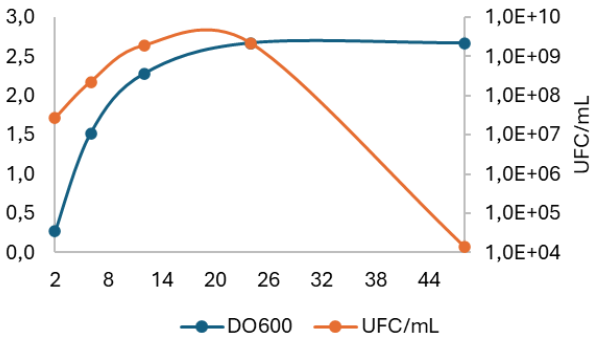
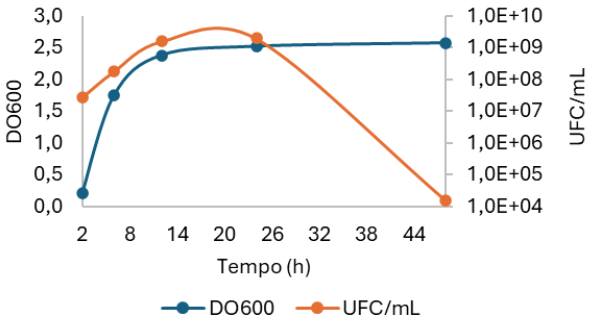


Figure 3. Cell growth of *L. plantarum* over time under cultivation with agitation at 150 rpm.



L. rhamnosus showed the best performance in terms of cell viability under agitation at 150 rpm, reaching 1.8 × 10^9 CFU/mL after 24 hours of cultivation. However, as also observed for *L. plantarum*, a significant reduction in viability occurred after this period (Graphic 4, 5 and 6).

Figure 4. Cell growth of *L. rhamnosus* over time under static cultivation.

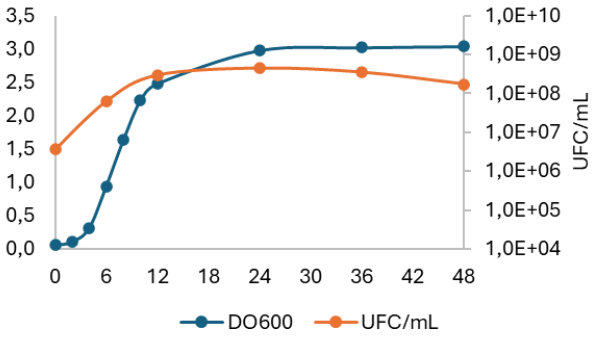


Figure 5. Cell growth of *L. rhamnosus* over time under cultivation with agitation at 100 rpm.

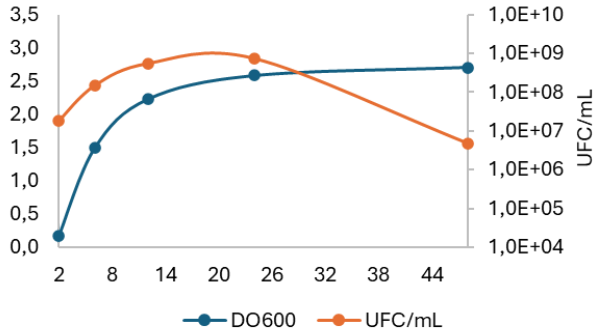
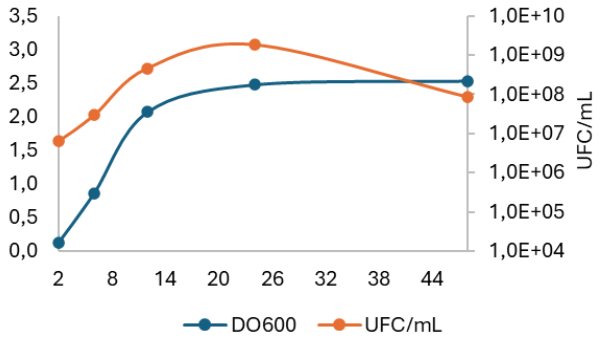


Figure 6. Cell growth of *L. rhamnosus* over time under cultivation with agitation at 150 rpm.



The highest viability of *L. acidophilus* (9.7×10^7 CFU/mL) was recorded under static conditions after 24 hours of cultivation, in agreement with previous reports [7] under similar conditions. A decline in cell viability over time was also observed in the agitated treatments (Graphs 7, 8, and 9).

Figure 7. Cell growth of *L. acidophilus* over time under static cultivation.

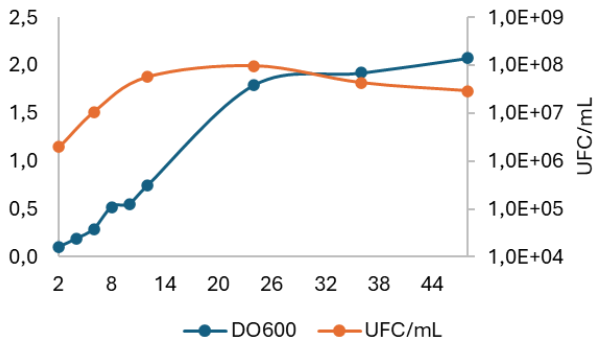


Figure 8. Cell growth of *L. acidophilus* over time under cultivation with agitation at 100 rpm.

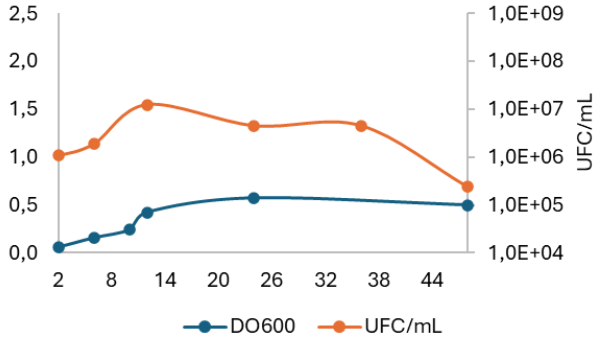
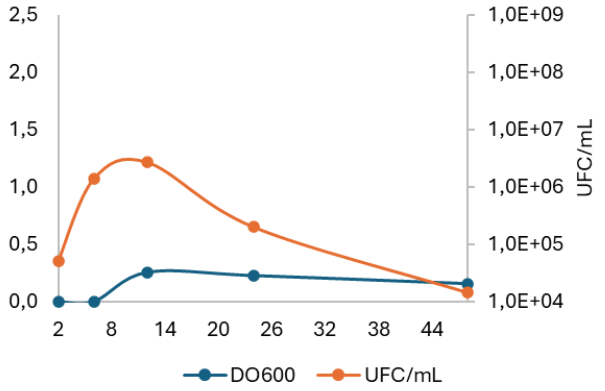


Figure 9. Cell growth of *L. acidophilus* over time under cultivation with agitation at 150 rpm.



Based on the data obtained in the conducted assays, it is recommended to use inocula cultivated under static conditions at 37 °C for a period of 8 to 16 hours for *L. plantarum* and *L. rhamnosus*, and 12 to 20 hours for *L. acidophilus*. This recommendation is based on the absence of significant differences in viability

values and on the fact that the ideal phase for inoculum addition in bioprocesses is the logarithmic phase, which is characterized by high metabolic activity, greater viability, cellular vitality, and better adaptation to the new culture medium [8].

Another relevant aspect is the morphological and physiological similarity between the strains *L. plantarum* and *L. rhamnosus*. In contrast, the *L. acidophilus* strain showed greater sensitivity to variations in culture conditions in the present study, as also demonstrated in a previous study [9].

Another relevant aspect is the morphological and physiological similarity (Figure 1, 2 e 3) between the strains *L. plantarum* and *L. rhamnosus*, which has also been demonstrated previously [9]. In their study, when evaluating different pH levels, NaCl, and sucrose concentrations, these strains showed similar specific growth rates. In contrast, the *L. acidophilus* strain exhibited a lower growth rate and greater sensitivity to variations in cultivation conditions, as observed in the present study.

4. Conclusion

Based on the results obtained under laboratory conditions, inoculum preparation under static conditions proved to be the most effective. The growth kinetics also revealed distinct differences between *Lactiplantibacillus plantarum* and *Lacticaseibacillus rhamnosus* in comparison to *Lactobacillus acidophilus*, underscoring the need for specific handling practices for the latter due to its unique physiological behavior. These differences highlight the importance of tailored strategies when working with *L. acidophilus*.

Acknowledgement

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