

Growth kinetics of probiotic lactobacilli strains cultivated in a laboratory bioreactor with stirring

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Abstract. Batch cultivation in a laboratory bioreactor with stirring of the lactobacilli strains with probiotic properties *Lactocaseibacillus casei* ssp. *casei* G17 and *Lactocaseibacillus casei* ssp. *rhamnosus* G16 isolated from pink blossom of *Rosa damascena* Mill was conducted. The changes in the concentration of viable cells were monitored. The growth kinetics was modeled applying the classic and modified logistic curve model and the maximum specific growth rate (μ_m) of the studied strains was determined. The classical model of the logistic curve showed higher μ_m for *Lactocaseibacillus casei* ssp. *casei* G17 - 0.133 h⁻¹, compared to *Lactocaseibacillus casei* ssp. *rhamnosus* G16 - 0.120 h⁻¹, while the modified logistic curve model predicted comparable maximum growth rates of 0.105 h⁻¹ and 0.101 h⁻¹ for *Lactocaseibacillus casei* ssp. *casei* G17 and *Lactocaseibacillus casei* ssp. *rhamnosus* G16, respectively. *Lactocaseibacillus casei* ssp. *casei* G17 was characterized by a shorter induction period ($\tau_a = 0.72$ h) and a higher adaptation rate constant ($k_0 = 0.390$ h⁻¹) compared to *Lactocaseibacillus casei* ssp. *rhamnosus* G16 ($\tau_a = 1.66$ h; $k_0 = 0.110$ h⁻¹). The established kinetic parameters show that *Lactocaseibacillus casei* ssp. *rhamnosus* G16 needs the addition of growth factors in the fermentation medium that will help to optimize its composition for scaling up the fermentation process.

1 Introduction

According to the modern interpretation, probiotics are live microorganisms that support or maintain a favorable balance of the autochthonous microbial population in the human gastrointestinal tract (GIT) [1-5]. According to the definition accepted by the Food Agriculture Organization (FAO) and WHO (2001), probiotics are live microorganisms that, when applied in adequate amount, have a beneficial effect on the human health [6]. Schrezenmeier and de Vrese (2001) complement and refine this definition as follows: probiotics are preparations or products containing adequate amount of live and species-specific microorganisms that change the microflora in certain areas of the host's body and thus exhibit a beneficial effect on the health of the given host [7].

Modern research shows that probiotic preparations are effective for the prevention and treatment of a number of diseases, such as diarrhea, diabetes, cancer, allergies, hypertension, genetic disorders, etc. [6, 8-15]. In addition, probiotics help the body's immune system, and also some representatives of *Lactiplantibacillus plantarum* possess the ability to degrade mycotoxins, patulin in particular [16].

Lactobacilli are some of the most widely used microorganisms for the preparation of probiotic concentrates. The most common species included in the composition of probiotics are *Lactobacillus acidophilus*,

Lactocaseibacillus casei ssp. *casei*, *Lactocaseibacillus paracasei*, *Lactocaseibacillus casei* ssp. *rhamnosus*, *Lactiplantibacillus plantarum*, *Lactobacillus johnsonii* [6, 17]. Probiotic microorganisms must meet a number of requirements: to be part of the natural microflora in humans and animals; to be able to adhere to and colonize the intestinal mucosa to compete with enteropathogenic bacteria for adhesion sites and nutrients; to survive and maintain their activity in the conditions of the gastrointestinal tract; to reproduce in the gastrointestinal tract, to have high antimicrobial activity, suppressing and expelling pathogenic and toxigenic microorganisms out of the biological niche [6, 8].

In addition, strains with pronounced probiotic properties should allow industrial cultivation and maintenance of high activity during the production process and during storage of the finished probiotic preparations. Therefore, batch cultivation processes of *Lactocaseibacillus casei* ssp. *casei* G17 and *Lactocaseibacillus casei* ssp. *rhamnosus* G16 were carried out. The study of the cultivation process is incomplete if the kinetics of growth of the strains are not known [18]. Mathematical modeling is a fundamental method for studying the cultivation process [18].

Knowledge of the kinetics of the fermentation process is essential when scaling up the process from laboratory scale to industrial scale, for optimization of the composition of the fermentation medium and cultivation conditions, for the design of the main equipment, as well

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as for determining the productivity of the bioreactor according to the following dependence:

$$G_X = \frac{X_K - X_0}{\tau_f} \quad (1)$$

where:

G_X - productivity of the bioreactor;
 X_K and X_0 – final and initial biomass concentration, cfu/cm³;
 τ_f – duration of the entire fermentation cycle, h.

In a batch cultivation process, only the exponential growth period is important. In the exponential growth period, the specific growth rate is equal to the maximum growth rate, from which the duration of the entire fermentation cycle will be calculated according to the following dependence:

$$\tau_f = \frac{1}{\mu_{max}} \cdot \ln \frac{X_K}{X_0} + \tau_{cont} \quad (2)$$

where:

τ_{cont} - non-productive time, which includes the lag phase, the time for filling and draining the bioreactor, the time for sterilization, for washing and preparing the bioreactor, the sterilization of the nutrient medium and the cooling to the fermentation temperature [19].

The aim of the present study was to conduct batch cultivation processes of *Lactocaseibacillus casei* ssp. *casei* G17 and *Lactocaseibacillus casei* ssp. *rhamnosus* G16 and to determine the fermentation process kinetics.

2 Materials and methods

2.1 Microorganisms

Lactocaseibacillus casei ssp. *casei* G17 and *Lactocaseibacillus casei* ssp. *rhamnosus* G16, isolated from rose blossom of *Rosa damascena* Mill, were used in the present study.

2.2 Media

LAPTg10 - agar. Composition (g/dm³): peptone – 15; yeast extract – 10; tryptone – 10; glucose - 10. pH was adjusted to 6.6 - 6.8 and Tween 80 - 1cm³/dm³, agar - 15 were added. Sterilization - 20 min at 121°C. The medium was used for the determination of the number of viable lactobacilli cells.

2.3 Cultivation of the investigated strains

The cultivation of the studied strains was carried out in a bioreactor with mechanical stirring and a working volume of 1.5 dm³ in 10% sterile skimmed milk at a temperature of 37 ± 1 °C.

2.4 Determination of the number of viable cells

The number of viable cells was determined by the tenfold dilution method in accordance with BSS ISO 7889:2005.

2.5 Modeling the fermentation process kinetics

To model the kinetics of the process, the classical logistic curve model (eq. 3 and the modified logistic curve model (eq. 4) were used, as well as a model for determining the induction period and the adaptation rate constant (eq. 5) [18, 20 - 21].

$$\frac{dX}{d\tau} = \mu_m X - \beta X^2 \quad (3)$$

$$\mu_m X - \frac{\mu_m}{X_{sp}} X^2 = \mu_m \left(1 - \frac{X}{X_{sp}} \right) X$$

$$\frac{dX}{d\tau} = (\mu_m X - \beta X^2)^n \quad (4)$$

$$\left(\mu_m X - \frac{\mu_m}{X_{sp}} X^2 \right)^n = \mu_m \left(1 - \frac{X}{X_{sp}} \right)^n X$$

$$\ln \frac{M}{N_0} = \mu_m \tau + \ln \left\{ \frac{k_0}{k_0 + \mu_m} \left[1 + \frac{\mu_m}{k_0} e^{-(k_0 + \mu_m)\tau} \right] \right\} \quad (5)$$

where:

μ_m - maximum specific growth rate, h⁻¹;
 X_n and X_{sp} - initial and final concentration of viable cells, cfu/cm³;
 β – internal population competition coefficient cfu/cm³.h;
 n – coefficient taking into account the influence of the composition of the medium and the conditions of cultivation on the growth of the studied strain;
 M – current biomass concentration, cfu/cm³;
 N_0 – initial biomass concentration, cfu/cm³;
 τ_a – induction period, h;
 k_0 – rate constant of adaptation of the cells to the medium and cultivation conditions, h⁻¹;

The logistic curve models (3) and (4) were solved numerically using the Runge-Kutta method of the 4th order, the identification of the parameters of the models (3) and (4) was done using the Solver function in Excel, by means of minimization of the square of the difference between the experimental data and those obtained from the corresponding model [22].

The parametric identification of model (3) was performed by non-linear regression using the software product *Curve Expert Professional*.

3 Results and discussion

Lactobacilli with probiotic properties must allow industrial cultivation, they also must grow well in the selected fermentation medium, and to accumulate high concentration of viable cells, which determines the activity and the effectiveness of the finished probiotic preparations. For this reason, batch cultivation processes of *Lactocaseibacillus casei* ssp. *rhamnosus* G16 and *Lactocaseibacillus casei* ssp. *casei* G17 in a bioreactor with mechanical stirring while maintaining an optimal pH

for the strains were studied. The dynamics of changes in the biomass of the studied strains were monitored and their growth kinetics were modeled. Fig. 1 shows the dynamics of changes in the biomass of the studied strains during the cultivation process. The duration of the lag phase for *Lacticaseibacillus casei* ssp. *rhamnosus* G16 and *Lacticaseibacillus casei* ssp. *casei* G17 was about 3 h. For a more complete study of the adaptability of the studied strains to the fermentation medium and the cultivation conditions, the induction period (i.e. the time from the lag-phase during which the cells begin to synthesize the necessary cellular structures and enzymes and pass from an unadapted to an adapted state) and the rate constant of adaptation k_0 , were determined [22]. This was only possible using mathematical modeling. The results of these studies are presented in Table 1.

The induction period in *Lacticaseibacillus casei* ssp. *casei* G17 was significantly shorter – 0.72 h, compared to that for *Lacticaseibacillus casei* ssp. *rhamnosus* G16. This indicates that *Lacticaseibacillus casei* ssp. *casei* G17 adapted faster to the culture medium and conditions compared to *Lacticaseibacillus casei* ssp. *rhamnosus* G16 (Table 1). This was also confirmed by the higher rate constant of adaptation in *Lacticaseibacillus casei* ssp. *casei* G17 – 0.390 h⁻¹, compared to the same parameter in *Lacticaseibacillus casei* ssp. *rhamnosus* G16 - 0.110 h⁻¹. It can be concluded that for *Lacticaseibacillus casei* ssp. *rhamnosus* G16 it is necessary to additionally add growth factors to the fermentation medium to improve the adaptation and growth of the strain (Table 1).

Table 1. Kinetic parameters of model (3).

Strain	τ_a, h	k_0, h^{-1}
<i>L. rhamnosus</i> G16	1.66	0.110
<i>L. casei</i> G17	0.72	0.390

After adaptation of the cultures to the culture medium and the cultivation conditions, their entry into the exponential growth phase was observed. It was characterized by a continuous increase in the concentration of active cells for both strains until the 24th hour, reaching comparable concentrations of viable cells - around 10¹³ cfu/cm³.

The growth rate of *Lacticaseibacillus casei* ssp. *rhamnosus* G16 was lower compared to that of *Lacticaseibacillus casei* ssp. *casei* G17 (Fig. 1).

Knowledge of the growth kinetics of the investigated strains is essential for scaling up and managing the process in industrial settings, as well as for optimizing the composition of the fermentation medium and the cultivation conditions. For this reason, the growth kinetics was modeled and basic bioprocess parameters of the fermentation process with *Lacticaseibacillus casei* ssp. *rhamnosus* G16 and *Lacticaseibacillus casei* ssp. *casei* G17 under appropriate culture conditions were determined (Table 2).

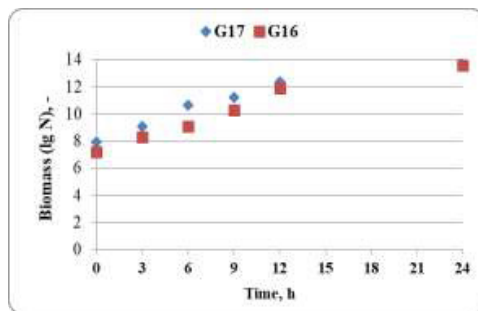


Fig. 1. Dynamics of changes in the biomass of *Lacticaseibacillus casei* ssp. *rhamnosus* G16 and *Lacticaseibacillus casei* ssp. *casei* G17 cultivated in a laboratory bioreactor with mechanical stirring at 37 °C

The models were characterized by a high value of the correlation coefficients, which varied from 0.9882 to 0.9978, as well as low identification errors, which varied in the range of 0.85 to 0.87 (Table 2).

Table 2. Kinetic parameters in the classic logistic curve model and the modified logistic curve model

Strain	Classic logistic curve model				
	μ_{max} h ⁻¹	β cfu/cm ³ .h	X_K cfu/cm ³	R ² -	e -
G16	0.120	0.0087	13.84	0.98	0.87
G17	0.133	0.0094	14.17	0.99	0.85
Modified logistic curve model					
G16	0.101	0.0074	13.72	0.98	0.87
G17	0.105	0.0076	13.78	0.99	0.86
n _{G16} =0.8707; n _{G17} =0.7510					

This shows that the chosen models were adequate and could be used to describe and predict the fermentation process carried out with the studied strains. The classical logistic curve model gave higher maximum specific growth rate values for both strains compared to the modified logistic curve model. Besides, the classical logistic curve model calculated a higher μ_{max} for *Lacticaseibacillus casei* ssp. *casei* G17 - 0.133 h⁻¹, compared to that for *Lacticaseibacillus casei* ssp. *rhamnosus* G16 - 0.120 h⁻¹. The modified logistic curve model gave a commensurate value of μ_{max} , but again the higher value was for *Lacticaseibacillus casei* ssp. *casei* G17 - 0.105 h⁻¹, while for *Lacticaseibacillus casei* ssp. *rhamnosus* G16 μ_{max} was 0.101 h⁻¹. The coefficient of intra-population competition for both studied strains had a low value, which varied in the range of 0.0074 cfu/cm³.h to 0.0094 cfu/cm³.h in the models (Table 2).

A comparison of the experimental data with those of the models was made. The results of these studies are presented in Fig. 2, Fig. 3, Fig. 4 and Fig. 5. The selected models agreed very well with the experimental results.

The changes in pH and the oxidation-reduction potential during the fermentation processes carried out with the two studied strains were monitored. The results of these studies are reflected in Fig. 6 and Fig. 7.

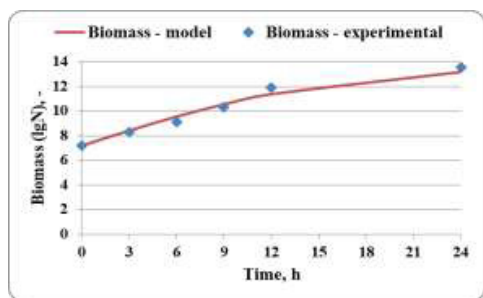


Fig. 2. Comparison of experimental data with data from the classic logistic curve model for *Lactocaseibacillus casei* ssp. *rhamnosus* G16 cultivated in a bioreactor with mechanical stirring at 37 °C

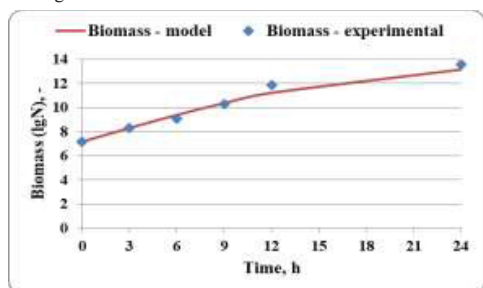


Fig. 3. Comparison of experimental data with data from the modified logistic curve model for *Lactocaseibacillus casei* ssp. *rhamnosus* G16 cultivated in a bioreactor with mechanical stirring at 37 °C

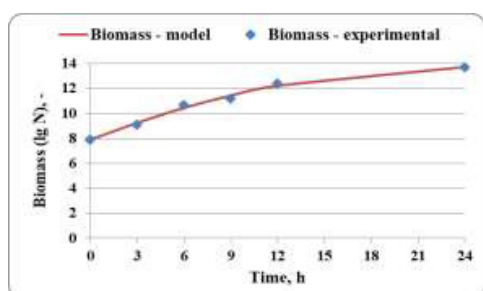


Fig. 4. Comparison of experimental data with data from the classic logistic curve model for *Lactocaseibacillus casei* ssp. *casei* G17 cultivated in a bioreactor with mechanical stirring at 37 °C

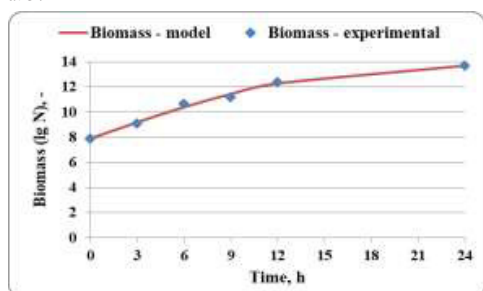


Fig. 5. Comparison of experimental data with that of the modified logistic curve model for *Lactocaseibacillus casei* ssp. *casei* G17 cultivated in a bioreactor with mechanical stirring at 37 °C

The oxidation-reduction potential increased from -298 mV to 3.7 mV during the lag phase, then followed a continuous decrease in the value of this indicator, more intensively until the 9th h, and then smoothly until the end of the process, where the redox potential value for *Lactocaseibacillus casei* ssp. *rhamnosus* G16 was -366 mV.

Lactocaseibacillus casei ssp. *casei* G17 was similar to *Lactocaseibacillus casei* ssp. *rhamnosus* G16 (Fig. 7), namely a rapid decrease until the 9th h from the start of the process, after which the automatic system was turned on again to maintain a constant optimal pH value for the cultivated strain.

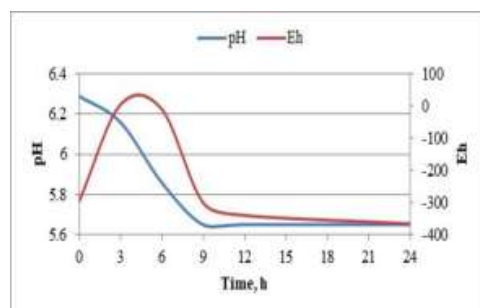


Fig. 6. Changes in the pH and the redox potential during the cultivation of *Lactocaseibacillus casei* ssp. *rhamnosus* G16 in a laboratory bioreactor with mechanical stirring at 37 °C

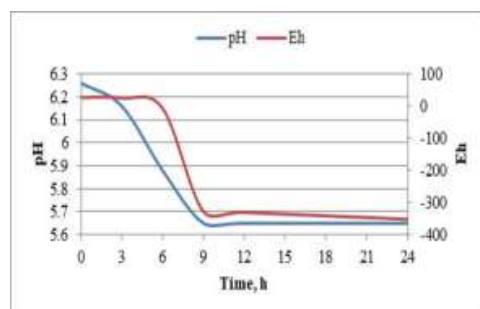


Fig. 7. Changes in the pH and the oxidation-reduction potential during the cultivation of *Lactocaseibacillus casei* ssp. *casei* G17 in a laboratory bioreactor with mechanical stirring at 37 °C

A different trend was observed in the changes of the redox potential in *Lactocaseibacillus casei* ssp. *casei* G17, namely during the lag phase, the value of the redox potential remained relatively constant, then its value began to decrease until the 9th h from the start of the process, reaching -328 mV and this value slightly changed to -352 mV at the end of the fermentation process.

The parameter *n* in the modified logistic curve model, showing the influence of culture conditions on the growth of *Lactocaseibacillus casei* ssp. *rhamnosus* G16 and *Lactocaseibacillus casei* ssp. *casei* G17, was 0.8707 and 0.7510, respectively. The higher value of the parameter *n* for *Lactocaseibacillus casei* ssp. *rhamnosus* G16, as well as the lower kinetic parameters (maximum specific growth rate and adaptation rate

constant), compared to those for *Lacticaseibacillus casei* ssp. *casei* G17, indicated that, regardless of the fact that the two studied strains were subspecies of the same species, to improve the growth kinetics of *Lacticaseibacillus casei* ssp. *rhamnosus* G16 it was necessary to add additional growth factors to the fermentation medium.

pH rapidly decreased until the 9th h from the start of the cultivation process of *Lacticaseibacillus casei* ssp. *rhamnosus* G16, after which the automatic system for maintenance of a constant optimal pH value for the cultured strain was activated (Fig. 6).

4 Conclusion

The kinetic parameters in the batch cultivation of *Lacticaseibacillus casei* ssp. *casei* G17 and *Lacticaseibacillus casei* ssp. *rhamnosus* G16 with probiotic potential were determined by modeling the dynamics of microbial growth. The studies have shown that both strains grew with relatively high maximum specific growth rates. Data from mathematical models show that *Lacticaseibacillus casei* ssp. *casei* G17 adapted more easily to fermentation conditions compared to *Lacticaseibacillus casei* ssp. *rhamnosus* G16. This leads to the conclusion that *Lacticaseibacillus casei* ssp. *rhamnosus* G16 requires growth factors in the fermentation medium. This necessitates modeling of the media composition for this strain prior to scaling up the fermentation process in semi-industrial conditions.

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