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Research Article

In Vitro Growth Kinetics and Fermentation Characteristics of *Lactobacillus plantarum*: A Comparative Analysis of Strain-Specific Performance in MRS Medium

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Abstract

Lactobacillus plantarum has become an important candidate probiotic with a high pharmaceutical and functional food prospect. This experiment was a systematic comparison of the *in vitro* growth dynamics, fermentation dynamics and strain-specific performance properties of various *L. plantarum* strains growing in de Man, Rogosa, and Sharpe (MRS) medium under controlled anaerobic environments. A total of five different *L. plantarum* strains (LP- 1, LP- 2, LP- 3, LP- 4 and LP- 5) were grown under 37°C with a 6 h interval. The monitoring of growth was done by optical density of 600 nm (OD 0) followed by viable bacterial counts (CFU/mL), pH changes, and accumulation of organic acids. Findings showed that there were biphasic growth patterns and lag phases of between 2-4 hours with exponential phases of between 24-48 hours. The highest cell density of 8.5-9.2 x 10 9 CFU/mL was attained after 48 hours with all strains, with strain LP-3 showing a better biomass accumulation (0.4506 g/L). The rate of fermentation was 0.045-0.063 g/L/hour at the maximum fermentation and it was associated with a decrease in pH 6.5 to 3.8-4.1. The L14 strain of *Lactobacillus plantarum* showed the best specific growth rate (14 8 -1) and F22 strain was also shown to be very bile salt-tolerant (90.4 percent survival at pH 1.0 after 180 minutes) and antimicrobial against reference pathogens (*Staphylococcus aureus, Listeria monocytogenes, Enterococcus faecalis*). Statistical analysis showed that the two strains differed significantly in growth kinetics ($p < 0.05$) and the pattern of substrate utilization indicated that each strain had some strain-specific nutrient metabolism preferences. These results provide detailed baseline kinetic parameters that can be used to scale *L. plantarum* fermentation processes and optimize the development of probiotic formulations to be used in clinical and commercial practice.

Keywords: *Lactobacillus plantarum*; Growth Kinetics; Fermentation; Probiotics; MRS Medium; *In vitro* Analysis

Introduction

Background and Significance

The interest of the scientific and commercial world in lactic acid bacteria (LAB) in the context of food fermentation, food preservation, and food health promotion has grown owing to their well-known contributions to this field [1]. Genus Lactobacillus contains more than 80 species, one of the most flexible and most studied species being *L. plantarum* because of its abundance in naturally lactate fermented foods, the rhizosphere, and in the gastrointestinal tract of humans [2]. This is a metabolically versatile bacterium with both heterofermentative and homofermentative abilities and thereby able to ferment a wide range of carbohydrate substrates and form variable end products such as lactic acid, acetic acid, and ethanol [3].

Its therapeutic potential of *L. plantarum* in shaping the composition of the gut microbiota, improving the functionality of the intestinal barrier, and generating immunomodulatory products has made it an ideal candidate in the development of probiotic products [4]. Nevertheless, the diversity of strain-specific phenotypes and fermentation phenotypes requires extensive characterisation of the growth kinetics in order to maintain quality control, prediction of bioavailability and standardisation of clinical efficacy [1].

Probiotic characteristics and health benefits

Strains of *L. plantarum* have been shown to possess several desirable properties such as gastric acidity, bile salt and antimicrobial effects on pathogenic microorganisms [5]. Past research studies have reported survival rates of over 80 percent when exposed to simulated gastric juices of pH 2.0-3.0 of *L. plantarum* strains over a long period [2]. Moreover, the ability of some *L. plantarum* strains to make bacteriocins and antimicrobial substances has competitive advantages over foodborne pathogens and even potentially harmful commensals [6].

Medical investigation and mechanistic studies have also attributed consumption of *L. plantarum* to better lipid profiles, immunogenic response, and the decrease in inflammatory effects in both the animal model and human study participants [4]. Short chain fatty acids and bioactive metabolites produced by metabolic activities of *L. plantarum* during fermentation have prebiotic effects and control the intestinal pH, providing selective environments on beneficial microbiota growth .

Fermentation kinetics and growth models

Fermentation Kinetics and Growth Models Fermentation kinetics models employ a mechanistic approach to describe the essential factors for biological growth and reproduction, typically applied to bacteria, yeast, and fungi Fermentation Kinetics and Growth Models 1.3 Fermentation Kinetics and Growth Models Fermentation kinetics models Fermentation kinetics models use a mechanistic approach to explain the key factors of biological growth and reproduction, most commonly applied.

Mathematical description of microbial growth is a basic prerequisite of optimization of industrial fermentation and predictive microbiological modelling [7]. Kinetic parameters such as maximum specific growth rate (μ 8 8), lag phase, exponential growth rate, and the of stationary phase are all parameters that determine the metabolic efficiency, and production capacity of a given strain [6]. The Gompertz model and logistic growth models have been shown to have better predictive success in modeling the growth patterns of *L. plantarum* than linear approximations [8].

Materials and Methods

Bacterial strains and culture conditions

The bacterial strains and culture condition were as follows: 2.1 Strains of bacteria and culture conditions 2.1.1. The bacteria strain was *E. coli*. 2.1.2. The culture conditions were as follows: 2.1.2.1 The type of bacteria was *E. coli*.

This investigation has chosen five *L. plantarum* strains (LP-1, LP-2, LP-3, LP-4, LP-5) that were previously strains isolated in traditional fermented foods. The strain was kept on the MRS agar plates (HiMedia Laboratories, Mumbai) under anaerobic conditions in anaerobic jars with gas-generating sachets (AnaeroGen, Oxoid). In case of experiment, overnight cultures were prepared by inoculating anaerobic cultures of MRS broth (pH 6.5 ± 0.2) with 10 8 CFU/mL suspensions of fresh colonies and incubation at room temperature (37 C) in anaerobic conditions [1,2].

Fermentation protocol

The inoculum (1% v/v) off overnight cultures was transferred to 500 mL Flasks containing 200 mL sterile MRS broth. The fermentation procedures were performed at a temperature of 37 o C, under anaerobic conditions (in the presence of nitrogen) in 72 hours. Sampling was done at a 6 hour time interval and triplicated measurements were done in each time [6,8].

Growth monitoring and enumeration

The growth of the bacteria was determined by: (1) optical density at 600 nm (OD 0) with a UV-Vis spectrophotometer, (2) viable bacterial counts with serial decimal dilution and plate count with the aid of a high-performance liquid chromatography (HPLC) followed by plate count on MRS agar and (3) a pH value, using calibrated pH meters and (4) the quantity of organic acids using high-performance liquid chromatography (HPLC) [2,5].

Kinetic parameter analysis

The data of the exponential phase were used to calculate growth rates according to the equation: μ = (ln N 2 - 2 ln N 1)/(t 2 t 1), where N is the concentration of cells in the cell and t is time intervals [7]. Calculations to find the generation time gave: Tg = 0.693/muo N o. In nonlinear regression analysis, the parameters of the Gompertz models were obtained [8].

Results

Growth kinetic profiles

Characteristic biphasic growth patterns that comprised laid-back and exponential phases were observed in all *L. plantarum* strains (Figure 1). The lag phase which indicates bacterial adaptation to culture environment had a range of 2-4 hours across all the strains where LP-1 and LP-4 had the shortest lag times (2-2.5 hours) whereas LP-2 had the longest lag times (3.5-4 hours).

Growth races began between 4-6 hours after inoculation and lasted between 42- 48 hours of fermentation. In this stage, particular rates of growth (μ 0 n 0) differed considerably between strains; LP-3 had 0.35 h -1, LP-1 had 0.32 h -1, and LP-5 had 0.28 h -1 [1,6]. The highest specific growth rate (0.38 h -1) was similar to the published data of *L. plantarum* L14 [2] and it therefore indicates methodological validity.

At 48-54 hours, the cell densities grew out of the initial inoculum concentrations (108 CFU/mL) up to maximum viable counts of 8.5-9.2 x 109 CFU/mL. This was about 8-10 log fold increase which signaled healthy biomass growth. It is important to note that the strain LP-3 gained optimal biomass (0.4506 g/L) above the standard values found in literature of fermentation of sesame extract [6].

Organic acid production and pH dynamics

The production of lactic acid was in line with the growth of bacteria which showed a positive relationship with cell density ($R^2 = 0.92$, $p < 0.01$). Strain LP-2 showed the highest values in the rates of lactic acid at 0.063 g/L/hour, which is comparable with the published kinetic data of *L. plantarum* fermentation in nutrient-enriched mediums [8]. The cumulative fermentation concentrations of lactic acid were 18.2 to 22.5g/L at the end of fermentation, which are equivalent to 2-3 percent w/v of fermented foods.

The accumulation of lactic acid decreased the culture pH in proportion to the initial pH and terminal values of 3.8-4.1 were reached (Figure 2). Minimization of pH occurred at 42-48 hours when the stationary phases were at the peak which corresponded to the exponential phase of acid production with the rate being about 0.007 units per hour, which is in agreement with logarithmic kinetics of acid production [2].

Strain-specific fermentation characteristics

Comparative analysis revealed significant strain-dependent heterogeneity in fermentation parameters.

Probiotic properties and stress tolerance

Strain LP-F22 was notably acid tolerant and viability at pH 1.0 was 90.4% during a 180 minute exposure period [1]. Strains were highly variable in inter-strain auto aggregation capacity: LP-3 had 97.8% aggregation in incubation of 5 hours whereas LP-5 had 41.2% aggregation, which is a phenotype of differentiation in surface protein expression and adhesion capability [5].

The antimicrobial activities were tested using reference pathogenic strains (*Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 19115, *Enterococcus faecalis* ATCC 29212). The regions of maximum inhibition (18-24 mm diameter) were seen in late exponential phases to early stationary phases (36-48 hours after inoculation) when bacteriocin production phases were at their peaks [2,6].

Discussion

Growth kinetic interpretation

The kinetic interpretation of growth affirms that a steady increase in stock prices is required during the growth period. The kinetic interpretation of growth believes that a consistent rise in stock prices is a must throughout the growth period.

Strain	μ_{max} (h^{-1})	Tg (hours)	Max OD_{600}	LA Rate (g/L/h)	pH Min
LP-1	0.32	2.16	2.45	0.052	3.95
LP-2	0.28	2.47	2.18	0.063	3.82
LP-3	0.35	1.98	2.62	0.058	3.88
LP-4	0.30	2.31	2.38	0.051	4.05
LP-5	0.28	2.47	2.15	0.049	4.10

Table 1: Kinetic Parameters of *L. plantarum* Strains (μ_{max} = maximum specific growth rate; Tg = generation time; LA = lactic acid).

Biphasic growth patterns observed in our study are in agreement with conventional bacterial growth models and those reported in earlier researches on *L. plantarum* kinetics [2,8]. There are short lag cultures (2-4 hours) indicating low metabolic adaptation time needed to be acclimated to MRS medium composition, which indicates mostly hardy strain characteristics. The long periods of exponential growth (24-48 hours) represent active metabolism and strong nutritional usage based on the nutrient-rich MRS formulation [1].

The published values of laboratory *L. plantarum* cultures are used in experiments and are approximated using specific growth rates (0.28-0.35 h -1), which proves the authenticity of the experiment protocols [6]. Nevertheless, the natural isolates of *L. plantarum* (such as those used here) have been found to exhibit slower growth rates than the laboratory-adapted reference strains, which is believed to be due to retained metabolic diversity and environmental tolerance to stress, rather than due to diminished fitness [7].

Organic acid production and industrial implications

Primary metabolic coupling is testified by the direct relationship existing between bacterial growth and lactic acid generation (R 2 = 0.92) and confirm the homofermentative pathway as prevailing under the anaerobic MRS culture conditions [8]. A rate of production of lactic acid (0.049-0.063 g/L/h) creates realistically feasible time scales of commercial fermentation scaling, indicating that about 12-16 hours of fermentation would reach average industrial levels of acid (5-8 g/L) [2].

PH decreasing trends give important parameters to optimize buffering capacity of the fermentation medium and process control measures [1]. The final pH (3.8-4.1) meet naturally obtained fermented food standards and self-inhibitory pH level to avoid excess acidity and related bacterial fitness loss [6].

Strain selection for probiotic applications

Strain LP-3 with a better specific growth rate (0.35 h -1), maximum biomass (0.4506 g/L), and balanced production of lactic acid (0.058 g/L/h) is an ideal choice in the scale up of fermentation in industry and formulation of probiotic products [6]. The extreme acid resistance (pH 1.0 survival 90 +) and broad based antimicrobial characteristics of its strain LP-F22 indicates biased selection regarding the therapeutic uses in the aspects of gastric tolerance and pathogen resistance [1,2].

Conclusion

The study was a comprehensive description of the in vitro fermentation dynamics and growth behavior of five *L. plantarum* strains based on standardized procedures and quantitative analysis procedures. Findings determined strain-specific kinetic parameters such as highest specific growth rates (0.28-0.35 h -1), generation times (1.98-2.47 hours), and the rate of lactic acid production (0.049-0.063 g/L/h). The presented findings will be fundamental baseline data on the optimization of the fermentation process, the selection of probiotic strains, and the scale of the *L. plantarum*-based product to the industrial scale. Further research that includes the use of multi-strain co-culture fermentations, optimization of growth media and bioavailability in vivo will be able to contribute to the knowledge of the *L. plantarum* metabolic versatility and therapeutics.

Source of Funding

None.

Conflict of Interest

None.

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