COMPARATIVE EVALUATION OF *LACTOBACILLUS PLANTARUM* STRAINS THROUGH MICROBIAL GROWTH KINETICS

Georgi Kostov*, Rositsa Denkova-Kostova**, Vesela Shopska*, Bogdan Goranov***, Zapryana Denkova***
*Department of Wine and Beer ** Department of Biochemistry and Molecular Biology, *** Department of Microbiology
University of Food Technologies, 4002, 26 Maritza Blvd., Plovdiv, Bulgaria
E-mail: george_kostov2@abv.bg; rositsa_denkova@mail.bg; vesi_nevelinova@abv.bg; goranov_chemistry@abv.bg; zdenkova@abv.bg

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ABSTRACT

The study of the growth kinetics of lactobacilli with pronounced probiotic properties in their batch cultivation is essential. Various models based on the logistic curve model, containing parameters showing the influence of the accumulating lactic acid on the biosynthesis of the product, as well as parameters showing the sensitivity of the cells to lactic acid were used to model the growth kinetics in the present work. The rate constant of adaptation of the studied strains to the used nutrient medium and the induction period were also determined. The kinetics of lactic acid synthesis was determined according to the Weibull model.

INTRODUCTION

The bacteria most commonly included as components of probiotic preparations are lactic acid bacteria (*Lactobacillus* sp., *Enterococcus* sp., *Pediococcus* sp., *Streptococcus* sp., *Lactococcus* sp., *Leuconostoc* sp.) and bifidobacteria. They are also used in the production of probiotic foods (Gibson, 2004), with the largest share being that of the lactobacilli.

Not all species of lactobacilli, as well as not all strains of the same species can be included in the composition of probiotics, but only those that have certain properties (Saarela et al., 2002): to be part of the natural microflora in humans and animals; to be able to adhere 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 be able to reproduce in the gastrointestinal tract; to have high antimicrobial activity in order to suppress and expel pathogenic and toxigenic microorganisms from the biological niche; to allow industrial cultivation - to maintain their activity during production and storage; to modulate the immune response and to be safe for clinical and nutritional use. *Lactobacillus plantarum* is a flexible and versatile species of lactic acid bacteria, which is often found in many probiotic, functional and fermented foods and beverages (cheeses, fermented milk, pasta, sausages and various vegetable juices) or is used as a probiotic (Gobbetti et . al., 1994a; Gobbetti et al., 1994b; Corsetti and Gobbetti, 2002; Guidone et al., 2014). This is due to its flexible metabolism, its ability to adapt to different environmental conditions and the wide range of antimicrobial activity it possesses (Di Cagno et al., 2009).

Along with its antimicrobial activity, the active cells of *L. plantarum* 13M5 have the ability to destroy the mycotoxin patulin at a concentration of 5 mg/dm^3 as a result of the synthesis of a bacteriocin called plantaricin (Todorov et al., 1999; Wei et al., 2020). *Lactobacillus plantarum* YJ7 shows antihyperglycemic potential and reduces insulin resistance, so it can be used in the composition of drugs targeted at people suffering from diabetes (Zhong et al., 2020). In experimental animals, *Lactobacillus plantarum* strains, and in particular *Lactobacillus plantarum* LP33, have been shown to reduce liver damage due to lead intoxication (Hu et al., 2020).

The main metabolite of lactic acid fermentation is lactic acid. It is known that its increasing concentration during fermentation has an inhibitory effect on the growth of the microbial population. The sensitivity to the accumulating lactic acid is strain-specific (Bouguettoucha et al., 2011; Gordeev et al., 2017). The selected mathematical models contain parameters characterizing the influence of lactic acid on lactobacilli. It is also important to determine both the induction period - the time from the lag phase, during which the cells begin to synthesize the necessary cellular structures and enzymes and to move from unadapted to adapted state to the composition of the medium and culture conditions, and the rate constant of adaptation (Warfolomeev and Gurevich, 1999). One of the important conditions for comparing the kinetic characteristics of the models is the initial conditions - inoculum and acidity of the medium – to be the same. Since this is difficult to achieve, it is necessary to measure the data of the biomass and the titratable acidity in the models (Tishin and Fedorov, 2016; Tishin and Golovinskaya, 2015). As a result of the above-mentioned features, the following mathematical models were chosen to model the kinetics of growth and acid formation:

$$\frac{dX_b}{d\tau} = \mu_{max}\left(1-\frac{P_b}{P_{bm}}\right)^c X_b \quad (1)$$

$$\frac{dX_b}{d\tau} = \mu_{max}\left(1-\frac{X_b}{X_{bm}}\right)^n X_b \quad (2)$$

$$\frac{dX_b}{d\tau} = \mu_{max}\left[\left(1-\frac{X_b}{X_{bm}}\right)^{n_1}\left(1-\frac{P_b}{P_{bm}}\right)^q\right] X_b \quad (3)$$

$$\ln\frac{M}{N_0} = \mu\tau + \ln\left\{\frac{k_0}{k_0+\mu}\left[1+\frac{\mu}{k_0}e^{[-(k_0+\mu)\tau]}\right]\right\} \quad (4)$$

$$K_T = a - be^{-(q_p\tau)^\delta} \quad (5)$$

where: μ_{max} - maximum specific growth rate, h^{-1}; X_b, P_b, X_{bm} and P_{bm} are the biomass, the lactic acid amount, the final concentration of the biomass and the lactic acid, respectively, in dimensionless form; M - current

biomass concentration, cfu/cm^3; N_0 - initial biomass concentration, cfu/cm^3; τ_a - induction period, h; k_0 - rate constant of cell adaptation to the medium and culturing conditions, h^{-1}; c - a parameter taking into account the inhibitory effect of the accumulating product (lactic acid) on the cell growth; n and n_1 - coefficients taking into account the influence of lactic acid on the cells, respectively showing the resistance (sensitivity) of the cells to the increasing concentration of the product; q - coefficient showing the inhibitory effect of the product, lactic acid, on its own synthesis; K_T - titratable acidity in dimensionless form; *a* - maximum value of the titratable acidity in dimensionless form; *b* - coefficient equal to the difference between the maximum and initial titratable acidity in dimensionless form; q_P - specific rate of acid formation, h^{-1}; δ - an indicator determining the change in the shape of the curve or the change in the rate of accumulation of lactic acid over time; τ - cultivation time, h.

The presented models make it possible to determine the parameters of the fermentation process analytically. Moreover, they allow the assession of the influence of cultivation conditions and the accumulation of lactic acid on the microbial population.

The aim of the present work was to study the lactic acid fermentation process with selected probiotic *Lactobacillus plantarum* strains by applying modified dependences of the logistic curve type and assessing the influence of acid formation on the lactic acid fermentation process.

MATERIALS AND METHODS

Microorganisms and cultivation conditions

The study was conducted with four different strains of *Lactobacillus plantarum*: *Lactobacillus plantarum* 4/17, *Lactobacillus plantarum* 3, *Lactobacillus plantarum* 10 and *Lactobacillus plantarum* 1/18, isolated from spontaneously fermented vegetables. The 4 strains were identified by molecular-genetic identification method – 16S rDNA sequencing – as representatives of the *Lactobacillus plantarum* species (unpublished data).

Cell cultivation was performed under static conditions in flasks using LAPTg10-broth medium. Samples were periodically taken to determine the titratable acidity of the medium and the number of viable lactobacilli cells.

Nutrient media

- LAPTg10-broth;
- MRS-agar;
- Saline solution.

Methods of analysis

- Determination of titratable acidity (ISO/TS 11869:2012);
- Number of viable lactobacilli cells (ISO 7889:2005).

Identification of the model parameters

The logistic curve models from 1 to 3 are solved numerically using the Runge-Kuta method of the 4th row, and the parametric identification in them is performed by minimizing the sum of the squares of the difference between the experimental data and the data obtained from the corresponding model in Microsoft Excel (Choi et al., 2014). The parametric identification of model 4 and the Weibull model was performed using the software Curve Expert Professional by nonlinear regression.

RESULTS AND DISCUSSION

Table 1 presents the data from the determination of the induction period and the rate constant of adaptation, determined according to equation 4.

Table 1: Induction period and rate constant of adaptation

Strain	τ_a, h	k_0, h^{-1}
L. plantarum 4/17	0.36	0.256
L. plantarum 3	0.73	0.253
L. plantarum 10	0.88	0.227
L. plantarum 1/18	1.43	0.103

The strains *L. plantarum* 4/17 and *L. plantarum* 3 have a significantly shorter induction period (0.36 h and 0.73 h, respectively) and higher values of the rate constant of adaptation (0.256 h^{-1} and 0.253 h^{-1}, respectively), compared to the other two strains studied (Table 1). The longest induction period of 1.43 h and the lowest rate constant of adaptation (0.103 h^{-1}) was observed for *L. plantarum* 1/18, while *L. plantarum* 10 occupied an intermediate place with an induction period of 0.88 h and a rate constant of adaptation of 0.227 h^{-1}.

From the studies conducted it can be concluded that *L. plantarum* 4/17 would most quickly adapt to the fermentation medium and cultivation conditions, followed by *L. plantarum* 3 and *L. plantarum* 1/18. This shows that the fermentation medium used for these strains has an optimal composition and is suitable for their growth. The slower adaptation of *L. plantarum* 10 compared to other strains may be due to the lack of some substrates in the medium, values of the redox potential and temperature regime different from the optimal ones for the specific strain studied. Another reason for the lower values of the rate constant of adaptation and the longer induction period is probably the static nature of the medium, which is characterized by a lack of surface aeration, which for some species of *L. plantarum* has certain stimulating effect, as many strains of this species are microaerophiles.

Table 2A presents the kinetic parameters of the used logistic curve models (equation 1 to equation 3). Table 2B presents the correlation coefficients and errors of the models. The comparison of the models with the experimental data is presented in Fig. 1 to Fig. 4. As a general conclusion, the three models used describe the experimental data from the cultivation of the four *L. plantarum* strains with very high accuracy. Similarly, the model used to describe the kinetics of lactic acid accumulation describes very well the experimental data (equation 5). This general conclusion can be explained by the fact that, unlike numerical methods for determining the kinetic parameters in the analytical solution of differential equations, the number of parameters in the model is minimized, and the obtained parameters have a

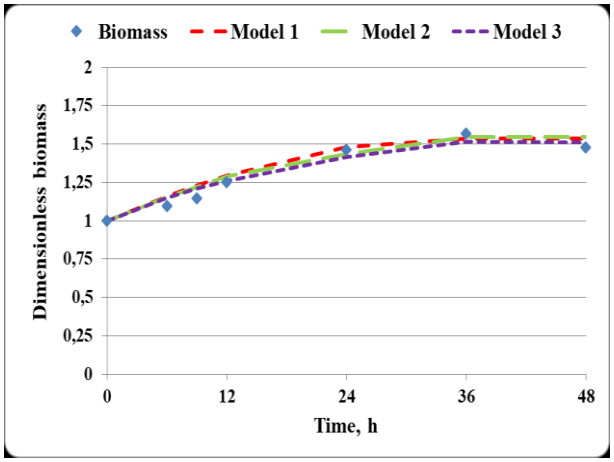
clear biological meaning. It is this biological meaning that we will demonstrate by interpreting the results for the four strains studied. Another reason for increasing the accuracy of the models is the dimensionless form of the biomass, which reduces the identification error. In this way, the influence of dimensionality and the influence of random errors in the enumeration of microorganisms according to the methodology for determining the concentration of viable cells is avoided.

Table 2A: Kinetic parameters in the different logistic curve models in the cultivation of the *Lactobacillus plantarum* strains

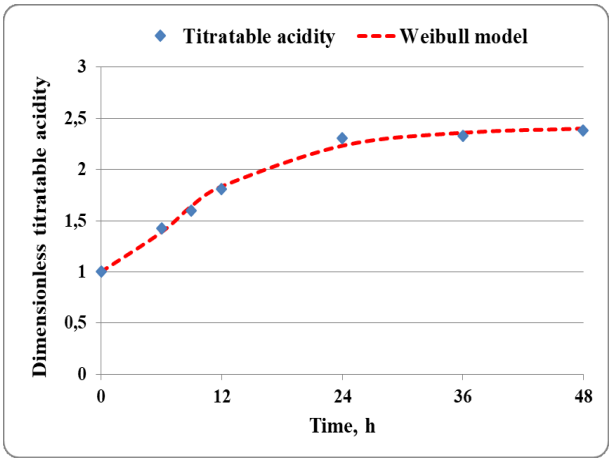
Strain	Mathematical model										
	Model 1 (eq.1)			Model 2 (eq.2)			Model 3 (eq.3)				
	μ_m, h^{-1}	c	P_m	μ_m, h^{-1}	n	X_m	μ_m, h^{-1}	n_1	q	P_m	X_m
4/17	0.0198	0.346	1.98	0.0210	3.661	2.41	0.0181	3.208	0.117	2.81	2.30
3	0.0190	0.329	2.48	0.0476	1.703	2.83	0.0383	1.257	0.116	2.78	2.54
10	0.0220	0.368	2.38	0.0740	1.135	2.16	0.0400	0.980	0.130	2.85	2.45
1/18	0.0190	0.448	2.83	0.0156	0.857	1.87	0.0470	0.478	0.621	1.91	2.64

Table 2B: Correlation coefficients and errors

Strain	Mathematical model					
	Model 1 (eq.1)		Model 2 (eq.2)		Model 3 (eq.3)	
	R^2	Error	R^2	Error	R^2	Error
4/17	0.9364	0.078	0.9405	0,078	0.9474	0.074
3	0.9115	0.174	0.9378	0.160	0.9461	0.159
10	0.9022	0.135	0.8200	0.155	0.9406	0.159
1/18	0.8853	0.153	0.9497	0.122	0.9495	0.123

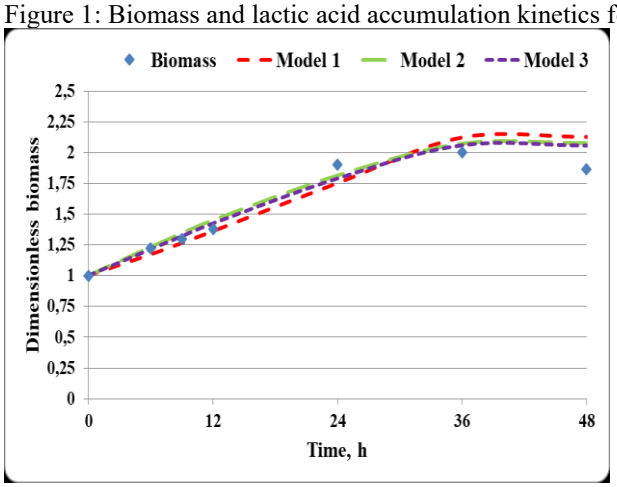


a) biomass

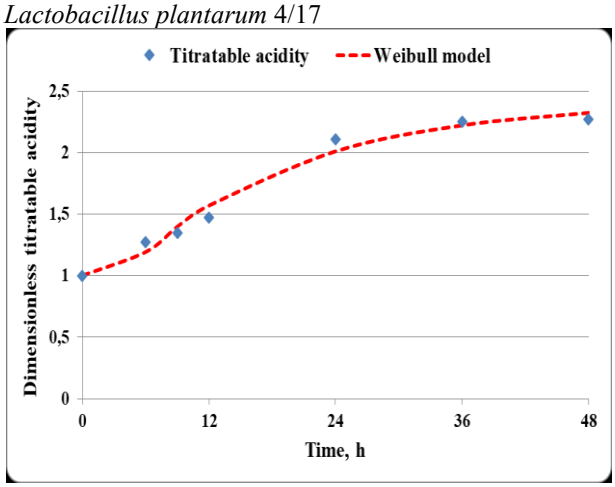


b) lactic acid

Figure 1: Biomass and lactic acid accumulation kinetics for *Lactobacillus plantarum* 4/17



a) biomass

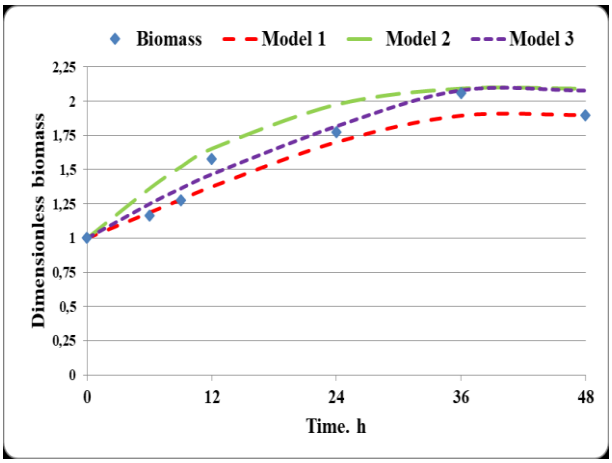


b) lactic acid

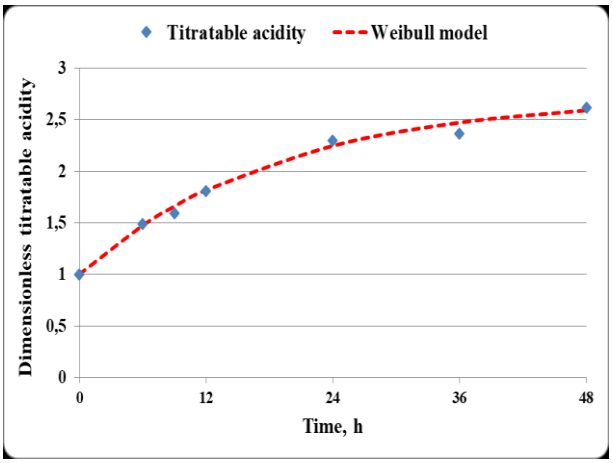
Figure 2: Biomass and lactic acid accumulation kinetics for *Lactobacillus plantarum* 3

In all four studied strains the increasing concentration of lactic acid would have a less pronounced inhibitory effect on the maximum specific growth rate (Table 2). This is underlined by the relatively low values of the parameter ***c*** in model 1. In *L. plantarum* 4/17, *L. plantarum* 3 and *L. plantarum* 10 this parameter has comparable values - 0.346, 0.329 and 0.368, respectively. A higher value of the parameter ***c*** is observed in *L. plantarum* 1/18 - 0.448. The observed higher value of the parameter can be explained by the higher concentration of lactic acid accumulated by the strain. This is evidenced by the value of the parameter **Pm**, which is 2.83 and its value is the highest one among the **Pm** values in all the four *L. plantarum* strains studied. The lowest final concentration of lactic acid in dimensionless form is observed for *L. plantarum* 4/17 - 1.98, while for *L. plantarum* 3 and *L. plantarum* 10 it has comparable values - 2.48 and 2.38, respectively. According to the data from model 1, *L. plantarum* 10 has the highest maximum specific growth rate of 0.0220 h^{-1}. The remaining three strains are characterized by lower and commensurable maximum specific growth rates, varying in the range from 0.0190 h^{-1} to 0.0198 h^{-1}.

It is interesting to determine the effect of lactic acid on the cultivation process. Model 2 in which the parameter **n** is subjected to identification is used to achieve this goal. This parameter shows the effect of lactic acid on the biomass, or, more precisely, the resistance of the cells to the accumulating lactic acid. The data are summarized in Table 2.

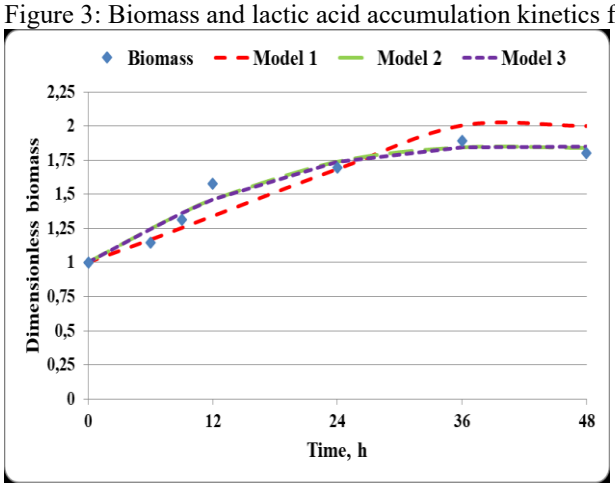


a) biomass

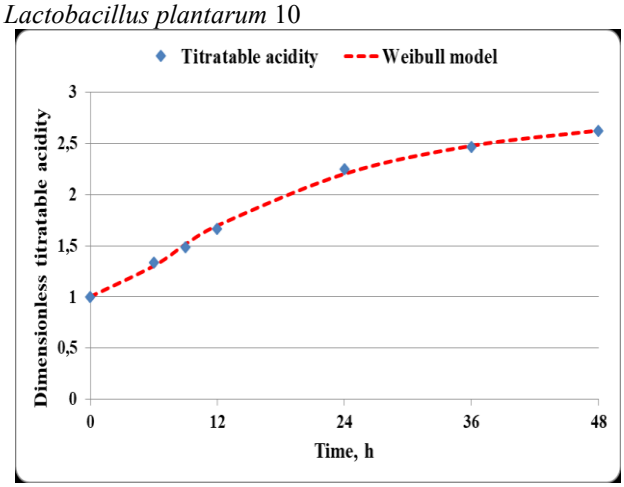


b) lactic acid

Figure 3: Biomass and lactic acid accumulation kinetics for *Lactobacillus plantarum* 10



a) biomass



b) lactic acid

Figure 4: Biomass and lactic acid accumulation kinetics for *Lactobacillus plantarum* 1-18

The cells of *L. plantarum* 4/17 (Table 2) show the highest sensitivity, respectively the lowest resistance to the increasing concentration of lactic acid. For this strain the parameter **n** is 3.661. *L. plantarum* 1/18 shows the lowest sensitivity to the increasing concentration of lactic acid (n = 0.857). *L. plantarum* 3 and *L. plantarum* 10 have intermediate resistance to the metabolic product, with the values of **n** being 1,703 and 1,135, respectively. According to model 2, the highest maximum specific growth rate was observed for *L. plantarum* 10 (μ_m = ***0.0740 h^{-1}***), followed by *L. plantarum* 3 (μ_m = ***0.0470 h^{-1}***). *L. plantarum* 4/17 and *L. plantarum* 1/18 have lower values of the maximum specific growth rate - μ_m = ***0.0210 h^{-1}*** and μ_m = ***0.0156 h^{-1}***, respectively.

According to the data from model 2, the highest final concentration of biomass in dimensionless form is achieved by *L. plantarum* 3 - 2.83. In *L. plantarum* 4/17 and *L. plantarum* 10 the maximum concentration of biomass in dimensionless form is 2.41 and 2.16, respectively, and in *L. plantarum* 1/18 it is 1.87.

The meaning of parameter $\mathbf{n_1}$ in Equation 3 is similar. According to this model, *L. plantarum* 4/17 is again characterized by the highest sensitivity to the accumulating lactic acid. In this strain $\mathbf{n_1}$ is 3.208. The next strain in terms of cell sensitivity to the increasing concentration of lactic acid is *L. plantarum* 3, which is also characterized by a high value of the parameter $\mathbf{n_1}$ (1.257). However, in *L. plantarum* 10 model 3 predicted a value of the parameter $\mathbf{n_1}$ below 1, namely 0.980 (the value of the analogous parameter **n** in model 2 is 1.135). This shows that according to model 3, the strain is characterized by increased resistance (reduced sensitivity) to the increasing concentration of lactic acid. The highest resistance to lactic acid is demonstrated by *L. plantarum* 1/18, characterized by the lowest value of the parameter $\mathbf{n_1}$ (0.478) (Table 2A).

The comparative assessment of the resistance of different *Lactobacillus plantarum* strains to lactic acid is important for the characterization of the strains. In general, it can be assumed that strains that have higher resistance to lactic acid would also have a higher survival rate at low pH in the human gastrointestinal tract. This is especially important for the selection of strains, to be included in the composition of probiotic preparations, because resistance to low pH is one of the most important requirements to potentially probiotic strains.

An important parameter in model 3 is the parameter **q**. It reflects the inhibitory effect of lactic acid on its synthesis rate. In *L. plantarum* 4/17, *L. plantarum* 3 and *L. plantarum* 10 this parameter has low values - 0.117, 0.116 and 0.130, respectively (Table 2). This in turn shows high intensity of acid formation and a relatively weak inhibitory effect of the acid on its synthesis rate.

In contrast, in *L. plantarum* 1/18 the value of q is 0.621. This means that the increasing acid concentration would have stronger inhibitory effect on the lactic acid synthesis rate. The high value of **q** characterizes this strain with lower energy of acid formation and therefore the process of acid formation would be more moderate, and the least amount of lactic acid in dimensionless form is accumulated in the medium - 1.91.

The other three strains are characterized by high values of the final concentration of lactic acid in dimensionless form – from 2.78 to 2.85. Unlike model 2, here the values of the biomass in dimensionless form at the end of the fermentation process vary in a relatively small range - from 2.30 to 2.64 (Table 2A).

In order to confirm the assumptions about the acid-forming ability of the studied strains, the specific rate of acid formation q_p and the degree of change in the intensity of lactic acid accumulation over time in general (δ) were calculated. The results of the conducted modeling are presented in Table 3 and Table 4 and the models for the studied strains are shown in real form.

The results in Table 3 once again confirm the conclusions made about the acid-forming ability of the studied strains. According to the Weibull model, *L. plantarum* 4/17, *L. plantarum* 3 and *L. plantarum* 10 have high values of the parameter δ - 2.55, 1.55 and 1.88, respectively. This indicates that in these strains the acid-formation process would proceed with a higher intensity over time in general, compared to *L. plantarum* 1/18. In *L. plantarum* 1/18 δ has a value less than 1, namely 0.99, which again confirms that in this strain the acid formation would be more moderate in time as a whole, although in this strain the Weibull model predicts a slightly higher rate of lactic acid synthesis (0.054 h^{-1}) compared to the other strains. In the other strains, the specific rate of acid formation occupies close values and varies in the range from 0.027 h^{-1} to 0.054 h^{-1} for the different strains.

Table 3: Kinetic parameters in the Weibull model in the cultivation of the *Lactobacillus plantarum* strains

Strain	a	b	q_p, h^{-1}	Δ	R^2	Error
4/17	2.42	1.82	0.037	2.55	0.9933	0.20
3	2.42	1.77	0.041	1.51	0.9803	0.20
10	2.81	2.04	0.027	1.88	0.9981	0.23
1/18	2.72	1.72	0.054	0.99	0.9900	0.22

Table 4: Weibull's mathematical models in real form

Strain	Models in real form
L. plantarum 4/17	$K_T = 2{,}42 - 1{,}82e^{(-0{,}032\tau)^{2{,}55}}$
L. plantarum 3	$K_T = 2{,}42 - 1{,}77e^{(-0{,}047\tau)^{1{,}51}}$
L. plantarum 10	$K_T = 2{,}81 - 2{,}04e^{(-0{,}027\tau)^{1{,}88}}$
L. plantarum 1/18	$K_T = 2{,}72 - 1{,}72e^{(-0{,}054\tau)^{0{,}99}}$

CONCLUSION

Some important conclusions can be drawn for the modeling of the fermentation processes and in particular lactic acid fermentation, from the obtained results. The data show that the use of only one kinetic model does not show all aspects of the lactic acid fermentation process. Combining several mathematical dependencies makes it possible to consider different aspects of the process. For example, equation 4 allows the estimation of the time for adaptation of the culture and the possibility for the real process to start faster. Equations 1 to 3 make it possible to assess the various aspects of the fermentation process - the accumulation of biomass, the influence of lactic

acid, both on the biomass growth and on the acid-formation rate.

The possibility of the models used to assess the sensitivity of the strains to their own metabolic product allows the selection of high-resistant strains to be used in the composition of probiotic preparations, but also the selection of strains that produce less lactic acid and can be used in food development and production.

Therefore, the modeling of the fermentation process must be done with at least two dependencies that reflect the different aspects of the modeled process. Thus, is it possible to achieve a complete interpretation of the various aspects of fermentation. In addition, the dependencies proposed in the present paper allow the estimation of kinetic parameters to be done through simple analytical dependencies. This allows for faster process management decisions.

The main purpose of the present study was to allow the evaluation of different strains of lactic acid bacteria with a view to their use in the production of different types of functional foods. Knowledge of the fermentation kinetics and the behavior of the strains under different cultivation conditions makes it possible to model the fermentation process, and hence the composition of the obtained functional foods.

In this regard, the results allow the strains to be divided into two groups - strains with high growth rate (strain 10), strains with moderate growth rate and high rate of acid formation (strain 1/18) and strains with moderate growth rate and moderate acid formation (strains 4/17 and 3). Depending on the specific food production, the choice may fall on different groups of strains. In some cases, the functional characteristics of a specific food product are determined by the high concentration of viable cells, while in other cases - by the lactic acid produced by the lactic acid bacteria strains and, hence, accumulated in the food product. In this sense, the combination of different models to describe the kinetics of microbial growth allows for improved options for selection and management of the process of functional food production.

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AUTHOR BIOGRAPHIES

GEORGI KOSTOV is Professor at the Department of Wine and Beer Technology at the University of Food Technologies, Plovdiv. He received his MSc degree in Mechanical Engineering in 2007, a PhD degreein Mechanical Engineering in the Food and Flavor Industry (Technological Equipment in the Biotechnology Industry) in 2007 at the University of Food Technologies, Plovdiv, and holds a DSc degree in Intensification of Fermentation Processes with Immobilized Biocatalysts. His research interests are in the area of bioreactor construction, biotechnology, microbial population investigation and modeling, hydrodynamics and mass transfer problems, fermentation kinetics, and beer production.

VESELA SHOPSKA is Head Assistant Professor at the Department of Wine and Beer Technology at the University of Food Technologies, Plovdiv. She received her MSc degree in Wine-making and Brewing Technology in 2006 at the University of Food Technologies, Plovdiv. She received her PhD in Technology of Alcoholic and Non-alcoholic Beverages (Brewing Technology) in 2014. Her research interests are in the area of beer fermentation with free and immobilized cells, yeast and bacteria metabolism and fermentation activity.

ROSITSA DENKOVA-KOSTOVA is Head Assistant Professor at the Department of Biochemistry and Molecular Biology at the University of Food Technologies, Plovdiv. She received her MSc degree in Industrial Biotechnologies in 2011 and a PhD degree in Biotechnology (Technology of Biologically Active Substances) in 2014. Her research interests are in the area of isolation, biochemical and molecular-genetic identification and selection of probiotic strains and development of starters for functional foods.

BOGDAN GORANOV is an assistant at the department of Microbiology at the University of Food Technologies, Plovdiv. He received his PhD in 2015 from the University of Food Technologies, Plovdiv. The theme of his thesis was "Production of Lactic Acid with Free and Immobilized Lactic Acid Bacteria and its Application in the Food Industry". His research interests are in the area of bioreactor construction, biotechnology, microbial population investigation and modeling, hydrodynamics and mass transfer problems, and fermentation kinetics.

ZAPRYANA DENKOVA is a professor at the department of Microbiology at the University of Food Technologies, Plovdiv. She received her MSc in "Technology of microbial products" in 1982, PhD in „Technology of biologically active substances" in 1994 and DSc on "Production and application of probiotics" in 2006. Her research interests are in the area of selection of probiotic strains and development of starters for food production, genetics of microorganisms, and development of functional foods.

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